Two Decades of TB Drug Discovery Efforts—What Have We Learned?

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Abstract: After several years of limited success, an effective regimen for the treatment of both drug-sensitive and multiple-drug-resistant tuberculosis is in place. However, this success is still incomplete, as we need several more novel combinations to treat extensively drug-resistant tuberculosis, as well newer emerging resistance. Additionally, the goal of a shortened therapy continues to evade us. A systematic analysis of the tuberculosis drug discovery approaches employed over the last two decades shows that the lead identification path has been largely influenced by the improved understanding of the biology of the pathogen Mycobacterium tuberculosis. Interestingly, the drug discovery efforts can be grouped into a few defined approaches that predominated over a period of time. This review delineates the key drivers during each of these periods. While doing so, the author’s experiences at AstraZeneca R&D, Bangalore, India, on the discovery of new antimycobacterial candidate drugs are used to exemplify the concept. Finally, the review also discusses the value of validated targets, promiscuous targets, the current anti-TB pipeline, the gaps in it, and the possible way forward.

Keywords: tuberculosis; Mycobacterium tuberculosis; drug discovery; drug development; target-based screening; phenotypic screening; antituberculosis agents; antimycobacterial; anti-TB drug pipeline; privileged targets; promiscuous targets; lead generation
reflected in the current set of drugs that are in late-stage development or have been recently introduced into the anti-TB regimen.

The anti-TB drugs currently in use, and those in the late stages of clinical development, can be broadly pooled into the following groups:

1. Serendipitous drug discovery—early chemotherapy;
2. Modification of drug scaffolds;
3. Revisiting targets that have clinically validated drugs against them (referred to as established targets);
4. Target-based screening;
5. Phenotypic screening.

1. Serendipitous Drug Discovery: Early Chemotherapy

The first successful chemotherapy and cure of an infectious disease is indeed the discovery and design of the ‘first line’ therapy for the treatment of tuberculosis—the design and development of which was completed in the 1960s. Even today, this regimen is the therapy of choice for treating drug-sensitive tuberculosis (DSTB). The drugs in the first-line treatment for DSTB, isoniazid, rifampicin, pyrazinamide, and ethambutol became anti-TB drugs based on their activity on *Mycobacterium tuberculosis* (MTB) cells in vitro, followed by testing in animal models and their rapid introduction into humans [1]. This progression was driven by the medical need, as no chemotherapy existed before these drugs were discovered.

It is interesting to note that two biological observations and a hypothesis on potential ‘chemical structures’ that may interfere with the observed biological process were the first starting points of anti-TB drug discovery. Aspirin was shown to be a potent stimulator of the TB bacilli’s ‘oxygen consumption’; analogs of aspirin were then postulated to be inhibitors of this process. This led to the synthesis of a number of aspirin-like structures, of which para-aminosalicylic acid (PAS) became a successful anti-TB drug [1–3]. The second observation was that niacin helped in the recovery of guinea pigs infected with MTB, as well as the observation that niacin helped in faster recovery of TB patients, raising the possibility that niacin was acting as a ‘vitamin’ [4,5]. Chemical synthesis focused on making derivatives of niacin led to the design of three anti-TB drugs, namely isoniazid (Inh), pyrazinamide (Pza) and ethionamide (Eth) [6]. Two of these are even today the most potent drugs for the treatment of TB. This early period of chemotherapy also included extensive search for natural products with antibacterial activity; Rifamycin and Streptomycin were natural products that showed potent activity against MTB cells and were also introduced into the treatment of TB.

In 1979, Mitchison observed that 10 to 12 drugs were available for the treatment of tuberculosis, which could be classified in terms of their effectiveness [7]. The choice of combinations was dictated by animal toxicity of the individual drugs and in human trials, which assessed time taken to the sputum negative state, cure as reflected by relapse rates, the emergence of resistant strains, and compliance. Once an effective combination had been proven, it became to be referred to as the ‘Short Course Chemotherapy’ and was adopted systematically all over the world [8]. This was the first successful conquering of an infectious disease. In about 20 years, TB patients went from complete helplessness to an effective cure achieved with drug treatment.

The key learning from this pioneering era was as follows:

1. In vitro MIC (Minimum Inhibitory Concentration) was insufficient to predict efficacy in humans. Requirement for a combination therapy of drugs.
2. The best regimen required six months of treatment to achieve cure.
3. Each of the drugs in the combination had a unique role to play in leading to the cure.

The cure was defined as the lack of relapse. The last two points remain, to date, the biggest challenge in finding and developing novel combinations. The era of 1950 to 1980 had 10 drugs, of which the most efficacious combination of four
drugs was identified through rapid testing in humans. In spite of more than two decades of sustained research into the biology of MTB, the traits of a new drug that could contribute to both the ‘cure’ and the shortening of therapy are still unknown.

2. Modification of Drug Scaffolds: Analogs of Known Drugs or the Literature Compounds

The initiation points for this approach were natural product scaffolds or scaffolds known to have activity against MTB cells in vitro but did not possess drug-like properties. Efforts to address this were mainly medicinal-chemistry driven, focused on understanding structure–activity relationship (SAR) on potency and animal toxicity. The advances in chemistry in terms of novel reactions and the use of combinatorial chemistry resulted in rapid diversification of key scaffolds to yield potent analogs. Some of the examples of scaffolds were the nitroimidazoles and several newer rifampicins, isoniazid, and ethambutol analogs [9–12].

2.1. Nitroimidazole as a Starting Point

Among the diverse scaffolds tested in this approach, the ‘nitroimidazole’ starting point has been the most successful. The antibiotic 5-nitroimidazole was used for treating bacterial infections of the gut and was also shown to be active on anaerobic bacteria [13]. Metronidazole, a drug still in use for the treatment of amoebic infections, was one of the first successful derivatives of 5-nitroimidazole. CGI-17341, a bicyclic imidazofuran, was one of the derivatives and was found to be a potent anti-Tb molecule [14]. Continued chemistry on this molecule led to several analogs, among which PA-824 (Pretomanid) [15], OPC-67683 (Delamanid) [16] have recently been registered as anti-TB drugs and are constituents of the current Multi drug resistant (MDR) regimen [15,16]. This progression is shown in Figure 1.

![Figure 1. The progression of 5-Nitroimidazole derivatives.](image)

2.2. Rifampicin Analogs

Rifampicin was obtained through the chemical modification of the natural product rifamycin [17]. Derivatives of rifampicin, like rifabutin, were synthesized and found to have favorable properties in terms of compatibility with anti-HIV drugs but could not overcome the cross-resistance with rifampicin [18].

2.3. Ethambutol Derivatives

Increased throughput in chemistry also contributed to finding new leads. A combinatorial library created around ethambutol led to the discovery of a clinical candidate SQ-109 [19,20]. SQ-109 was found to be active even on ethambutol-resistant strains of MTB. SQ109 has been shown to target the
Mycobacterial Membrane Protein Large 3 (Mmpl3) [21]. It is interesting to note that ethambutol does not inhibit Mmpl3.

2.4. Isoniazid Analogs

Among the many Inh analogs synthesized, Sudoterb (LL 3858) was successfully progressed to Phase 1 [22,23]. Inh, because of its simplicity of structure, remains an attractive starting point for analog-based discovery.

The approach involving modifications of existing drug scaffolds has two important aims: firstly, to discover novel analogs that are active on MTB strains resistant to the parent drug, and secondly, to design ‘drug-friendly’ molecules. This approach has yielded two, drugs and a third is in development: Pretomanid (Pre), Delamanid (Del), and SQ-109, respectively.

The key learning from these examples is that sustained effort can lead to useful drugs, even though the starting scaffolds have issues.

3. Revisiting Established Targets: Revisiting Targets Proven as Druggable by Using Broad-Spectrum Compounds

TB was declared a global emergency by WHO in 1996 [24]. The emergence of drug-resistant TB and the complete lack of drugs capable of treating patients with MDR TB led the drug development community to investigate alternate approaches to rapidly induct novel drugs into the regimen. This prompted investigations into the feasibility of introducing the existing broad-spectrum antibacterial drugs into the TB treatment regimen. Several antibacterial classes that have been shown to be active on MTB in vitro were investigated in clinical trials.

This approach, which is now classified as ‘repurposing’, has successfully delivered new options for TB treatment. The key classes that have added to the anti-TB portfolio are the following:

3.1. Protein-Synthesis Inhibitors

Streptomycin, a protein-synthesis inhibitor and a well-established anti-TB drug, has been shown to be effective in treating MTB patients, but its use is limited because of it not being an oral drug and the toxicities associated with it for prolonged use [25]. Several novel protein-synthesis inhibitors that have been approved as antibacterials were also tested for their antmycobacterial activity. The oxazolidinone [26] class of compounds, despite its limitations of myelotoxicity, hold a significant position in the treatment of MDR and Extensively drug-resistant tuberculosis (XDR)TB in the current anti-TB treatment regimen. Linezolid [27,28] is currently a part of the drug regimen for the treatment of MDR TB, XDR and non-responding TB (NRTB), while newer oxazolidinones like Posizolid [29–31] and Sutezolid [32,33] have been tested in advanced clinical trials. Newer oxazolidinones like the Delpazolid [34] and Conteozolid [35] are also in clinical trials.

3.2. Beta Lactams as Antimycobacterials

Several broad-spectrum antibacterials like meropenem, a beta-lactam, have also been shown to have activity against MTB in in vitro models, as well as in studies measuring Early Bactericidal Activity (EBA) in humans [36].

The key ‘unknowns’ in the development of broad-spectrum antibiotics as anti-TB treatment are twofold:

- Priming of resistance against the antibiotic among normal gut bacteria due to the long-term treatment required for TB. This priming could lead to the selection of resistant mutants and subsequently spread of resistance to other pathogens in the gut.
- Effect of the antibiotic on ‘latent MTB bacteria’: Latent bacteria could also be primed, leading to probability of a drug-resistant infection on reactivation.
Despite these concerns, even after several years of the use of rifampicin for the treatment of Drug sensitive (DS) TB and moxifloxacin for the treatment of MDR TB, the extent of ‘priming’ caused is not clear, and some of the fears could well be unfounded. This could also be a reflection of the use of these drugs only in combinations or our inability to monitor the impact systematically.

The key learning from this approach of including broad-spectrum antibiotics into the combination regimen to treat MTB patients has been as follows:

- Drugs with several new targets, like the ones discussed above, can be introduced into novel combinations, thus enabling the treatment of drug resistant (DR) MTB patients.
- The effectiveness of drugs like moxifloxacin or linezolid establish the vulnerability of the target, thus promoting the search for new compounds that can inhibit the same target. This approach of revisiting ‘established/vulnerable targets’ continues to be explored by using several newer assets, like new libraries, which are novel screening formats, including those enabled by the availability of the molecular structures. Two novel compounds that have entered clinical development, GSK070 shown in Figure 2a [37] and SPR20 shown in Figure 2b [38], are examples of this approach. GSK 3036656 (GSK-070) belongs to the oxaborole class of compounds and has been shown to be a Leucine tRNA synthase inhibitor. The compound is currently in Phase 2 clinical trials [37]. SPR 720 is a GyrB ATPase inhibitor that belongs to the benzimidazole class. The molecule is also in Phase 2 clinical trials [38,39]. A very recent report shows that SPR 720 obtained an orphan disease status from FDA to treat non-tuberculosis mycobacteria (NTM) [40].

![GSK 3036656 (GSK 070)](image1)

![SPR720 (VXc-100, VXc-486)](image2)

**Figure 2.** Two novel compounds that have entered clinical development (a) GSK070 and (b) SPR20.

### 3.3. Gyrase Inhibitors

The fluoroquinolone class (Moxifloxacin, Levofloxacin, Ofloxacin, and Gatifloxacin) of compounds are potent inhibitors of the DNA gyrase enzyme and are proven antibacterials. Several of these were shown to be active on the MTB bacilli in vitro. Researchers at the National Tuberculosis Institute, India, tested the usefulness of ofloxacin as a part of the anti-TB regimen and showed it to be effective in the clinical trial [41]. Multiple members of this class of compounds have undergone clinical trials as part of an anti-TB regimen; moxifloxacin [42] is now a part of the standard regimen to treat drug-resistant TB infections. Section 3.4 covers the target-based TB drug discovery efforts at AstraZeneca, with major emphasis on gyrase inhibitors.

### 3.4. The AstraZeneca India (AZI) Effort

**Gyrase as a target:** One of the favorite targets for anti-TB drug discovery is the ‘gyrase enzyme’. This is because of the multiple steps involved in the mechanistic of the ‘negative supercoiling’ enzyme reaction, several steps of which have been shown to be inhibitable [43]. Additionally, the availability of the several crystal structures of the enzyme has also helped in developing diverse screening approaches, as well as in building SAR of the identified inhibitors.
AZI employed multiple ‘hit’ generation approaches like high-throughput screening (HTS) of the AZ library, fragment library screening, targeted library screening, and pharmacophore-based screening, as well as virtual screening, in the quest for robust novel inhibitors. Shirude and Hameed [44] reviewed the features of the diverse set of inhibitors identified by the different groups. Several novel chemical entities were identified and are being investigated further (Table 1).

Table 1. Gyrase inhibitors identified at AstraZeneca (AZ).

| Approach                        | Target | Inhibitor Series       | Mechanism of Inhibition                        | Reference |
|---------------------------------|--------|------------------------|-----------------------------------------------|-----------|
| Following known series          | GyrB   | Pyrrolamides           | ATPase inhibitor                               | [45]      |
| Pharmacophore based library     | GyrB   | Thiazolopyridine ur eas | ATPase inhibitor                               | [46]      |
| Pharmacophore based library,   | GyrB   | Thiazolopyridone ur eas| ATPase inhibitor                               | [47]      |
| scaffold morphing               |        |                        |                                               |           |
| High throughput screening       | GyrB   | Aminopyrazinamides      | ATPase, MTB gyrase specific. Novel binding mode.| [48]      |
| Focused library screening       | GyrB   | Aminopiperidine         | Non-ATP site binders, different from FQs       | [49]      |
| Scaffold hopping                | GyrB   | Benzimidazoles          | Non-ATP site binders, different from FQs       | [50]      |

The key learning from these extensive efforts on ‘revisiting gyrase as an established target’ are as follows:

- A variety of novel chemical structures could be identified as potent starting points (Table 1).
- Several of these enzyme inhibitors showed an IC_{50}-MIC correlation.
- The inhibitors worked through different mechanisms; hence, they have a potential to avoid cross-resistance.
- These inhibitors were shown to have a higher potency against the MTB enzyme target, as compared to other bacterial gyrase, that translated into selectivity in their antimicrobial activity.

4. Target-Based Screening

The target-based lead identification approach was given a major impetus because of the following developments:

- The availability of the MTB genome sequence in 1998 [51] promised a new era both for studying the ‘biology’ of the pathogen and investigating novel pathways suitable for drug development. Several publications appeared, proving the essentiality of biochemical targets based on gene knockout studies in vitro, as well as investigations on the survival of the gene knockouts of MTB in the mouse model, confirming the essentiality of a variety of metabolic targets in vivo [52].
- Chris Lipinski et al. published the ‘Lipinski rule of 5’ for oral drugs [53]. The poor physiochemical properties of lead compounds vis a vis the ‘Lipinski rule of 5’ was shown to be the leading reason for the failure of potent compounds in the clinical trials, which was the direct result of poor pharmacokinetics. This led to the understanding of the concept of ‘lead-like compounds’ [54]. These rules served as guidelines for the selection/prioritization of ‘hits’ with a higher probability of being converted to drugs with favorable pharmacokinetics.
• This period also saw an enhanced efficiency in solving crystal structures; the macromolecular structures of several mycobacterial enzymes were solved and became key tools for the rationale design of novel inhibitors. A consortium of structural genomics groups was formed for facilitating the determination and analysis of structures of proteins from MTB [55].

• In addition to these facilitators of drug development, several public–private partnership consortia were also formed, which propelled interactions between the pharmaceutical industry and academic laboratories to engage in the TB drug discovery process. Examples include ‘The Action TB program’ (1996); the ‘Global Alliance for TB’ (GATB), which was launched in 2000; and the EU FW6/7 program entitled ‘New Medicines for Tuberculosis’ (NM4TB), which was launched in 2005, followed by the ‘More Medicines for Tuberculosis’ (MM4TB) program. Several pharmaceutical companies turned to target-based screening to find novel ‘lead compounds’ with a potential to inhibit the growth of MTB cells [56].

• Examples of efforts at AZI toward finding potent inhibitors by using target-based screening are summarized in Table 2. Though there are several publications on these efforts, it is interesting to view them in the context of the learning and the impact. A few of these programs were also in collaboration between AZI and partners of the NM4TB and MM4TB, the EU FW6, and seven programs, as well as with the Global Alliance for TB.

### Table 2. Target-based efforts at AstraZeneca.

| Approach         | Target          | Pathway                             | Inhibitor Class                  | Status Reached | Remarks                   | Reference |
|------------------|-----------------|-------------------------------------|----------------------------------|----------------|---------------------------|-----------|
| Known inhibitors | Acetolactate    | Branched chain amino acid biosynthesis | Triazolopyrimidine              | IC₅₀=30 nM MIC=0.5 µg/mL | Possible auxotrophy        | [57,58]  |
| Virtual screening | MtSK            | Aromatic amino acid biosynthesis    | Pyrazolones                      | IC₅₀=0.6 µM MIC=0.5 µg/mL | Possible promiscuity        | [59,60]  |
| Targeted Library | Pkn B           | Cell division                       | Bis anilinopyrimidines (BAP)    | IC₅₀=40 pM MIC=8 µg/mL | Possible redundancy         | [61]      |
| HTS              | PanK            | Coenzyme A (CoA) biosynthesis pathway | Triazoles (ATP competitive)    | Nanomolar IC₅₀, not translating into microbial inhibition. | Possible Target vulnerability | [62–64]  |
| HTS              | PanK            | Coenzyme A (CoA) biosynthesis pathway | Quinolones (ATP competitive)    | Nanomolar IC₅₀, not translating into microbial inhibition. | Possible Target vulnerability | [62–64]  |
| HTS              | PanK            | Coenzyme A (CoA) biosynthesis pathway | Biarylacetic acids (ATP non-competitive) | Nanomolar IC₅₀, not translating into microbial inhibition. | Possible Target vulnerability | [62–64]  |
| HTS              | TMK             | DNA synthesis                       | 3-Cyanopyridine                 | Nanomolar IC₅₀, not translating into microbial inhibition. | Possible redundancy         | [65]      |
| Fragment screening | TMK            | DNA synthesis                       | 1,6-Naphthyridinone             | Nanomolar IC₅₀, not translating into microbial inhibition. | Possible redundancy         | [65]      |
| HTS              | NdH2            | Energy Metabolism                   | Quinolyl pyrimidines            | NdH2 IC₅₀: 96 nM MIC: <0.5 µg/mL | In progress                | [66]      |

The key learning derived from these extensive studies are as follows:

a. The patterns obtained throw important light on both the characteristics of the target and the characteristics of the inhibitor.

b. The question of target vulnerability: Gene knockout studies for PanK demonstrated the essentiality of the gene product. However, elegant studies with the PanK target showed that modulation of enzyme levels (inferred based on regulating gene expression) altered the sensitivity of the MTB cell to inhibitors of the enzyme [62]. This change in vulnerability of the target was the reason attributed to the failure to obtaining bactericidal effects with the potent inhibitors.
c. Type of inhibition: Most of the inhibitors described in Table 2 were ‘competitive’ in nature, competing with the ATP site for binding. High-throughput screening mostly identifies ‘competitive inhibitors’ because of the design of the assay. The inhibition brought about by this type of inhibition is influenced by the ‘substrate concentration’, resulting in the modulation of target vulnerability [65].

d. Promiscuous targets: These types of targets are discussed later, in the lead generation approaches.

e. Established target: As described under Section 3.4, several inhibitors that have progressed to anti-TB drugs establish the ‘vulnerability’ of a target; ATP synthase (ATPS) is one such target. Bedaquiline, a non-competitive inhibitor of ATPS, is a front-line treatment for MDR TB [67,68].

5. Phenotypic Screening

This is a biology-driven effort where compound libraries are screened for antimycobacterial activity against mycobacterial cells in culture, and the ‘hits’ are progressed based on the potency both in vitro and in vivo [69]. This cell-based approach has been successful from the early days of TB discovery, and most of the frontline drugs were progressed based on the structure–activity relationship that was evaluated directly on the whole cells. Current-day phenotypic-screening approaches include the ability to identify inhibitors that are active against MTB cells growing in a variety of microenvironments that represent the replicating, the non-replicating, or bacilli in different physiological states [70]. Genetic probes have also facilitated the unravelling of the biological targets of the inhibitors.

Some of the examples of phenotypic-screening-derived compounds that have become drugs, or those in late clinical development, are discussed here.

a. Bedaquiline: Approved in 2012 for the treatment of MDR TB, Bedaquiline (TMC-207, R207910, shown in Figure 3) has the reputation of being the first FDA approved TB drug in more than four decades. It was discovered by Johnson & Johnson by screening more than 70,000 compounds against Mycobacterium smegmatis (M.sm). The compound was first described in 2004, at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). The target of TMC-207 was later conclusively proven to be the MTB ATP synthase [68]. Subsequently, in 2012, Bedaquiline became an essential part of the MDR regimen [71,72]. Several combinations of Bedaquiline are also being investigated for their potential to reduce the duration of therapy [73]. Discovery of Bedaquiline and the target link has made MTB ATP synthase an attractive validated target.

![Bedaquiline](image1)

Figure 3. Structure of Bedaquiline (TMC-207, R207910).

b. Benzothiazinone (BTZ) and Macozinone (PBTZ): BTZ was discovered at the Russian academy of science and developed with NM4TB, and it is presently being clinically evaluated under the Innovative Medicines for Tuberculosis (iM4TB). The target for BTZ has been shown as decaprenylphosphoryl-β-d-ribose-2’-oxidase (DprE1) by genetic and biochemical studies [74,75]. Based on SAR explorations, a piperazine derivative, PBTZ-169 (Macozinone), which has superior pharmacokinetics (PK), as compared to that of BTZ [76], was discovered and is currently in Phase 2 clinical trials, in addition to BTZ 043. Structures of BTZ and PBTZ are shown in Figure 4.
The compound had an MIC but was not bactericidal. Scaffold morphing efforts on this compound at AZ Bangalore in a collaboration with TB alliance led to the identification of TBA 7371 [79–81], a potent DprE1 inhibitor with non-covalent binding. TBA 7371, jointly owned by Global Alliance for TB and the Foundation for Neglected Disease Research (FNDR), was developed by GATB through Phase 1 safety trials in humans. Recently, GATB has licensed TBA 7371 to Gates Medical Research Institute (GMRI); the news was disclosed in Union World conference of lung health at Hyderabad, India, in November 2019. GMRI is currently testing TBA7371 in Phase 2A clinical trials in TB patients [82]. The progression is depicted in Figure 5.

c. **Azaindoles:** These compounds emerged from a literature search for compounds similar to Q-203 [77,78]. The compound had an MIC but was not bactericidal. Scaffold morphing efforts on this compound at AZ Bangalore in a collaboration with TB alliance led to the identification of TBA 7371 [79–81], a potent DprE1 inhibitor with non-covalent binding. TBA 7371, jointly owned by Global Alliance for TB and the Foundation for Neglected Disease Research (FNDR), was developed by GATB through Phase 1 safety trials in humans. Recently, GATB has licensed TBA 7371 to Gates Medical Research Institute (GMRI); the news was disclosed in Union World conference of lung health at Hyderabad, India, in November 2019. GMRI is currently testing TBA7371 in Phase 2A clinical trials in TB patients [82]. The progression is depicted in Figure 5.

d. **Q203:** Telacebec (Q203) is a compound that was discovered by the researchers at Institute Pasteur, Korea, and is structurally very similar to the one discussed above [77,78]. This compound inhibits the cytochrome bc1 complex of MTB that is critical for the electron transport chain. The compound is in Phase 2 clinical trials [83]. Structure of Telacebec is shown in Figure 6.
e. **OPC-167832**: First discussed in 2016 at the 47th Union World Conference on Lung Health in Liverpool, UK, this is a carbostyril derivative that inhibits DprE1. The structure shown in Figure 7 represents a balance of hydrophobic and hydrophilic residues. The compound is currently in Phase 2 clinical trials [84]. Structure of OPC-167832 is shown in Figure 7.

![Structure of OPC-167832](image)

**Figure 7. Structure of OPC-167832.**

**Phenotypic-Screening Efforts at AstraZeneca India**

The continued success of phenotypic screening as a viable alternative to discover novel inhibitors of MTB was based on its ability to circumvent the failure to convert enzyme inhibition into antibacterial activity. The advent of ‘rapid sequencing technology’ provided an impetus to this approach because of the ability to rapidly sequence the genome to elucidate mechanism of action. This led AZ India to focus on phenotypic screening for lead generation. A diversity library of 100,000 compounds was assembled and used for screening directly against both *M. smegmatis* or *Mycobacterium bovis* BCG cells as surrogates for MTB. Several novel scaffolds and their cognate targets were identified, confirming the validity of this approach; a short list of the results obtained is shown in Table 3. Many of the novel scaffolds identified were found to target DprE1 or the ATP synthase in target enzyme assays.

| Screening Mode | Target      | Compound                     | Comments               | Reference          |
|----------------|-------------|------------------------------|------------------------|--------------------|
| HTS            | DprE1       | Benzothiazole                | Promiscuous target     | [85,86]            |
| HTS            | DprE1       | 4-Aminoquinoline piperidine amides | Promiscuous target     | [87]               |
| HTS            | DprE1       | Pyrazolopyridones           | Promiscuous target     | [88]               |
| HTS            | DprE1       | Azaindoles                  | Promiscuous target     | [79,80]            |
| Scaffold hopping| DprE1     | Benzimidazole                | Promiscuous target     | [81]               |
| HTS            | ATPS        | Squaramide                  | Established/Validated Target | [89]            |
| HTS            | ATPS        | Imidazopyridine             | Established/Validated Target | [89]            |
| Scaffold morphing | ATPS  | Diaminoquinazoline          | Established/Validated Target | [90]            |

**Table 3. Phenotypic screening-based efforts at AstraZeneca India (AZI).**

6. **The Anti-TB Pipeline—The Learning and the Gaps**

6.1. **The ‘Evolution’ of the Anti-TB Drug Pipeline**

The anti-TB drug portfolio went from a ‘no treatment’ option to a successful therapy in ~20 years. The main properties of drugs in the regimen that was labeled ‘Short Course Chemotherapy’ were potency against the microbe, the ability to prevent relapse, and compatibility in terms of safety. The next phase, ~1980s, was the search for a compound that could shorten the course of the treatment. In the absence of ‘physiologically’ relevant in vitro and in vivo models to progress compounds for this property, it was indeed a ‘black box’ experimentation. However, with the spread of MDR TB, this focus turned to finding compounds ‘active on MDR TB’ and compatibility with the ‘patient comorbidities’,...
like HIV infection [91]. The concept of introducing ‘broad-spectrum antibiotics’ for the treatment of TB was a direct consequence of this shift; even the original trial that included ‘ofloxacin’ into the regimen was for reducing the duration of therapy [25]. Studies in animal models with a combination containing moxifloxacin showed that the combination had a potential to reduce the duration of therapy [92], and this was one of the end points in the trials with moxifloxacin in humans. However, the final results showed that, while the drug was effective, the combination did not reduce the duration of therapy, although it was efficacious on MDR, as well as on DS TB patients. The drug was introduced into the regimen to treat MDR TB [93].

The need for efficacious drugs for treating MDR TB became the ‘new end point’ for a drug. Both Bedaquiline and Delamanid fulfilled this criterion and are now parts of the new regimen [67,94].

There has been a concerted effort to understand the ‘persister’ population that is hypothesized to be difficult to eradicate and hence the need for prolonged duration of therapy [95,96]. The non-replicating persister (NRP) state has been the subject of intense investigations and several models representing this state have been developed. Both Bedaquiline and Delamanid are active against MTB bacilli growing under such conditions [16,67]. Further trials to investigate if this property of the new drugs will contribute to the reduction in therapy duration needs to be performed. In parallel, the portfolio is sure to see ‘adjunct therapy’ compounds that modulate the host response [92–95]. Repurposed candidate drugs offer faster development options and are also expected to enrich the TB portfolio [97–103]. These could also be weapons against the ‘persister’ population.

6.2. Lead Generation Approaches

The evolution of the anti-TB drug regimen, including the current portfolio, has followed a traceable pattern of periods. These periods are recognizable by the ‘classes’ of compounds that were introduced into the regimen. The different lead generation approaches and their accelerators are shown in Figure 8. Accelerators are defined as new knowledge or global exigencies. Each of these accelerators influenced lead generation methodology, to follow a certain approach during a certain period.

![Figure 8. Evolution of lead generation approaches.](image)

What is the current trend of preclinical ‘hit’ molecules being identified by using phenotypic screening? Mdluli et al. [104] have discussed this trend which indicates that the majority of these ‘hits’ act on the targets, namely DprE1, MmpL3, and Cytochrome bcc complex. This has led to the coining of the term ‘promiscuous targets’. The AstraZeneca ‘hit’ compound list also shows the same trend (Table 3). Why are these targets prone to get hit in phenotypic screens? The ultimate consequence of the so called ‘promiscuity’ on drug discovery will be fully answered by the clinical trial outcome of the compounds TBA737, BTZ043, PBTZ-169, OPC 167832 (DprE1 and MmpL3), and Q203 (bcc1 complex) hitting such targets [105].
Another interesting trend is the increased number of compounds in later stages of development that are inhibitors of ‘established targets’. This again adds to the concept of ‘privileged targets’ of MTB (privileged because the inhibition of these targets converts to an antimicrobial effect on MTB). Perhaps the so-called ‘promiscuous targets’ can also be classified as ‘privileged targets’.

6.3. Has the Pipeline Evolved Based on the Significant Increase in the Knowledge of the ‘Biology’ of MTB?

In 1996, Mitchison in his Garrod lecture postulated the presence of different populations of MTB in the lungs of the TB patient [106]. Mitchison postulated the presence of the rapidly multiplying and the slow-growing populations of MTB, as well as the intracellular and extracellular niches of the organism. The last two decades have uncovered several shifts in the physiological state of the pathogen that are responses to both the immune mechanism of the host or the ‘niche’ in which the pathogen is proliferating [107]. The newer drugs like Bedaquiline, Pretomanid, and Delamanid have been shown to be active against MTB cells in the NRP state in vitro, as well as against the MTB bacilli multiplying within the macrophage [108,109]. However, these drugs were initially identified as inhibitors of the rapidly multiplying pathogen under in vitro culture conditions. It is generally accepted that the MTB population in the human host is a heterogeneous collection of the multiple ‘physiological states’ [69] that could indeed be a dynamic interchanging state. This raises two questions:

1. What is the value of having novel drugs that are active predominantly on one of the physiological states like the hypoxic or the intracellular state? Metronidazole is a known antiparasitic drug that is also active on MTB growing under oxygen-limiting conditions [110]. The addition of metronidazole to a cocktail of drugs for the treatment of MDR TB did not show clinical benefit [111]. On the other hand, several inhibitors of the intracellular survival of the pathogen in the macrophage have been identified, have been shown to be active in animal models of infection [34,67,77,83,112], and are undergoing clinical evaluation. These inhibitors potentially will facilitate the ‘killing’ of the pathogen within the macrophage, which in turn may reflect in ‘faster’ cure.

2. How do we develop molecules with activity against only specific subpopulations? The accepted path through the regulatory system is by showing superiority of the new combination over the existing standard of care (SOC) combination—a steep bar to cross. Until the beginning of this decade, very few efficacious drugs were available for the treatment of MDR TB patients. This provided the opportunity for newer efficacious drugs to show non-inferiority against the poorly active regimen, a bar, which was reasonable to go across. However, the current combination regimen for MDR TB treatment is efficacious, and newer compounds will have to demonstrate a clear advantage over the current regimen. Identification of novel biomarkers that can be evaluated during Phase 2a (Early Bactericidal Activity trials) could help in positioning these compounds that are active on specific populations to the best advantage.

Undoubtedly, there is a rapid expansion in the knowledge of the pathogen biology and its interaction with the host. Additionally, several novel inhibitors for pathways of the host that influence the survival of the bacilli have been identified. However, translational research must be supported to evaluate the potential of these compounds to become anti-TB drugs.

6.4. Combination Therapy: Finding Novel Combinations

What constitutes an ‘ideal’ combination? Faster time for sputum conversion, faster cure, tolerability, lower rates of drug resistance, lower relapse rates, combination suitable for treating pulmonary and extrapulmonary TB, and other properties, like compatible with comorbidities, etc. The concept of searching for novel combinations is recent; it was first advocated by the GATB [113] by studying a variety of combinations in humans using EBA. The two recently approved drugs, Bedaquiline and Delamanid, were progressed as add-ons to a cocktail of second-line drugs. Pretomanid, in combination
with Bedaquiline and linezolid, has been approved for the treatment of XDR TB patients for whom there is very limited choice [73].

Another question is, can compounds active on the same target be compatible in the same combination? It is possible if the two inhibitors bind to different sites of the same targets, like in the examples of BTZ, PBTZ, and TBA7371. If the resistant mutants for the individual compounds do not confer cross-resistance, can such an approach bring additional advantage to the treatment? This can be relevant in the context of the so-called ‘promiscuous targets’ in TB and the diverse chemical inhibitors of these targets reported in recent years, e.g., benzothiazinones, azaindoles, and Q 203.

7. Going Forward

It is interesting to view the current anti-TB portfolio as a glass ‘half full’. Novel efficacious compounds are in the late stages of clinical development [114–116]. This will offer the opportunity of designing new combinations. Several novel molecules are in the late discovery phase that will diversify the repertoire of potential anti-TB molecules. The challenge is to find simpler paths through the regulatory system that can demonstrate advantages with the molecule, as well as ensure safety. This will need concerted discussion with multiple stakeholders, which is already happening [117,118].

The secret to ‘shortening therapy’ needs to be unraveled, whether this will continue to be a hit-and-miss experimentation or further knowledge of the biology of the pathogen and will allow rational experimentation is not clear. Can adjunct therapy with immuno-modulators help, or will compounds acting on subpopulations that represent the ‘difficult to treat’ cells facilitate faster cure? Such questions need to be evaluated. This requires an urgent need to find novel translational approaches compatible with the regulatory framework to achieve this shift that, in turn, can create a paradigm shift in our modus operandi of how we treat this affliction.

8. Conclusions

Finding new drugs for the treatment of tuberculosis has been and continues to be a challenge despite the increased efforts over the last two decades. Lead generation approaches for MTB drug discovery have undergone several changes, mostly driven by an increased understanding of the biology of the pathogen, as well as the rapidly expanding knowledge on the biology of the interaction of the pathogen with the human host. This review chronicles the advances in a systematic study, starting from ‘serendipitous discovery to phenotypic screening’. The technological advance mirrors the increased understanding of the biology of the pathogen. Interestingly, much of this increased understanding is also a consequence of the introduction of newer drugs for the treatment of tuberculosis.

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References
1. TB Facts.org, Information about Tuberculosis. Available online: https://tbfacts.org/history-of-tb-drugs/ (accessed on 13 April 2020).
2. Lehmann, J. Para-aminosalicylic acid in the treatment of Tuberculosis. *Lancet* 1946, 247, 15–16. [CrossRef]
3. Lehmann, J. Twenty years afterwards, Historical notes on the Discovery of the Antituberculosis Effect of Para-Aminosalicylic Acid (PAS) and the First Clinical Trials. *Am. Rev. Respir. Dis.* 1964, 90, 953–956.
4. Lehmann, E. Nicotinic acid in therapy of pulmonary tuberculosis; preliminary therapeutic report. *Dtsch. Med. Wochenschr.* 1952, 77, 1480–1481. [CrossRef]
5. Murray, F.M. Nicotinamide: An Oral Antimicrobial Agent with Activity against Both Mycobacterium tuberculosis and Human Immunodeficiency Virus. *Clin. Infect. Dis.* 2003, 36, 453–460. [CrossRef]

6. Thayer, J.D.; Seligman, R.B. The anti-tuberculous activity of some derivatives of p-aminosalicylic acid, nicotinic acid, and isonicotinic acid. *Antibiot. Chemother. (Northfield)* 1955, 5, 129–131.

7. Mitchison, D.A. Treatment of tuberculosis. The Mitchell lecture 1979. *J. R. Coll. Physicians Lond.* 1980, 14, 91.

8. Aquinas, M. Short-course therapy for tuberculosis. *Drugs* 1982, 24, 118–132. [CrossRef]

9. Mohammad, A. Rifampin and Their Analogs: A Development of Antitubercular Drugs. *World J. Org. Chem.* 2013, 1, 14–19.

10. Ramani, A.V.; Monika, A.; Indira, V.L.; Karyavardhi, G.; Venkatesh, J.; Jeankumar, V.U.; Manjashetty, T.H.; Yogeesswari, P.; Sriram, D. Synthesis of highly potent novel anti-tubercular isoniazid analogues with preliminary pharmacokinetic evaluation. *Bioorg. Med. Chem. Lett.* 2012, 22, 2764–2767. [CrossRef]

11. Yamamoto, S.; Toida, I.; Watanabe, N.; Ura, T. In vitro Antimycobacterial Activities of Pyrazinamide Analogs. *Antimicrob. Agents Chemother.* 1995, 39, 2088–2091. [CrossRef]

12. Häusler, H.; Kawakami, R.P.; Mlaker, E.; Severn, W.B.; Stütz, A.E. Ethambutol Analogues as Potential Antimycobacterial Agents. *Bioorg. Med. Chem. Lett.* 2001, 11, 1679–1681. [CrossRef]

13. Goldman, P. The development of 5-nitroimidazole for the treatment and prophylaxis of anaerobic bacterial infections. *J. Antimicrob. Chemother.* 1982, 10, 23–33. [CrossRef]

14. Ashtekar, D.R.; Costa-Perira, R.; Nagarajan, K.; Vishwanathan, N.; Bhatt, A.D.; Rittel, W. In vitro and in vivo activities of the nitroimidazole CGI 17341 against Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* 1993, 37, 183–186. [CrossRef]

15. Stover, C.K.; Warrener, P.; Van Devanter, D.R.; Sherman, D.R.; Arain, T.M.; Langhorne, M.H.; Anderson, S.W.; Towell, J.A.; Yuan, Y.; McMurray, D.N.; et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* 2000, 405, 962–966. [CrossRef]

16. Liu, Y.; Matsumoto, M.; Ishida, H.; Ohguro, K.; Yoshitake, M.; Gupta, R.; Geiter, L.; Hafkin, J. Delamanid: From discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). *Tuberculosis* 2018, 111, 20–30. [CrossRef]

17. Sensi, P. History of the development of rifampin. *Rev. Infect. Dis.* 1983, 5, S402–S466. [CrossRef]

18. Janin, Y.L. Antituberculosis drugs: Ten years of research. *Bioorg. Med. Chem.* 2007, 15, 2479–2513. [CrossRef]

19. Bogatcheva, E.; Hanrahan, C.F.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Barbosa, F.; Einck, L.; Nacy, A.C.A.; Protopopova, M. Identification of new diamine scaffolds with activity against Mycobacterium tuberculosis. *J. Med. Chem.* 2006, 49, 3045–3048. [CrossRef]

20. Yendapally, R.; Lee, R.E. Design, synthesis, and evaluation of novel ethambutol analogues. *Bioorg. Med. Chem. Lett.* 2008, 18, 1607–1611. [CrossRef]

21. Kapil, T.; Regina, W.; David, B.K.; Kriti, A.; Vinod, N.; Elizabeth, F.; Barnes, S.W.; John, R.W.; David, A.; Clifton, E.B., III; et al. SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* 2012, 56, 1797–1809.

22. Sinha, N.; Jain, S.; Tilekar, A.; Upadhyayaya, R.S.; Kishore, N.; Jana, G.H.; Arora, S.K. Synthesis of isonicotinic acid N’-arylidene-N-2-oxo-2-(4-aryl-piperazin-1-yl) ethyl-hydrazides as antituberculosis agents. *Bioorg. Med. Chem. Lett.* 2005, 15, 1573–1576. [CrossRef]

23. Sinha, R.K.; Arora, S.K.; Sinha, N.; Modak, V.M. In vivo activity of LL4858 against Mycobacterium tuberculosis. In Proceedings of the 44th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC-2004), Washington, DC, USA, 30 October–2 November 2004.

24. World Health Organization. *Tuberculosis—A Global Emergency Case Notification Update: February 1996*; WHO Reference Number: WHO/TB/96.197; WHO: Geneva, Switzerland, 1996.

25. Hinshaw, H.; Pyle, M.M.; Feldman, W.H. Streptomycin in tuberculosis. *Am. J. Med.* 1947, 2, 429–435. [CrossRef]

26. Schecter, G.F.; Scott, C.; True, L.; Raftery, A.; Flood, J.; Mase, S. Linezolid in the Treatment of Multidrug-Resistant Tuberculosis. *Clin. Infect. Dis.* 2010, 50, 49–55. [CrossRef]

27. Maartens, G.; Benson, C.A. Linezolid for Treating Tuberculosis: A Delicate Balancing Act. *BioMedicine* 2015, 2, 1568–1569. [CrossRef]

28. Lee, M.; Song, T.; Kim, Y.; Jeong, I.; Cho, S.N.; Barry, C.E., III. Linezolid for XDR-TB—Final Study Outcomes. *N. Engl. J. Med.* 2015, 373, 290–291. [CrossRef]
29. Balasubramanian, V.; Solapure, S.; Shandil, R.; Gaonkar, S.; Mahesh, K.N.; Reddy, J.; Deshpande, A.; Bharath, S.; Kumar, N.; Wright, L.; et al. Pharmacokinetic and pharmacodynamic evaluation of AZD5847 in a mouse model of tuberculosis. *Antimicrob. Agents Chemother.* 2014, 58, 4185–4190. [CrossRef]

30. Balasubramanian, V.; Solapure, S.; Iyer, H.; Ghosh, A.; Sharma, S.; Kaur, P.; Deepthi, R.; Subbulakshmi, V.; Ramya, V.; Ramachandran, V.; et al. Bactericidal activity and mechanism of action of AZD5847, a novel oxazolidinone for treatment of tuberculosis. *Antimicrob. Agents Chemother.* 2013, 58, 495–502. [CrossRef]

31. Werngren, J.; Wijkander, M.; Perskvist, N.; Balasubramanian, V.; Sambandamurthy, V.K.; Rodrigues, C.; Hoffner, S. In vitro activity of AZD5847 against geographically diverse clinical isolates of Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* 2014, 58, 4222–4223. [CrossRef]

32. Williams, K.N.; Brickner, S.J.; Stover, C.K.; Zhu, T.; Ogden, A.; Tasneem, R.; Tyagi, S.; Grosse, J.H.; Nuermberger, E.L. Addition of PNU-100480 to First-Line Drugs Shortens the Time Needed to Cure Murine Tuberculosis. *Am. J. Respir. Crit. Care Med.* 2009, 180, 371–376. [CrossRef]

33. Lanoix, J.P.; Nuermberger, E. Sutezolid: Oxazolidinone antibacterial treatment of tuberculosis. *Drugs Future* 2013, 38, 387–394. [CrossRef]

34. Choi, Y.; Lee, S.W.; Kim, A.; Jang, K.; Nam, H.; Cho, Y.L.; Yu, K.-S.; Chung, J.-Y. Safety, tolerability and pharmacokinetics of 21 day multiple oral administration of a new oxazolidinone antibiotic, LCB01-0371, in healthy male subjects. *Antimicrob. Chemother.* 2018, 73, 183–190. [CrossRef]

35. Shoen, C.; DeStefano, M.; Hafkin, B.; Cynamon, M. In vitro and in vivo activity of contezolid (MRX-I) against M. tuberculosis. *Antimicrob. Agents Chemother.* 2018, 62, e00493-18. [CrossRef]

36. ClinicalTrials.gov. Phase 2 Trial to Evaluate the Early Bactericidal Activity, Safety and Tolerability of Meropenem Plus Amoxycillin/CA and Faropenem Plus Amoxycillin/CA in Adult Patients with Newly Diagnosed Pulmonary Tuberculosis. Available online: https://clinicaltrials.gov/ct2/show/NCT02349841 (accessed on 15 March 2020).

37. Palencia, A.; Li, X.; Bu, W.; Choi, W.; Ding, C.Z.; Easom, E.E.; Feng, L.; Hernandez, V.; Houston, P.; Liu, L.; et al. Discovery of Novel Oral Protein Synthesis Inhibitors of Mycobacterium tuberculosis That Target Leucyl-tRNA Synthetase. *Antimicrob. Agents Chemother.* 2016, 60, 6271–6280. [CrossRef]

38. Shoen, C.; DeStefano, M.; Pucci, M.; Cynamon, M. Evaluating the Sterilizing Activity of SPR720 in Combination Therapy against Mycobacterium Tuberculosis Infection in Mice, ASM Microbe 2019. Session 336. Poster 43. Available online: https://www.newtbdrugs.org/pipeline/presentation/compound/spr720 (accessed on 19 March 2020).

39. Shoen, C.; Pucci, M.; DeStefano, M.; Cynamon, M. Efficacy of SPR720 and SPR750 Gyrase Inhibitors in a Mouse Mycobacterium tuberculosis Infection Model, ASM Microbe 2017. Session 336. Poster 43. Available online: https://www.abstractsonline.com/pp8/#I4358/presentation/6167 (accessed on 25 March 2020).

40. Spero Therapeutics Receives FDA Orphan Drug Designation for SPR720 for the Treatment of on tuberculosis Mycobacterial (NTM) Infection. Available online: https://www.globenewswire.com/news-release/2020/03/11/1998722 (accessed on 6 April 2020).

41. Narayanan, P. Shortening short course chemotherapy: A randomised clinical trial for treatment of smear positive pulmonary tuberculosis with regimens using ofloxacin in the intensive phase. *Indian J. Tuberc.* 2002, 49, 27–38.

42. Stephen, H.G. The role of moxifloxacin in tuberculosis therapy. *Eur. Respir. Rev.* 2016, 25, 19–28.

43. 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]

44. Shirude, P.S.; Hameed, S. Nonfluoroquinolone-Based Inhibitors of Mycobacterial Type II topoisomerase as Potential Therapeutic Agents for TB. *Annu. Rep. Med. Chem.* 2012, 47, 319–330.

45. Solapure, S.; Mukherjee, K.; Nandi, V.; Waterson, D.; Shandil, R.; Balganesh, M.; Sambandamurthy, V.K.; Raichurkar, A.K.; Deshpande, A.; Ghosh, A.; et al. Optimization of Pyrrolamides as Mycobacterial GyrB ATPase Inhibitors: Structure-Activity Relationship and In Vivo Efficacy in a Mouse Model of Tuberculosis. *Antimicrob. Agents Chemother.* 2014, 58, 61–70.

46. Kale, M.G.; Raichurkar, A.; Waterson, D.; McKinney, D.; Manjunatha, M.R.; Kranthi, U.; Koushik, K.; Jena, L.K.; Shinde, V.; Rudrapatna, S.; et al. Thiazolopyridine Ureas as Novel Antitubercular Agents Acting through Inhibition of DNA Gyrase B. *J. Med. Chem.* 2013, 56, 8834–8848.
47. Kale, R.R.; Kale, M.G.; Waterson, D.; Raichurkar, A.; Hameed, S.P.; Manjunatha, M.R.; Reddy, B.K.; Malolanarasimhan, K.; Shinde, V.; Koushik, K.; et al. Thiazolopyridoneureas as DNA gyrase B inhibitors: Optimization of antitubercular activity and efficacy. *Bioorg. Med. Chem. Lett.* 2014, 24, 870–879. [CrossRef]

48. Shirude, P.S.; Madhavapeddi, P.; Tucker, J.A.; Murugan, K.; Patil, V.; Basavarajappa, H.D.; Raichurkar, A.V.; Humnabadkar, V.; Hussein, S.; Sharma, S.; et al. Aminopyrazinamides: Novel and Specific GyrB Inhibitors that Kill Replicating and Nonreplicating Mycobacterium tuberculosis. *ACS Chem. Biol.* 2013, 8, 519–523.

49. Hameed, P.S.; Patil, V.; Solapure, S.; Sharma, U.; Madhavapeddi, P.; Raichurkar, A.; Chinnapattu, M.; Manjrekar, P.; Shanbhag, G.; Puttur, J.; et al. Novel N-Linked Aminopiperidine-Based Gyrase Inhibitors with Improved hERG and in Vivo Efficacy against Mycobacterium tuberculosis. *J. Med. Chem.* 2014, 57, 4889–4905.

50. Hameed, P.S.; Raichurkar, A.; Madhavapeddi, P.; Menasinakai, S.; Sharma, S.; Kaur, P.; Nandishaih, R.; Panduga, V.; Reddy, J.; Sambandamurthy, V.K.; et al. Benzimidazoles: Novel Mycobacterial Gyrase Inhibitors from Scaffold Morphing. *ACS Med. Chem. Lett.* 2014, 5, 820–825. [CrossRef]

51. Cole, S.T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S.; 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]

52. Vashisht, R.; Bhat, A.G.; Kushwaha, S.; Bhardwaj, A.; OSDD Consortium; Brahmachari, S.K. Systems level mapping of metabolic complexity in Mycobacterium tuberculosis to identify high-value drug target. *J. Transl. Med.* 2014, 12, 263. [CrossRef]

53. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 1997, 23, 3–25. [CrossRef]

54. Teague, S.J.; Davis, A.M.; Leeson, P.D.; Oprea, T. The Design of Lead like Combinatorial Libraries. *Angew. Chem. Int. Ed. Eng.* 1999, 28, 3743–3748. [CrossRef]

55. Terwilliger, T.C.; Park, M.; Waldo, G.; Berendzen, J.; Hung, L-W.; Kim, C.-Y.; Smith, C.; Sacchettini, J.; Bellinzone, M.; Bossi, R.; et al. The TB structural genomics consortium: A resource for Mycobacterium tuberculosis biology. *Tuberculosis* 2003, 83, 223–249. [CrossRef]

56. Yuan, T.; Sampson, N.S. Hit Generation in TB Drug Discovery: From Genome to Granuloma. *Chem. Rev.* 2018, 118, 1887–1916. [CrossRef]

57. Patil, V.; Kale, M.; Raichurkar, A.; Bhaskar, B.; Prahlad, D.; Balganes, M.; Nandan, S.; Shahul Hameed, P. Design and synthesis of triazolopyrimidineacylsulfonamides as novel anti-mycobacterial leads acting through inhibition of acetohydroxyacid synthase. *Bioorg. Med. Chem. Lett.* 2014, 24, 2222–2225. [CrossRef]

58. Balganes, M.; Nandan, S. Combination Chemotherapy for Tuberculosis by Synergistic Action of Rifampicin as the RNA Polymerase Inhibitors with Acetolactate Synthase Inhibitors. PCT International Application WO 2007132189 A1 20071122, 22 November 2007.

59. Bandodkar, B.S.; Naik, M.; Ghorpade, S.; Kale, M.; Shanbhag, G.; Patil, V.; Solapure, S.; Balganes, M.; Shandil, R.K.; Balasubramanian, B.; et al. Lead Generation via Virtual Screening: Discovery of Pyrazolones as Potent Antimycobacterial Leads through structure based virtual screening of shikimate kinase. In Proceedings of the ICAAC 2009, San Francisco, CA, USA, 12–15 September 2009.

60. Bandodkar, B.S.; Schmitt, S. Pyrazolone Derivatives for the Treatment of Tuberculosis. PCT International Application No. WO/2007/020426 A1 20070204, 22 February 2007.

61. Bandodkar, B.S. Oral presentation, “A decade of learning”. In Proceedings of the CSIR-NM4TB Symposium, Bangalore, India, 14 December 2009.

62. Venkatraman, J.; Bhat, J.; Solapure, S.M.; Sandesh, J.; Sarkar, D.; Aishwarya, S.; Mukherjee, K.; Datta, S.; Malolanarasimhan, K.; Bandodkar, B.; et al. Screening, Identification, and Characterization of Mechanistically Diverse Inhibitors of the Mycobacterium Tuberculosis Enzyme, Pantetheine Kinase (CoaA). *J. Biomol. Screen.* 2012, 17, 293. [CrossRef] [PubMed]

63. Reddy, B.K.K.; Landge, S.; Ravishankar, S.; Patil, V.; Shinde, V.; Tantry, S.; Kale, M.; Raichurkar, A.; Menasinakai, S.; Mudugal, N.V.; et al. Assessment of Mycobacterium tuberculosis Pantetheine Kinase Vulnerability through Target Knockdown and Mechanistically Diverse Inhibitors. *Antimicrob. Agents Chemother.* 2014, 8, 3312–3326. [CrossRef]
83. Pethe, K.; Bifani, P.J.; Jang, J.; Kang, S.; Park, S.; Ahn, S.; Jiricek, J.; Jung, J.; Jeon, H.K.; Cechetto, J.; et al. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat. Med.* 2013, 19, 1157–1160. [CrossRef]

84. Otsuka Awarded Grant to Advance Development of Novel Anti-Tuberculosis Compound OPC-167832 with Delamanid. Available online: https://www.businesswire.com/news/home/20180129005737/en/ (accessed on 11 April 2020).

85. Landge, S.; Mullick, A.B.; Nagalapur, K.; Neres, J.; Subbulakshmi, V.; Murugan, K.; Ghosh, A.; Sadler, C.; Fellows, M.D.; Humnabadkar, V.; et al. Discovery of benzothiazolines as antitubercular agents: Synthesis, structure–activity relationships and binding studies with Mycobacterium tuberculosis decaprenylphosphoryl-b-D-ribose 20-oxidase. *Biorg. Med. Chem.* 2015, 23, 7694–7710. [CrossRef]

86. Landge, S.; Ramachandran, V.; Kumar, A.; Neres, J.; Murugan, K.; Sadler, C.; Fellows, M.D.; Humnabadkar, V.; Vachaspati, P.; Raichurkar, A.; et al. Nitroarenes as Antitubercular Agents: Stereoelectronic Modulation to Mitigate Mutagenicity. *Chemmedchem* 2016, 11, 331–339. [CrossRef] [PubMed]

87. Naik, M.; Humnabadkar, V.; Tantry, S.J.; Markad, S.D.; Gupta, A.K.; Narayan, A.; Guptha, S.; Panduga, V.; Manjrekar, P.; Jena, L.K.; Koushik, K.; et al. 4-Aminoquinolone Piperidine Amides: Noncovalent Inhibitors of DprE1 with Long Residence Time and Potent Antimycobacterial Activity. *J. Med. Chem.* 2014, 57, 5419–5434.

88. Panda, M.; Ramachandran, S.; Ramachandran, V.; Shirude, P.S.; Humnabadkar, V.; Nagalapur, K.; Sharma, S.; Kaur, P.; Guptha, S.; Narayan, A.; et al. Discovery of Pyrazolopyridones as a Novel Class of Noncovalent DprE1 Inhibitor with Potent Anti-Mycobacterial Activity. *J. Med. Chem.* 2014, 57, 4761–4771. [PubMed]

89. Tantry, S.J.; Markad, S.D.; Shinde, V.; Bhat, J.; Balakrishnan, G.; Gupta, A.K.; Ambady, A.; Raichurkar, A.; Kedari, C.; Sharma, S.; et al. Discovery of Imidazo[1,2-alpyridine Ethers and Squaramides as Selective and Potent Inhibitors of Mycobacterial Adenosine Triphosphate (ATP) Synthesis. *J. Med. Chem.* 2017, 60, 1379–1399. [PubMed]

90. Tantry, S.J.; Shinde, V.; Balakrishnan, G.; Markad, S.D.; Gupta, A.K.; Bhat, J.; Narayan, A.; Raichurkar, A.; Jena, L.K.; Sharma, S.; et al. Scaffold morphing leading to evolution of 2,4-diaminoquinolines and aminopyrazolopyrimidines as inhibitors of the ATP synthesis pathway. *MedChemComm* 2016, 7, 1022–1032. [CrossRef]

91. Gandhi, N.R.; Nunn, P.; Dheda, K.; Schaal, H.S.; Zignol, M.; Van Soolingen, D.; Jensen, P.; Bayona, J. Multidrug-resistant and extensively drug-resistant tuberculosis: A threat to global control of tuberculosis. *Lancet* 2010, 375, 1830–1843. [CrossRef]

92. Nuermerberger, E.; Yoshimatsu, T.; Tyagi, S.; O’Brien, R.J.; Vernon, A.N.; Chaisson, R.E.; Bishai, W.R.; Grosset, J. 2004. Moxifloxacin-containing Regimen Greatly Reduces Time to Culture Conversion in Murine Tuberculosis. *Am. J. Respir. Crit. Care Med.* 2003, 169, 421–426. [CrossRef]

93. Gillespie, S.H.; Crook, A.M.; McHugh, T.D.; Mendel, C.M.; Meredith, S.K.; Murray, S.R.; Pappas, F.; Phillips, P.P.; Nunn, A.J. Four-Month Moxifloxacin-Based Regimens for Drug-Sensitive Tuberculosis. *N. Engl. J. Med.* 2014, 371, 1577–1587. [CrossRef]

94. Mabhula, A.; Singh, V. Drug-resistance in Mycobacterium tuberculosis: Where we stand. *MedChemComm* 2019, 10, 1342–1360. [CrossRef]

95. Muñoz-Elías, E.J.; Timm, J.; Botha, T.; Chan, W.T.; Gomez, J.E.; McKinney, J.D. Replication Dynamics of Mycobacterium tuberculosis in Chronically Infected Mice. *Infect. Immun.* 2005, 73, 546–551. [PubMed]

96. Mitchison, D.A. Role of individual drugs in the chemotherapy of tuberculosis. *Int. J. Tuberc. Lung Dis.* 2004, 8, 796–806.

97. Rayasam, G.V.; Balganesh, T.S. Exploring the potential of adjunct therapy in tuberculosis. *Trends Pharmacol. Sci.* 2015, 36, 506–513. [CrossRef] [PubMed]

98. Zumla, A.; Chand, B.; Hoelscher, M.; Neres, J.; Humnabadkar, V.; et al. Towards host-directed therapies for tuberculosis. *Nat. Rev. Drug Discov.* 2015, 14, 511–512. [CrossRef] [PubMed]

99. Zumla, A.; Rao, M.; Wallis, R.S.; Kaufmann, S.H.; Rustomjee, R.; Mwaba, P.; Vilaplana, C.; Yeboah-Manu, D.; Chakaya, J.; Ippolito, G.; et al. Host-directed therapies for infectious diseases: Current status, recent progress and future prospects. *Lancet Infect. Dis.* 2016, 16, e47–e63. [CrossRef]
101. Singhal, A.; Jie, L.; Kumar, P.; Hong, G.S.; Leow, M.K.-S.; Paleja, B.; Tsenova, L.; Kurepina, N.; Chen, J.; Zolezzi, F.; et al. Metformin as adjunct antituberculosis therapy. *Sci. Transl. Med.* **2014**, *263*, 263ra159. [CrossRef]

102. Mishra, R.; Kohli, S.; Malhotra, N.; Bandypadhyay, P.; Mehta, M.; Munshi, M.; Adiga, V.; Ahuja, V.K.; Shandil, R.K.; Rajmani, R.S.; et al. Targeting redox heterogeneity to counteract drug tolerance in replicating Mycobacterium tuberculosis. *Sci. Transl. Med.* **2019**, *11*, eaaw6635. [CrossRef]

103. Padmapriyadarsini, C.; Bhavani, P.K.; Natrajan, M.; Ponnuraja, C.; Kumar, H.; Gomathy, S.N.; Guleria, R.; Jawahar, S.M.; Singh, M.; Balganesh, T.; et al. Evaluation of metformin in combination with rifampicin containing antituberculosis therapy in patients with new, smear-positive pulmonary tuberculosis (METrif): Study protocol for a randomised clinical trial. *BMJ Open* **2019**, *9*, e024363. [CrossRef]

104. Mdluli, K.; Kaneko, T.; Upton, A. The Tuberculosis Drug Discovery and Development Pipeline and Emerging Drug Targets. *Cold Spring Harb. Perspect. Med.* **2015**, *5*, a021154. [CrossRef]

105. Roy, K.K.; Wani, M.A. Emerging opportunities of exploiting electron transport chain pathway for drug resistant tuberculosis drug discovery. *Expert Opin. Drug Discov.* **2020**, *15*, 231–241. [CrossRef]

106. Mitchison, D.A. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle* **1985**, *66*, 219–225. [CrossRef]

107. Jindani, A.; Doré, C.J.; Mitchison, D.A. The bactericidal and sterilising activities of antituberculosis drugs during the first 14 days. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1348–1354. [CrossRef]

108. Hernandez-Pando, R. Persistence of DNA from Mycobacterium tuberculosis in superficially normal lung tissue during latent infection. *Lancet* **2004**, *356*, 2133–2137. [CrossRef]

109. Mitchison, D.A. The search for new sterilizing anti-tuberculosis drugs. *Front. Biosci.* **2004**, *9*, 1059–1072. [CrossRef] [PubMed]

110. Waynel, L.G.; Hilda, A. Metronidazole is bactericidal to dormant cells of Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **1994**, *38*, 2054–2058. [CrossRef] [PubMed]

111. Carroll, M.W. Efficacy and safety of metronidazole for pulmonary multidrug resistant tuberculosis. *Antimicrob. Agents Chemother.* **2013**, *57*, 3903–3909. [CrossRef] [PubMed]

112. Tyagi, S.; Nuermberger, E.; Yoshimatsu, T.; Williams, K.; Rosenthal, I.; Lounis, N.; Bishai, W.; Grosset, J. Bactericidal activity of the nitroimidazopyran PA-824 in a murine model of tuberculosis. *Antimicrob. Agents Chemother.* **2005**, *49*, 2289–2293. [CrossRef]

113. Dooley, K.E.; Hanna, D.; Mave, V.; Eisenach, K.; Savic, R.M. Advancing the development of new tuberculosis treatment regimens: The essential role of translational and clinical pharmacology and microbiology. *PLoS Med.* **2019**, *16*, e1002842. [CrossRef]

114. Nimmo, C.; Naidoo, K.; O’Donnell, M.; Bolhuis, M.S.; Van Der Werf, T.S.; Akkerman, O.W.; Conradi, F.; Everitt, D.; Crook, A.M. Treatment of Highly Drug-Resistant Pulmonary Tuberculosis. *N. Engl. J. Med.* **2020**, *382*, 893–902.

115. Diacon, A.H.; Dawson, R.; Von Groote-Bidlingmaier, F.; Symons, G.; Venter, A.; Donald, P.R.; Van Niekerk, C.; Everitt, D.; Winter, H.; Becker, P.; et al. 14-Day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: A randomised trial. *Lancet* **2012**, *380*, 986–993. [CrossRef]

116. McHugh, T.D.; Honeyborne, I.; Lipman, M.; Zumla, A. Revolutionary new regimens for multidrug resistant tuberculosis. *Lancet* **2018**, *19*, 233–234. [CrossRef]

117. Working Group on New TB Drugs. Available online: https://www.newtbdrugs.org/ (accessed on 13 April 2020).

118. TB Facts.org, New-Tb-Drugs. Available online: https://tbfacts.org/new-tb-drugs/ (accessed on 13 April 2020).

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