Pyrimidine derivatives with antitubercular activity

Vladimir Finger[a,b], Martin Kufa[a,b], Ondrej Soukup[b], Daniele Castagnolo[c], Jaroslav Roh[a,*], Jan Korabecny[b,*]

[a] *Faculty of Pharmacy in Hradec Kralové, Charles University, Akademika Heyrovskeho 1203, 50005 Hradec Kralove, Czech Republic*

[b] *Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic*

[c] *Department of Chemistry, University College London, 20 Gordon Street, WC1H 0AJ London, United Kingdom*

* corresponding authors: Jaroslav Roh, ORCID ID: 0000-0003-4698-8379, e-mail: jaroslav.roh@faf.cuni.cz; Jan Korabecny, ORCID ID: 0000-0001-6977-7596, e-mail: jan.korabecny@fnhk.cz

Abstract: Small molecules with antitubercular activity containing the pyrimidine motif in their structure have gained more attention after three drugs, namely GSK 2556286 (GSK-286), TBA-7371 and SPR720, have entered clinical trials. This review provides an overview of recent advances in the hit-to-lead drug discovery studies of antitubercular pyrimidine-containing compounds with the aim to highlight their structural diversity. In the first part, the review discusses the pyrimidine compounds according to their targets, pinpointing the structure-activity relationships of each pyrimidine family. The second part of this review is concentrated on antitubercular pyrimidine derivatives with a yet unexplored or speculative target, dividing the compounds according to their structural types.

Keywords: *Mycobacterium tuberculosis*; pyrimidine; tuberculosis; drug development; structure-activity relationships

1   Introduction

Tuberculosis (TB) is a widespread infectious disease caused by the pathogenic bacteria *Mycobacterium tuberculosis* (*Mtb*). The disease is most frequently transmitted through inhalation of small aerosolized droplets which settle in the lungs of the patients. The common symptoms of the active form of TB include persistent cough with sputum, chest pain, weight loss, fever, and night sweats. Mycobacteria can also affect other parts of the body, such as the lymph nodes, bones, brain, or heart. Such extrapulmonary forms of the disease, most commonly diagnosed in comorbidity with HIV/AIDS, may not be infectious, but if untreated, it can be as fatal as the pulmonary form of TB. However, the presence of mycobacteria in the body does not necessarily have to turn into active TB. About one-quarter of the world's population is infected with TB and suffers from the so-called latent form of TB infection (LTBI). LTBI is characterized by the absence of symptoms and limited transmissibility. However, LTBI turn into active TB when the immune system of the infected person is weakened.

TB is ranked among the top 10 causes of death in low and middle-income countries, representing a serious economic burden. In 2017, the costs of TB treatment was estimated at 10.9 billion USD, ranking the disease third worldwide.[1] For decades, TB has been considered the leading cause of death from a single infectious agent, and only recently, it has been overcome by COVID-19 infections. The World Health Organization (WHO) report data from 205 countries regarding TB prevalence, diagnosis, and progress, has established epidemiological plan by setting "Sustainable Development Goals (SDGs)" and "End TB Strategy". In the report from 2020, WHO estimated 10 million new cases and 1.5 million annual deaths from TB. Geographically, the most affected regions are those with less affordable healthcare or a lower standard of living, specifically some countries from Asia (44%) and Africa (24%). India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh, and South Africa are the most affected. Insufficient health care, poor supply of anti-TB drugs, and patients' non-compliance with the treatment regimens led to the development of multidrug-resistant tuberculosis (MDR-TB), which is characterized by the resistance to at least two most powerful anti-TB drugs, isoniazid (INH) and rifampicin (RIF). MDR-TB was first recognized in the 1980s, when new cases of TB not responding to standard regimens emerged. Therefore, the view that TB is fully curable and the disease itself is under control is no longer valid. Moreover, the COVID-19 pandemic reduced the number of patients supplied with drug-resistant TB treatment by approximately 15%, with only one in three people having access to the treatment in 2020. Globally, 3-4% of newly diagnosed TB infections is classified as MDR-TB. More strikingly, the portion of MDR-TB diagnosed patients reaches more than 18 % in the previously TB-treated group. More than half of the cases of MDR-TB (484,000) annually occur in India, China and Russia, of which 30000 cases further develop into extensively drug-resistant tuberculosis (XDR-TB).[2] XDR-TB strains are resistant to all first-line drugs, fluoroquinolones and at least to one of the injectable anti-TB drugs. TB treatment is a long-term and expensive process with different medical guidelines for each country. Due to the high cost of the treatment, it is very important to start the therapy quickly, preventing the disease from spreading in the population.

Overall, TB represents a huge unmet medical challenge to be tackled. Several research groups worldwide are involved in the drug discovery process, trying to find a new cure and to overcome the issue associated with drug resistance in MDR/XDR-TB strains.

1.1 Treatment of tuberculosis

In the initial phase of treating a TB infection, the patient has to be stabilized by reducing the number of actively growing bacilli, which results in a significant health improvement, allowing the patient to cease to be infectious. In an ideal case, the therapy is continued until complete eradication of the bacillus, preventing relapse and possible evolvement of resistance to drugs used during therapy. The choice of chemotherapy is related to the form of TB the patient is infected.

The standard treatment of drug-susceptible tuberculosis (DS-TB) has not changed for several decades, so the treatment regimens postulated by the WHO still hold true. In general, the WHO guideline for the treatment of TB has been established after long-term experience gained from clinical trials to minimize drug toxicity and enhance the likelihood of successful recovery. Indeed, DOTS (directly observed therapy, short course) is a patient-centered case management approach effective in 82% of TB patients. According to these guidelines, patients are treated by so-called first-line therapeutics for two months (Fig. 1). First-line drugs are represented by four antimicrobials - INH, RIF, pyrazinamide (PZA), and ethambutol (EMB). After two months of treatment, patients become non-infectious and continue receiving INH and RIF only for another four months. The treatment regimen does not distinguish between pulmonary and extrapulmonary TB, but this does not apply to central nervous, bone, and joint TB. In such cases, prolonged therapy is inevitable.
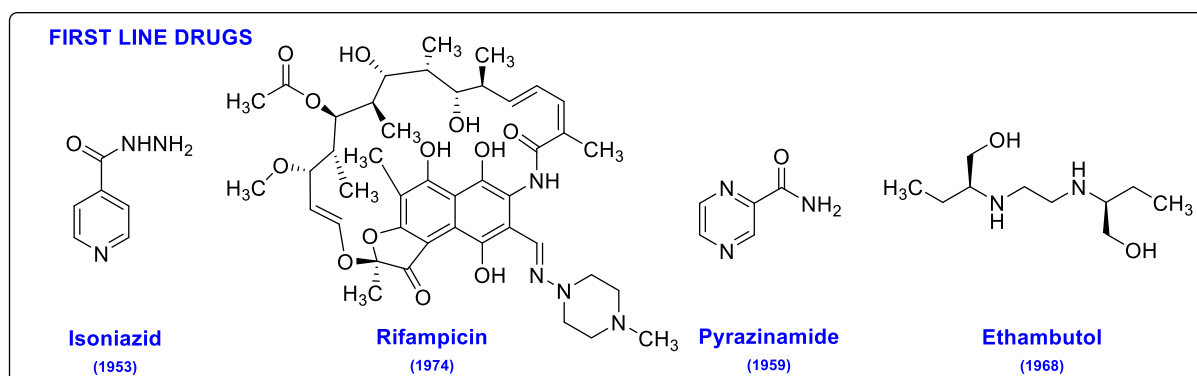

Fig. 1. First-line drugs in the therapy of TB. The year of their approval by FDA is displayed in parenthesis.
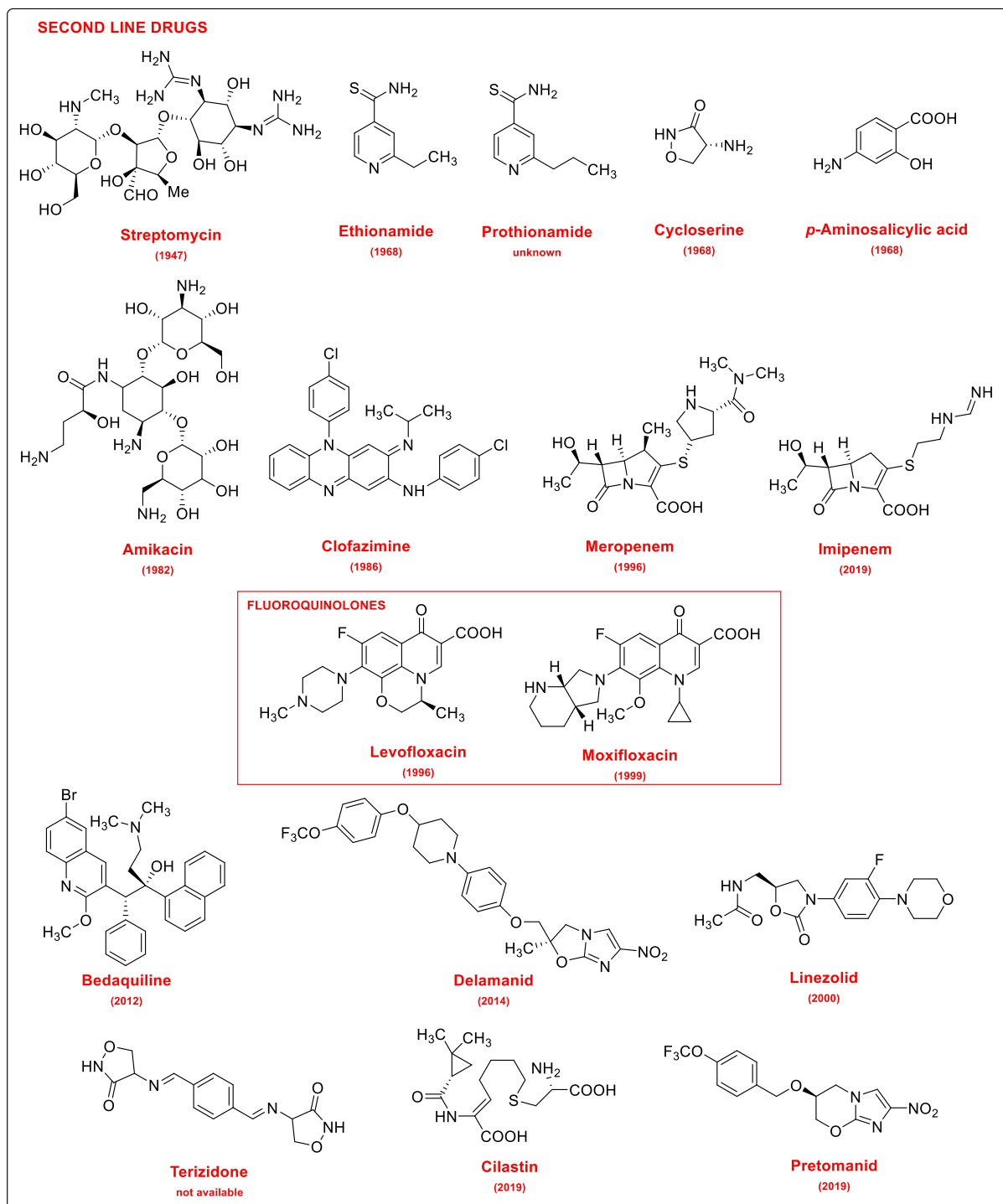
Fig. 2. Second-line drugs intended for the therapy of MDR-TB. The year of their approval by FDA is displayed in parenthesis.

Multidrug-resistant TB (MDR-TB) is caused by mycobacterium strains that resist the two most effective first-line drugs, INH and RIF. MDR-TB is treated by extensive chemotherapy with the use of second-line drugs (Fig. 2). According to the WHO classification, second-line drugs and new anti-MDR-TB drugs are sub-divided into three groups, classified into groups A-C (Table 1).

Combinations of these drugs are used in the treatment of MDR-TB according to the WHO guidelines.

Table 1. Drugs used for the treatment of MDR-TB are sub-divided into three groups by the WHO.[3] For each of the compounds, its mechanism of action (MoA) is displayed.

Group	Drug name	Mechanism of action
A	Levofloxacin/Moxifloxacin[4]	Inhibition of DNA gyrase, which catalyzes the negative supercoiling of DNA
A	Bedaquiline[5]	Targeting the energy metabolism by inhibiting ATP synthase
A	Linezolid[6]	Inhibition of protein synthesis in rRNA
B	Clofazimine[7]	Unknown MoA
B	Cycloserine/terizidone[8,9]	Blocking the synthesis of peptidoglycan by inhibiting D-Ala-D-Ala ligase
C	Pyrazinamide[10–12]	Inhibition of 1) fatty acid synthetase 2) ribosomal protein S1 (RpsA) 3) aspartate decarboxylase (PanD)
C	Ethambutol[13]	Disrupting the synthesis of arabinogalactan and lipoarabinomannan via inhibition of arabinosyltransferases (EmbA, EmbB, EmbC)
C	Amikacin[14] Streptomycin[15]	Inhibiting protein synthesis by binding to the mycobacterial ribosome
C	Ethionamide/prothionamide[16]	Disrupting the synthesis of mycolic acid biosynthesis by inhibiting InhA
C	Imipenem-cilastin/Meropenem[17]	Inhibiting cell wall synthesis by binding to penicillin-binding proteins (PBPs)
C	p-aminosalicylic acid[18]	Inhibition of folic acid synthesis
C	Delamanid[19]	Inhibition of methoxy- and ketomycolic acid synthesis

XDR-TB was first described in 2006. This is the most severe form of TB defined as a rare form of MDR-TB resistant to INH, RIF, some fluoroquinolones, and at least one of the three injectable second-line drugs (capreomycin, amikacin, or kanamycin). Patients are treated with a combination of up to eight drugs, with some of them being injected daily for up to two years. The XDR-TB patient's prognosis survival and full recovery is relatively low, culminating at around 34%. The breakthrough in the therapy of XDR-TB has been represented by the approval of pretomanid in August 2019. Along with delamanid and bedaquiline, it is the third approved drug in the last 40 years and the only anti-TB agent developed by a non-profit organization. From a chemical point of view, pretomanid belongs to a group of

nitroimidazooxazines. All these approved drugs bring new hope in the therapy of MDR/XDR-TB resistant forms.

2 Pyrimidine derivatives with antitubercular activity

Pyrimidine (also known as 1,3-diazine, according to Hantzsch-Widman nomenclature) is a six-membered heterocyclic compound with less basic properties and a lower solubility profile than pyridine. Substituted pyrimidines can be found in many natural substances, including nucleobases (cytosine, thymine and uracil) or vitamin B_1 and also in many marketed drugs with different biological targets.[20–23] These drugs (Fig. 3) are used as antimicrobial, anti-HIV, antihistaminic, anticancer or antipsychotic agents.[24–28]
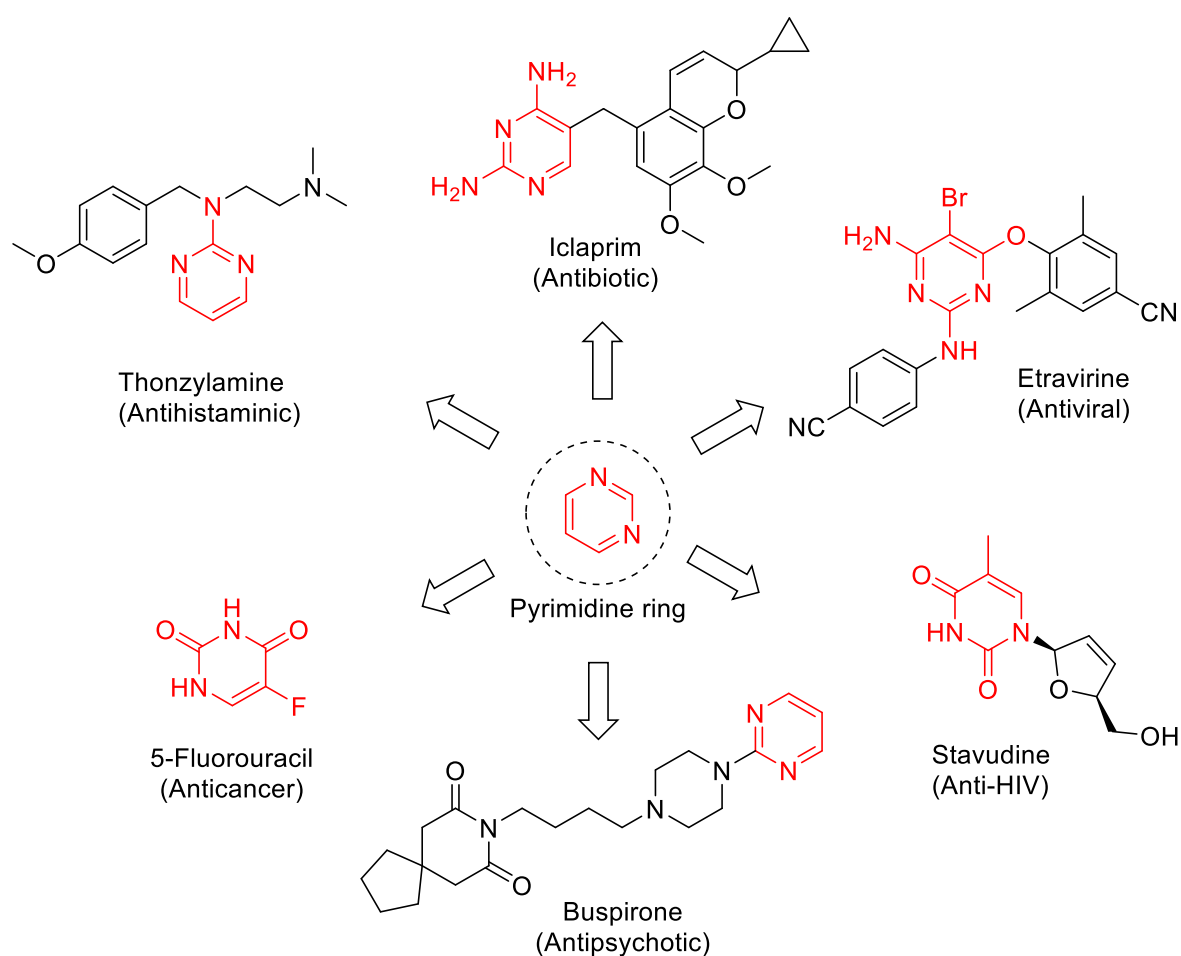

Fig.3. Examples of marketed drugs containing a pyrimidine ring.

In the context of TB, some pyrimidine derivatives have been broadly inspected for more than two decades. Most of them are predominantly substituted pyrimidine nucleosides, inhibiting the thymidine monophosphate kinase. A review devoted to this class of anti-TB agents has been released in 2013 by Shmalenyuk *et al.*.[29] The authors discussed the antitubercular properties of several pyrimidine nucleosides and compared their *in vitro* activity against the mycobacterial susceptible strain $H_{37}R_v$. Since then, many articles have been published with pyrimidine as a core scaffold endowed with antitubercular properties. Most importantly,

three derivatives containing a pyrimidine core in their structure are currently being investigated in clinical trials (Fig. 4), namely the azaindole derivative TBA-7371 (clinical trial identifier: NCT04176250), the GSK 2556286 (NCT04472897), and the benzimidazole SPR720 (NCT05496374). The compound GSK 2556286 (GSK-286), interfering with mycobacterial cholesterol catabolism, is currently in the first phase of clinical trials, while compounds TBA-7371 inhibits the decaprenylphosphoryl-β-D-ribose-2′-epimerase (DprE1), a critical enzyme in the synthesis of the mycobacterial cell wall. SPR720, which inhibits the bacterial DNA gyrase (GyrB), is also undergoing Phase II clinical trials.[30–33]

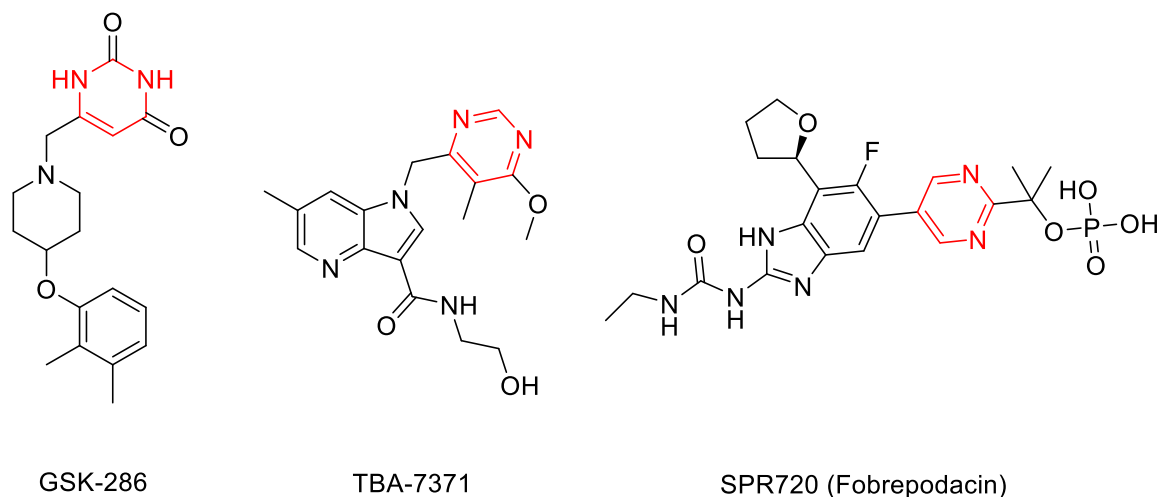| GSK-286 | TBA-7371 | SPR720 (Fobrepodacin) |

Fig. 4. Clinical candidates GSK-286, TBA-7371 and SPR720 bearing pyrimidine core (highlighted in blue) in their structure currently investigated in clinical trials.

2.1 Pyrimidine derivatives with defined target

2.1.1 Thymidine monophosphate kinase (TMPK) inhibitors

Thymidine monophosphate kinase (TMPK; EC 2.7.4.9) is the enzyme essential for human (TMPKh) and mycobacteria (TMPKmt) DNA replication. TMPK catalyzes reversible phosphorylation of thymidine monophosphate (dTMP) to thymidine diphosphate (dTDP) in the presence of Mg^{2+} ions.[34] Most inhibitors of this enzyme contain structurally modified thymidine nucleosides and nucleotides in their structure. Other inhibitors are no longer based on nucleotides and nucleosides and therefore can be divided into thymine-containing and thymine-free inhibitors.

Inhibitors containing thymine in their structure can be further sub-divided into thymine nucleoside and thymine non-nucleoside derivatives.

In 2003, the Van Calenbergh group developed a series of thymidine nucleotides as potent inhibitors of TMPKmt.[35] The authors followed the results of Li de la Sierra *et al.*, suggesting that the azido group shifts the magnesium cation in the active site of the enzyme.[36] This subsequently leads to conformational changes that abolish the catalytic activity of the enzyme. For this reason, the authors focused on the C3' position, with the idea that a suitable substitution at this position could lead to more effective inhibitors (Fig. 5).

The most potent inhibitors of TMPKmt are those derivatives substituted in the position C3' (Fig. 5) with azidomethyl (**1**; K_i = 12 µM), aminomethyl (**2**; K_i = 10.5 µM) and fluoromethyl (**3**; K_i = 15 µM) groups. The selectivity between TMPKh and TMPKmt in this class of compounds was rather poor, although compound **2** displayed a higher selectivity to TMPKh over TMPKmt (Fig. 5).[35] Nucleotide **2** emerged as a promising lead with a low K_i for TMPKmt (**2**; K_i = 10.5 µM). Due to the low permeability of the phosphorylated derivatives **1-3**, the authors switched to nucleoside analogues in the follow-up study. The resulting derivative **4** (Fig. 5) combined low K_i values (40 µM) with a favorable selectivity profile for TMPKmt. Based on the conformational analysis, nucleosides combining the substituents of the highlighted compounds from the first study, i.e. C3´-aminomethyl and C3´-azidomethyl, and a halogen at the C2 (chlorine, fluorine) were developed. Unfortunately, all compounds turned inactive (K_i = > 165 µM). Ongoing efforts led to the discovery of bicyclic nucleosides (compounds **5**, **6**; Fig. 5) and one single-bridged nucleoside **7** (Fig. 5), all formed as synthetic by-products. The derivative **5** showed good affinity to the TMPKmt enzyme (K_i = 3.5 µM) and also good selectivity profile (SI, expressed as ratio between K_iTMPKh and K_iTMPKmt) toward TMPKmt (K_iTMPKh/K_iTMPKmt = 200). The latter can be attributed to better fitting into the cavity of the TMPKmt enzyme as demonstrated by the docking experiments.[37]

The following study was designed on single-bridged nucleoside **7** with the activity against TMPKmt (K_i = 37 µM). The authors structurally optimized one of the thymidines at position C3' by variously substituted phenyl rings (Fig. 5). The choice of substituents on the phenyl was guided by the Topliss tree scheme.[38] Thus, a series of substituted ureas was developed and extended to thiourea derivatives as well because of the known thiocarlide anti-TB activity.[39] The derivative **8** (Fig. 5) showed promising activity against TMPKmt (K_i = 5 µM), while the most potent compound **9** (Fig. 5) in this study displayed submicromolar inhibition activity of TMPKmt (K_i = 0.6 µM) and a SI value of 600 (TMPKh, K_i = 362 µM). Compound **9** substituted in the position C5' exhibited moderate growth inhibition of *Mycobacterium bovis* with MIC$_{99}$ = 20 µg/ml, with no cytotoxicity on Vero cell cultures (MCC > 100 µM).[40]
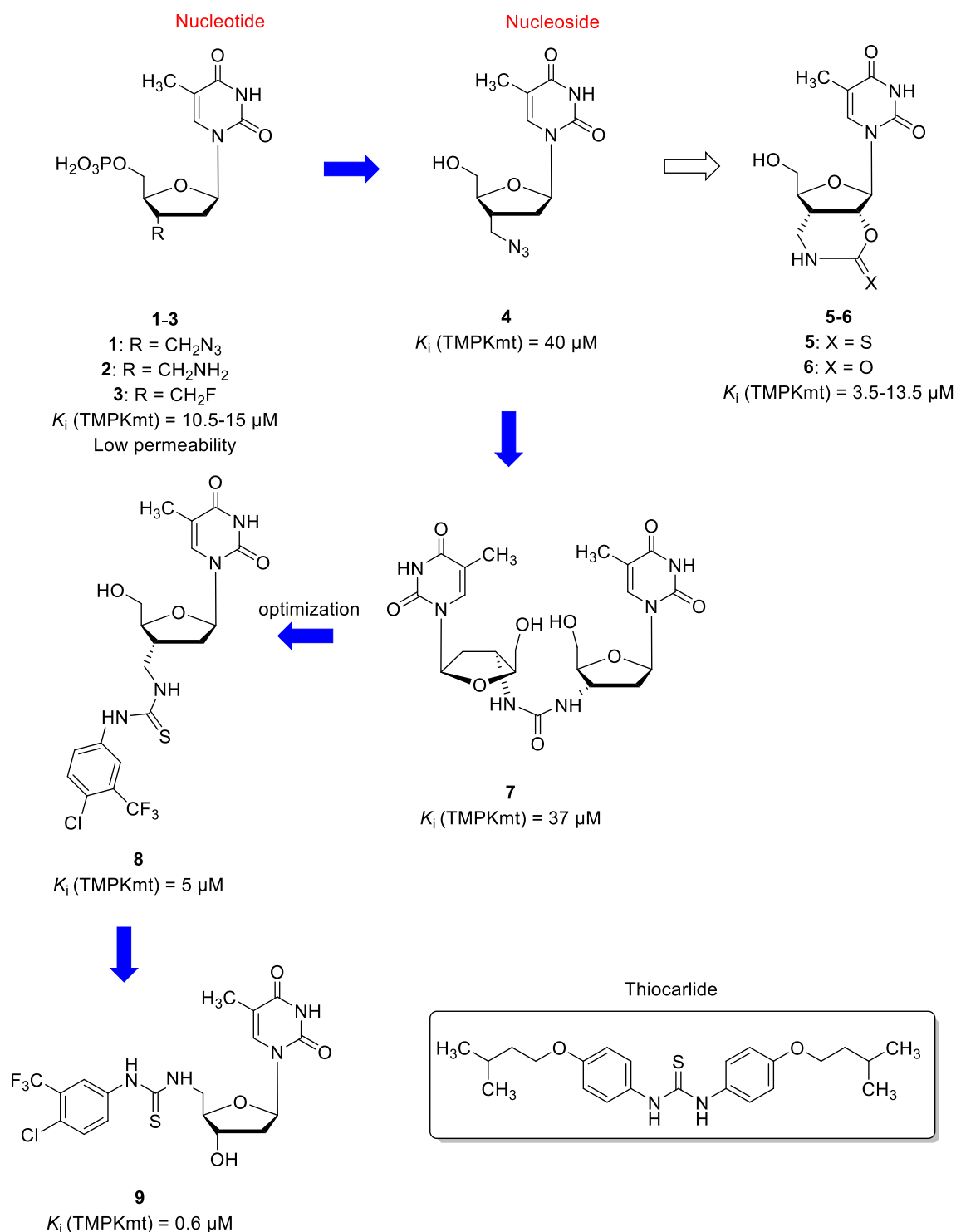
Fig. 5. Potent inhibitors of TMPKmt (**1-9**) developed by Van Calenbergh *et al*. The blue arrows indicate a positive change in terms of activity improvement.[35,37,40]

Further studies shifted from nucleoside inhibitors to non-nucleoside-based TMPKmt inhibitors.[41,42] New TMPKmt inhibitors were derived from already existing highly potent inhibitors of thymidylate kinase of *Staphylococcus aureus*. In this subset, deoxyribose was replaced by five-membered or six-membered aromatic rings (Fig. 6).[43] Van Calenbergh *et al.* followed up the work of Martínez-Bottela *et al.*[43] and resynthesized highly potent inhibitor **10** (Fig. 6) and tested its inhibition against TMPKmt (K_i = 1.5 µM). The separation of individual

enantiomers revealed that *S*-isomer **11** (Fig. 6) is the eutomer with a higher enzyme affinity to TMPKmt (K_i = 0.8 µM) than *R*-isomer (K_i = 8.8 µM).[44] In another work, the simplification of compound **11** and replacement of 3-substituted piperidine by 4-substituted-piperidine led to other TMPKmt derivative **12** (Fig. 6). Accordingly, quinoline-2-yl-containing analogues were discovered, out of which **13** and **14** (both Fig. 6) showed good inhibition activity to TMPKmt with IC_{50} = 1.1 µM and IC_{50} = 0.95 µM, respectively. For these compounds, minimal inhibitory concentration values (MIC) have been measured, making it possible to compare the antitubercular efficacy of these compounds with other anti-TB standards. The MIC values of compounds **13** (MIC = 15.6-31.25 µg/mL) and **14** (7.8-15.6 µg/mL) against the drug-susceptible $H_{37}R_v$ strain were rather negligible. The authors hypothesized that poor mycobacterial activity is due to low cell wall uptake or efflux mechanisms. Thus, the study exploited a potential combination of **13** and **14** with efflux pump inhibitor verapamil. In this combination, slightly better efficiency was achieved for derivative **13** (*Mtb* $H_{37}R_v$ MIC = 7.8 µg/mL) and also for **14** (*Mtb* $H_{37}R_v$ MIC = 3.9-7.8 µg/mL) indicating potential synergism.[45]

10
K_i (TMPKmt) = 1.5 µM

Isolation of *S*-enantiomer

11
K_i (TMPKmt) = 0.8 µM

Replacement of
3-substituted piperidine

12
K_i (TMPKmt) = 9 µM

13: R = H; K_i (TMPKmt) = 1.1 µM; MIC (Mtb) = 15.6-31.25 µg/mL
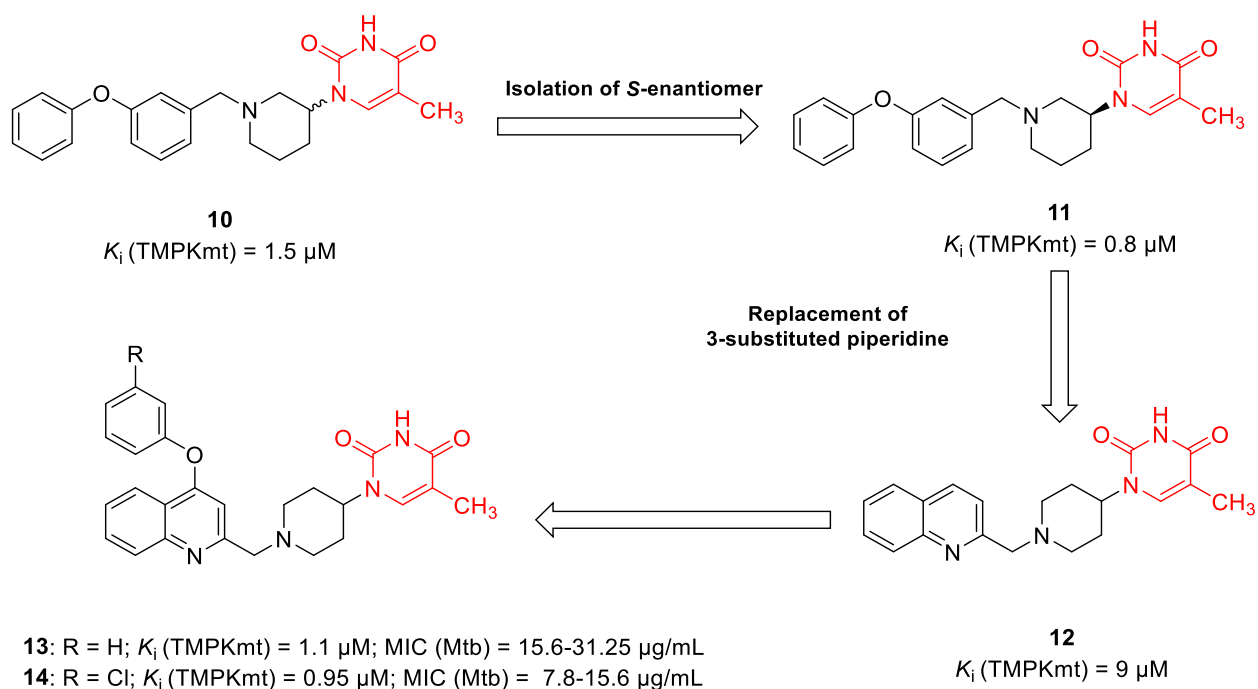**14**: R = Cl; K_i (TMPKmt) = 0.95 µM; MIC (Mtb) = 7.8-15.6 µg/mL

Fig. 6. Inhibitors of TMPKmt prepared by Van Calenbergh *et al*. The blue arrows indicate a positive change in terms of antitubercular activity increment.

Derivatives prepared in previous studies by Calenbergh *et. al* showed excellent activity against TMPKmt, but whole-cell activity was moderate or absent, documenting the frequently cited issue of target-based drug discovery approaches. Therefore, they sought to further structurally modify the TMPKmt inhibitor **15a**, preserving the 4-(thymin-1-yl)piperidine moiety responsible for inhibition of TMPK and attaching fragments known from the literature for their high whole-cell antitubercular activity such as parts of the Q203 (currently in the Phase II of clinical trials) and 3,5-dinitrobenzamide (covalent inhibitor of DprE1).[46,47] [48–51] Furthermore, the TMPKmt inhibitor **15a** was modified also with siderophores (catecholes, salicylic acid, 8-

hydroxyquinoline, 2,2´-bipyridine, hydroxamate and 3-(2-hydroxybenzylidene)dithiocarbazate) capable of chelating iron to increase the whole-cell activity and preserve the inhibitory effect on the TMPKmt enzyme.

The derivative **16** containing imidazopyridine core and the benzamides **17** and **18** showed good *in vitro* activity against $H_{37}R_v$ *Mtb* strain (Fig. 7). This activity was affected by cultivation media, where benzamides showed better activity in iron-deficient media. Moreover, compounds **16**, **17** and **18** were non-cytotoxic against MRC-5 fibroblasts (IC_{50} for all > 64 µM). The effect of the iron-chelating group was observed for derivative **19** containing 3-(2-hydroxybenzylidene)dithiocarbazate group, as it was inactive in 7H9/glucose culture medium (*Mtb* $H_{37}R_v$ MIC > 100 µM) but improved activity in Fe-deficient GAST medium (*Mtb* $H_{37}R_v$ MIC = 25 µM). Compound **20** showed the highest inhibitory activity against TMPKmt (IC_{50} = 0.14 µM) but with no whole-cell activity (*Mtb* $H_{37}R_v$ MIC > 100 µM). However, both compound **19** and **20** showed high *in vitro* cytotoxicity (MRC-5 CC_{50} (**19**) = 6.39 µM, MRC-5 CC_{50} (**20**) = 1.45 µM).[52]
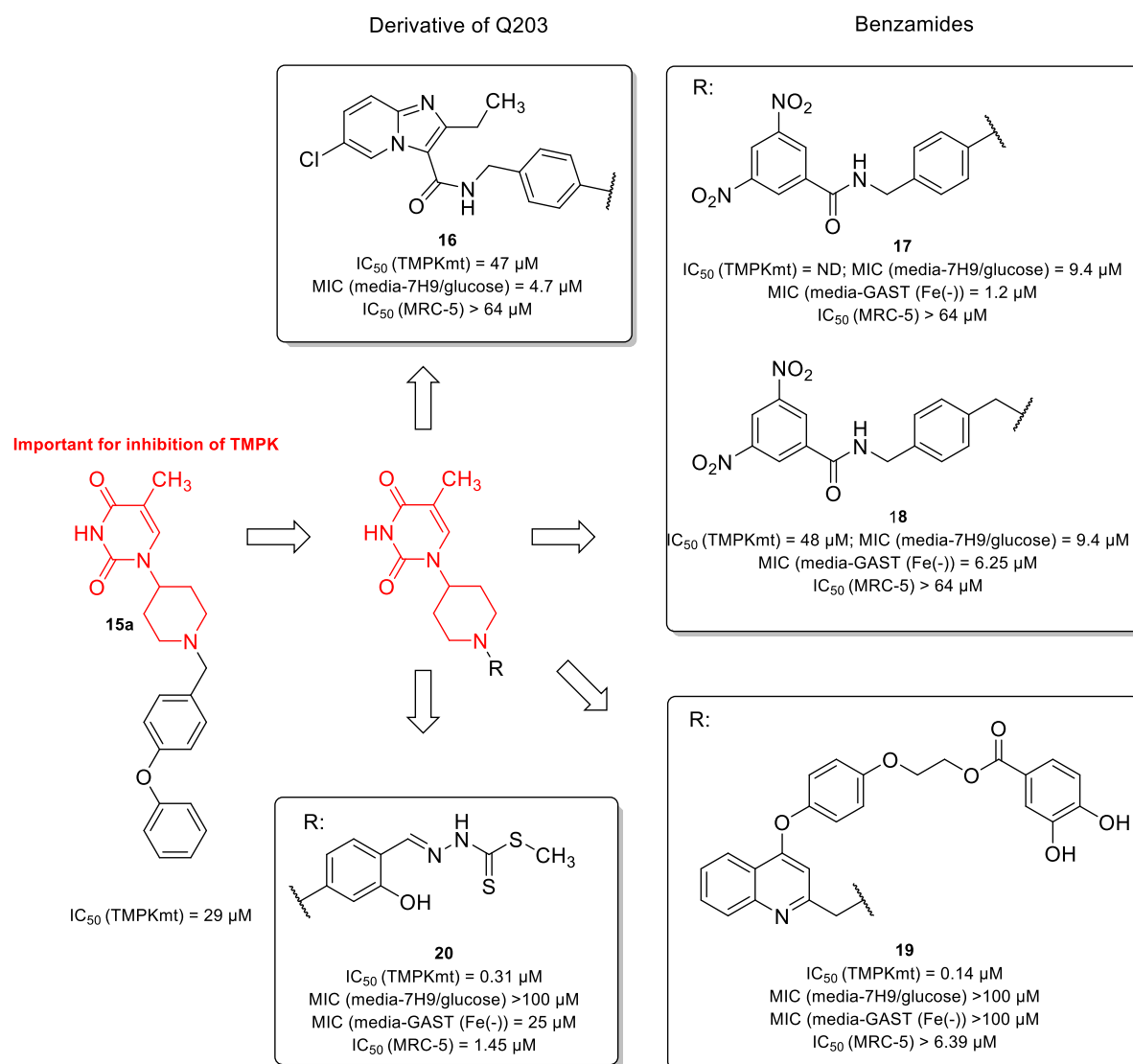

Fig. 7. Novel derivatives bearing 4-(thymin-1-yl)piperidine moiety responsible for inhibition of TMPKmt in combination with the key fragments of highly active antitubercular agents-benzamides or Q203. ND stands for not determined, due to precipitation in media. [52]

Another study by Song *et al.* was inspired by compound **15b**.[53] In this work the 4-(thymin-1-yl)piperidine moiety was preserved (Fig. 8),[45] while the structure optimization emphasis was placed on both phenyl rings of **15b**. Initially, authors attempted to incorporate pyridine into the structure generating compounds with better water solubility and significantly reduced cytotoxicity against MRC-5 (EC_{50} > 64 µM for all pyridine-containing derivatives). According to the cocrystal structure of **21** with TMPKmt, the presence of the pyridine in **21** contributed to the inhibitory properties (IC_{50} = 2.5 µM) via electronic repulsion between the pyridine and the phenoxy group oxygen, causing a conformational change of the compound **21** in the enzyme. However, no whole-cell activity was observed for compound **21** (*Mtb* $H_{37}R_v$ MIC > 50 µM). Authors thus turned their attention to phenoxy moiety modification following the Topliss tree scheme.[38] The most active compounds of this series were derivatives **22**-**25** containing chlorine substituents in different phenyl ring positions (Fig. 8). These derivatives showed good inhibitory activity of TMPKmt (IC_{50} = 0.12-6.9 µM), also retaining moderate cell activity (*Mtb* $H_{37}R_v$ MIC = 12.5 µM). On the other hand, the cytotoxicity profile of compounds **22**-**25** was substantially aggravated.[53]
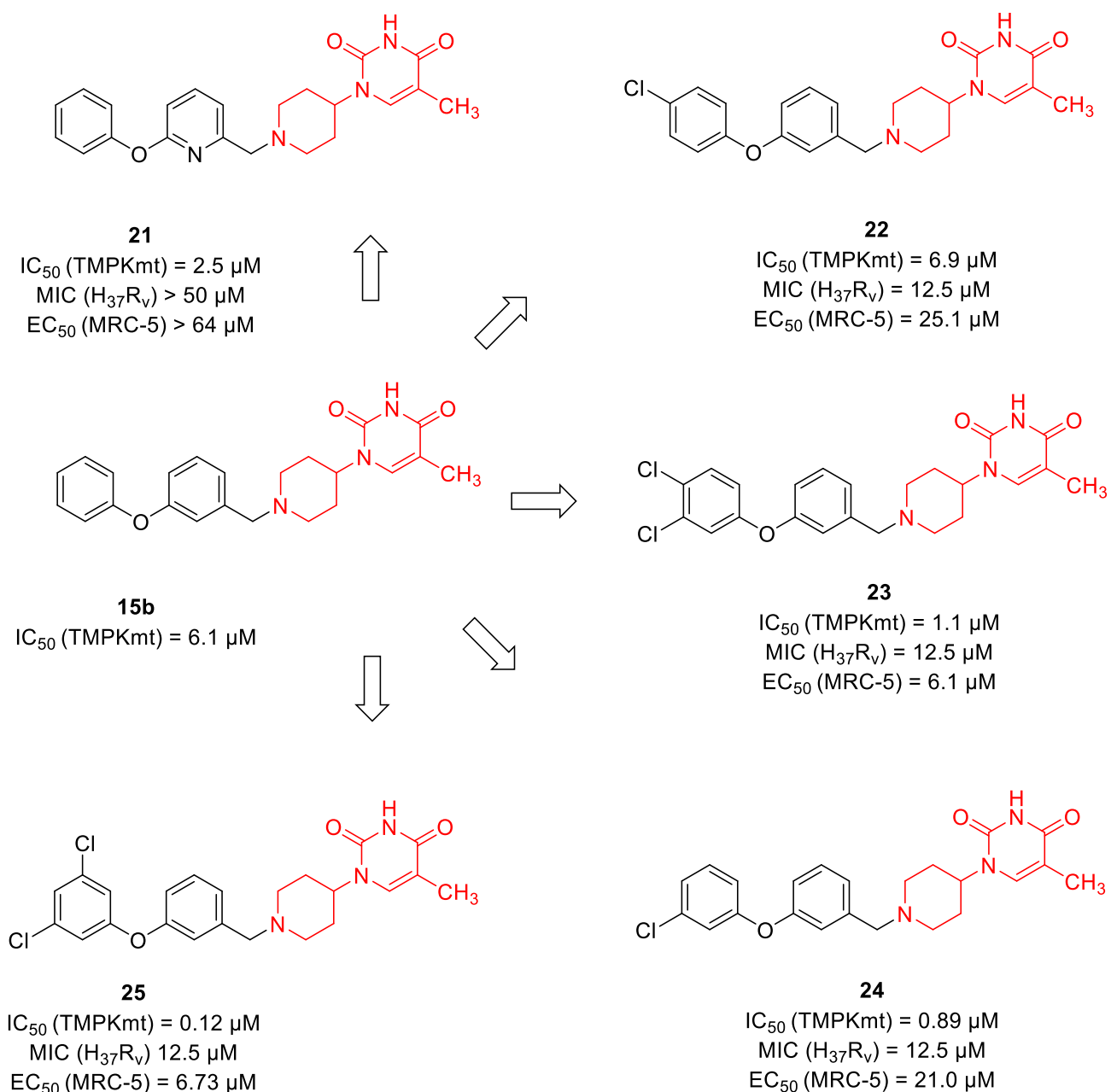
Fig. 8. Inhibitors of TMPKmt **21-25** based on derivative **15b** from the previous study. 4-(Thymin-1-yl)piperidine moiety is highlighted in red, being a key scaffold for interactions with TMPKmt active site. The incorporation of halogen into the phenoxy part of the molecule led to the compounds with whole-cell antitubercular activity.

Gasse *et al.* discovered substituted benzyl-pyrimidines as inhibitors of TMPKmt with a K_i = 6.5 μM for the most active compound **26** (Fig. 9).[54] Naphtosultam **27** (Fig. 9) is another potent TMPKmt inhibitor with K_i = 0.27 μM.[55] The major drawback of **27** is the poor water solubility. Modification of the naphthalene region of **27** did not yield a more potent derivative. The presence of thymine in **27** was crucial for preserving the activity against TMPKmt.[56]
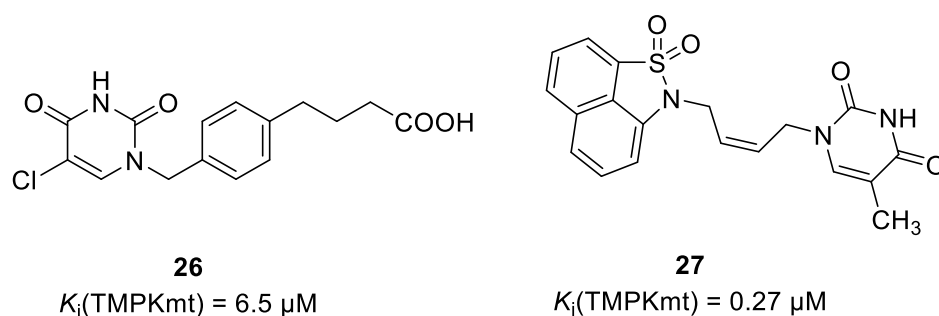
26
K_i(TMPKmt) = 6.5 μM

27
K_i(TMPKmt) = 0.27 μM

Fig. 9. Thymine-based non-nucleoside inhibitors of TMPKmt **26** and **27**.

2.1.2 Flavin-dependent thymidylate synthase inhibitors

Thymidine monophosphate, which is an essential DNA precursor found in different species (plants, bacteria and eukaryotic cells), is *de novo* synthesized from 2′-deoxyuridin-5′-monophosphate (dUMP) by reductive methylation (Fig. 10) at position C5 catalyzed by the enzyme thymidylate synthase (ThyA; EC 3.1.1.98). Compound N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate (CH$_2$THF) acts as methylene and hydride donor, while releasing 7,8-dihydrofolate (DHF). There are two other underlying processes associated with this methylation, namely the reduction of DHF to tetrahydrofolate-THF (catalyzed by dihydrofolate reductase) and re-methylation by serine hydroxymethyl-transferase.

The bacteria can also synthesize thymidylate using flavin-dependent thymidylate synthase (ThyX; EC 2.1.1.148) without using ThyA. ThyX contains FAD, which mediates hydride transfer from NADPH during the catalytic cycle. In the ThyX catalyzed reaction, the carbon donor is also CH$_2$THF converting into tetrahydrofolate (THF) (in ThyA-catalysis, DHF is formed). ThyA and ThyX enzymes are both presented in mycobacteria, rarely in eukaryotes, and are absent in humans, making them targets of high interest.[57–59]
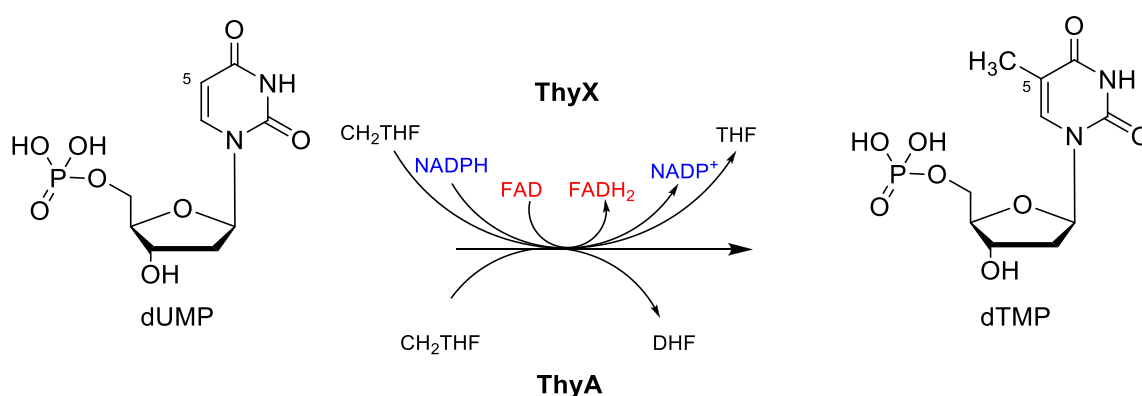

Fig. 10. Alternative *de novo* synthesis of dTMP from dUMP catalyzed by ThyX with the use of CH$_2$THF as the carbon donor.

Herdewijn *et al.* prepared a series of 5-alkynyl dUMP derivatives. These compounds were tested for inhibitory activity against ThyX and ThyA. The most potent compound **28** with an IC$_{50}$ (ThyX) = 0.91 μM behaved like a selective ThyX inhibitor (Fig. 11). It can be speculated that the presence of a phosphate group decreases the mycobacteria cell wall entrance given its

hydrophilic features. Therefore, the whole cell activity of **28** was not evaluated,[57] leaving the space for further structural optimization via phosphate protection strategies known from medicinal chemistry of antivirals.
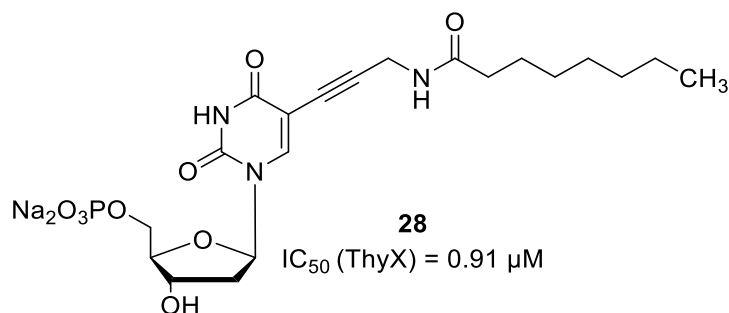

28
IC_{50} (ThyX) = 0.91 µM

Fig. 11. Selective inhibitor of ThyX as a potential antimycobacterial agent.

2.1.3   Inhibitors of the cytochrome B subunit of the cytochrome bc1 complex

The cytochrome B subunit of the cytochrome bc1 complex (QcrB; EC 7.1.1.8) is a recently discovered mycobacterial target associated with the highly effective anti-TB drug telacebec (Q203; Fig. 12), which is currently investigated in Phase II clinical trials.[60–63] Bc1 complexes are membrane proteins that catalyze the oxidation of ubihydroquinone and the reduction of cytochrome C in the respiratory chains of mycobacteria. Electron transport chains impose an attractive target because their inhibition interrupts the respiration of mycobacteria, thus preventing the synthesis of ATP.
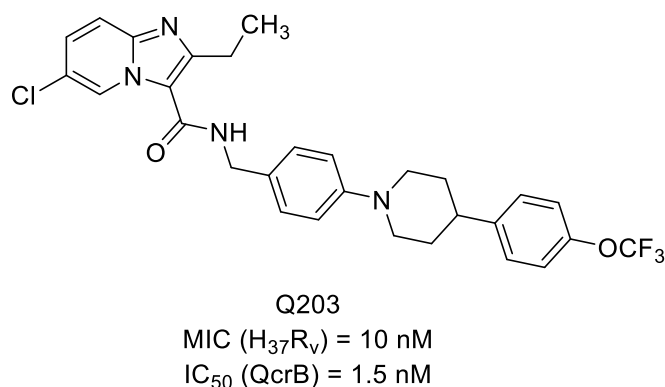

Q203
MIC ($H_{37}R_v$) = 10 nM
IC_{50} (QcrB) = 1.5 nM

Fig. 12. Clinical candidate Q203 inhibiting mitochondrial QcrB.

The screening of 78 commercially available small molecules led to the discovery of substituted 4-amino-thieno[2,3-*d*]pyrimidine derivatives suppressing the growth of the *Mycobacterium smegmatis* via QcrB inhibition. Removal of the carboxyl group (Fig. 13) from hit compound **29** led to mycobacterial cell wall permeability enhancement and an increase in anti-TB activity. The study highlighted **30** as the most promising compound (Fig. 13), confirming QcrB as a target using whole genome sequencing of resistant mutants.[64]
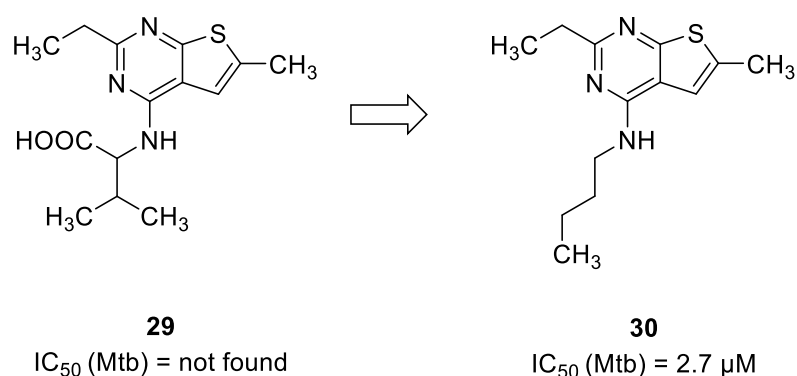
29
IC$_{50}$ (Mtb) = not found

30
IC$_{50}$ (Mtb) = 2.7 µM

Fig. 13. Hit compound **29** and top-ranked QcrB inhibitor **30** from thieno[2,3-*d*]pyrimidines family.

2.1.4   Inhibitors of decaprenylphosphoryl-β-D-ribose-2′-epimerase

Decaprenylphosphoryl-β-D-ribose-2′-epimerase (DprE1; EC 1.1.98.3) is a widely investigated target in mycobacteria species. This flavoprotein was discovered in 2005.[65] It catalyzes the oxidation of decaprenylphosphoryl-D-ribose (DPR) to decaprenylphophoryl-2-keto ribose (DPX), which is reduced to decaprenyl-D-arabinose (DPA) by DprE2 (Fig. 14). DPA is a crucial fragment for the synthesis of mycobacterium cell-wall polysaccharides lipoarabinomannan and arabinogalactan.[66–68]
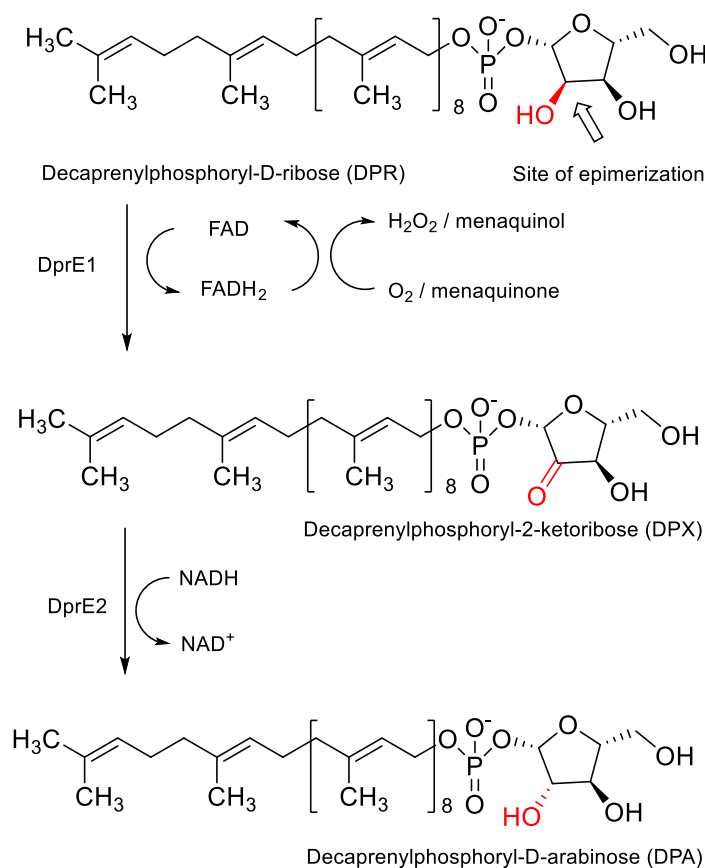

Decaprenylphosphoryl-D-ribose (DPR)   Site of epimerization

Decaprenylphosphoryl-2-ketoribose (DPX)

Decaprenylphosphoryl-D-arabinose (DPA)

Fig. 14. Epimerization of DPR to DPA. Adapted from ref.[68,69]

DprE1 inhibition strongly affects cell wall integrity and functioning.[66,70,71] DprE1 is considered a highly interesting target as four drugs inhibiting DprE1 are currently ongoing clinical trial testing (Fig. 15). Out of them, benzothiazinones BTZ043 (*Mtb* $H_{37}R_v$ MIC = 1 ng/mL) and PBTZ169 (*Mtb* $H_{37}R_v$ MIC = ≤ 0.19 ng/mL) are classified as covalent DprE1 inhibitors, being one of the most effective anti-TB drugs developed so far. The mechanism of benzothiazinone action is nitro group-dependent; it starts with the reduction of nitro to nitroso group that covalently bound to Cys387 in the active site of DprE1 forming semimercaptal adduct.[66,67,72] The process ultimately leads to DprE1 inactivation. The second group of compounds acts as noncovalent DprE1 inhibitors, including TBA-7371 and OPC-167832.[73,74]
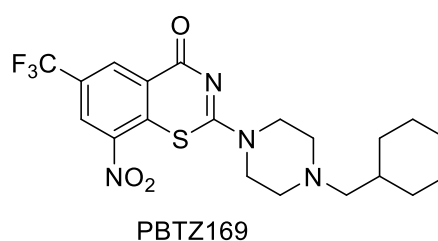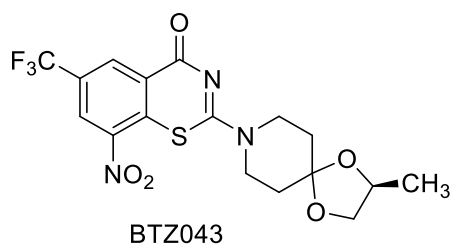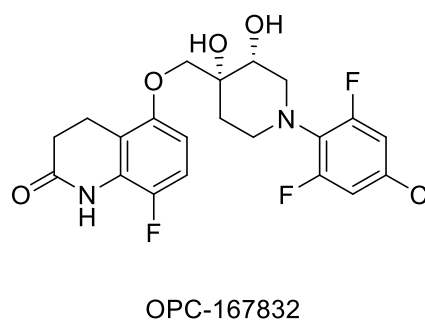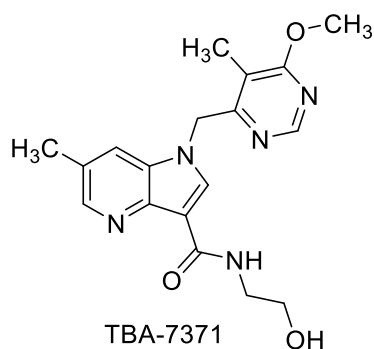
Fig. 15. Inhibitors of DprE1 in clinical trials with noncovalently and covalently binding representatives.

A series of substituted pyrimidines were developed based on the chemical similarity, applying the Tanimoto score to pyrrolothiadiazoles (Fig. 16). Pyrrolothiadiazole **31** was one of 177 potent anti-TB compounds discovered by high-throughput screening by GlaxoSmithKline.[75,76] Scaffold hopping was applied to improve the physicochemical properties of the initial pyrrolothiadiazole **31**, mainly to improve water solubility and *in vivo* efficacy, switching to pyrimidine congeners.[77] Both groups of compounds (**31**-**34**) were found to be DprE1 noncovalent inhibitors.

Fig. 16. Initial hit compound **31** and member of 4,6-disubstituted pyrimidine (**32-34**) family developed by scaffold hopping, identified as DprE1 noncovalent inhibitors.

To understand the structure-activity relationships (SAR), the authors gradually replaced each part of **32** to identify compound **33** as the most potent derivative exerting high activity against mycobacteria (*Mtb* $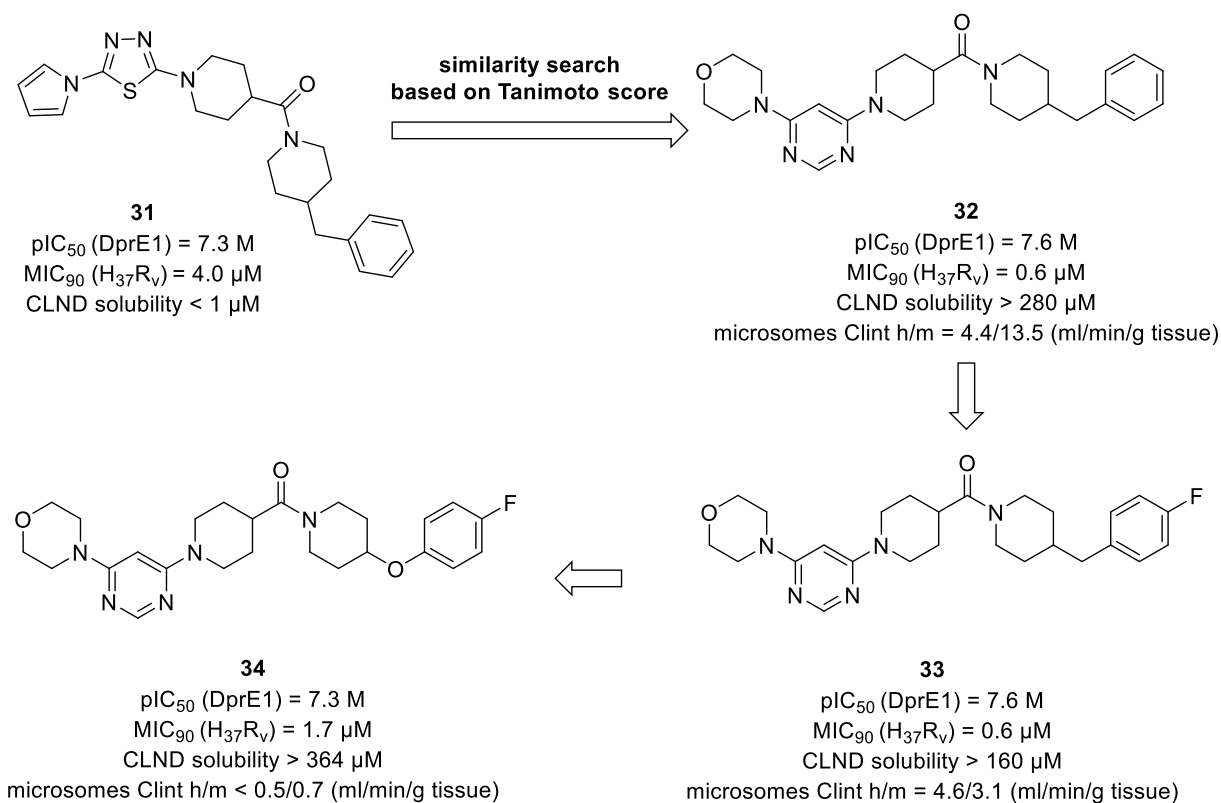H_{37}R_v$ MIC_{90} = 0.6 µM), as well as in the intracellular species using macrophage model (*Mtb* $H_{37}R_v$ MIC_{90} = 0.07 µM). The selectivity for *Mycobacterium* species was confirmed by testing the activity on a panel of 19 bacteria showing no activity against these bacteria (MIC > 128 µg/mL).

Compound **34** was developed following structural modifications, displaying an acceptable MIC value (*Mtb* $H_{37}R_v$ MIC_{90} = 1.7 µM), and excellent aqueous solubility (>364 µM, CLND – chemiluminescent nitrogen detection). Derivative **33** (4.6 mL/min/g tissue) and derivative **34** (0.5 mL/min/g tissue) exhibited moderate and low microsomal clearance for mouse microsomes, respectively. In contrast, derivative **33** (3.1 mL/min/g tissue) and derivative **34** exhibited high and moderate (0.7 mL/min/g tissue) microsomal clearance for human microsomes, respectively. In the mouse pharmacokinetics studies, both compounds had good oral bioavailability (100 % for **33** and 79 % for **34**), but short half-lives (0.45 h for **33** and 1.0 h for **34**).

The *in vivo* antimycobacterial efficacy of **33** and **34** was evaluated in a rapid acute assay on mice C57BL/6. Compounds **33** and **34** were orally administrated for eight days to the TB infected mice (*Mtb* $H_{37}R_v$). Compounds **33** and **34** were highly effective in the treatment of mice (ED_{99} = 29 mg/kg for derivative **33** a ED_{99} = 30 mg/kg for **34**), reaching the efficacy of the commercially available anti-TB drugs in this assay (ED_{99} = 28 mg/kg for linezolid and ED_{99} = 27.7 mg/kg for moxifloxacine).[78]

Noncovalent DprE1 inhibitors derived from *N*-alkyl-5-hydroxypyrimidinone carboxamides were identified from high-throughput screening of a small compound library by St. Jude Children's Research Hospital.[79] Structural similarity to the antiretroviral drug raltegravir **35** (Fig. 17), an anti-HIV-1 drug inhibiting integrase through chelation of the magnesium ion, gave hope that initial hit **36** would significantly affect phosphoryl transferase in mycobacteria.[80–82] The efficacy of **36** on the *Mtb* growth was tested in several culture media. The compound was active in all media, albeit exerting low activity in nitrate/butyrate medium due to acidic pH and nitrosative stress. All sites of the template molecule have been broadly inspected and structurally modified, leading to 18 novel derivatives. A detailed study was performed only for raltegravir **35**. For the other derivatives, only the MIC values in the different culture media were established.
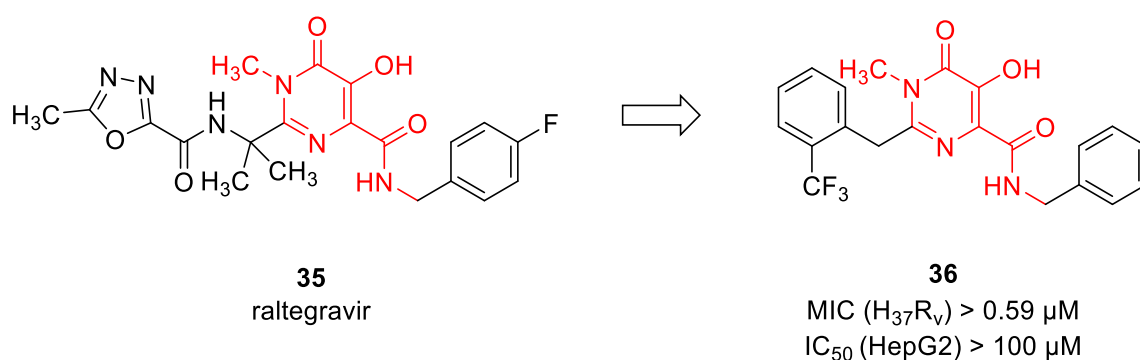

35
raltegravir

36
MIC (H$_{37}$R$_v$) > 0.59 µM
IC$_{50}$ (HepG2) > 100 µM

Fig. 17. 5-Hydroxypyrimidinone carboxamides derived from raltegravir. The *N*-methyl-5-hydroxypyrimidinone carboxamide core fragment is highlighted in red. [82]

Compound **36** revealed good water solubility, low cytotoxicity against HepG2 cell line (IC$_{50}$ > 100 µM), and favorable pharmacokinetics after oral administration to C57BL/6 mice at a dose of 10 mg/kg (C$_{max}$ = 0.25 µg/mL; T$_{max}$ = 1.0 h; T$_{1/2}$ = 4.1 h). MoA was found to be unrelated to magnesium regulation, but the DprE1 was confirmed and validated as a target of this class based upon the whole genome sequencing of resistant mutants.[82]

Chikhale *et al.* discovered a series of substituted benzothiazolylpyrimidine-5-carboxamides **37-40** (Fig. 18), showing an excellent activity *in vitro* against H$_{37}$R$_v$ strain of *Mtb* (MIC = 0.8-3.1 µM). The most active derivatives were substituted in the *para-* position of the phenyl ring directly attached to the pyrimidine core. MIC values ranged from 0.08 to 0.09 µM (*Mtb* H$_{37}$R$_v$; MIC (**37**, **38**) = 0.08 µM; MIC (**39**, **40**) = 0.09 µM). All of these compounds were found selective inhibitors of DprE1. Encouraged by their excellent activity, compounds underwent pharmacokinetic profile determination. Accordingly, compounds **37-40** were administered intravenously (10 mg/kg) and orally (20 mg/kg) to the Sprague Dawley rats. The best pharmacokinetic profile showed the most active compound **37** with 52.66 % oral availability (T$_{max}$ = 0.5 h; t$_{1/2}$ = 5.5 h; C$_{max}$ = 1.8 µg/ml and 0.59 µg/ml after i.v. and p.o administration, respectively). Derivative **38** showed comparable oral availability (52.70 %). Despite the good pharmacokinetic properties, the *in vivo* antitubercular activity was not determined. [69]
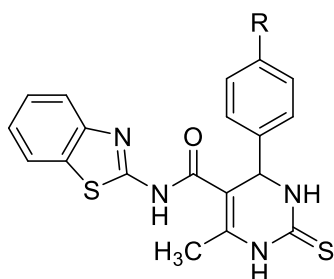
37-40
37: R = H; MIC ($H_{37}R_v$) = 0.08 µM; IC_{50} DprE1 = 7.7 µM
38: R = N(Me)$_2$; MIC ($H_{37}R_v$) = 0.08 µM; IC_{50} DprE1 = 10.3 µM
39: R = F; MIC ($H_{37}R_v$) = 0.09 µM; IC_{50} DprE1 = 9.2 µM
40: R = CF$_3$; MIC ($H_{37}R_v$) = 0.09 µM; IC_{50} DprE1 = 11.1 µM

Fig. 18. Benzothiazolylpyrimidine-5-carboxamides as DprE1 noncovalent inhibitors.[69] Structure modifications were performed in the phenyl ring at position C4.

2.1.5 Adenosine kinase inhibitors

Adenosine kinase (AdK; EC 2.7.1.20) is an enzyme involved in adenosine metabolism by phosphorylating it to adenosine 5′-monophosphate (AMP). Hocek group developed low cytotoxic and highly selective pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) inhibitors of AdK, displaying a high preference for mycobacteria AdK over the corresponding human enzyme (Fig. 19). Most of the compounds bearing chlorine and fluorine atom at position C2 of the 7-deazapurine moiety showed sub-micromolar or even nanomolar inhibition potency against AdK, highlighting compound **41** as the most potent one (IC_{50} = 1 nM). However, all members of the family were found nearly inactive under *in vitro* conditions against *Mtb* (strain My331/88). Only benzofuryl (**41**, Fig. 19) and dibenzofuryl (**42**; Fig. 19) nucleosides showed moderate activity (MIC value for **42** was 32 µM), while other compounds were inactive (*Mtb* My331/88 MIC > 125 µM). The reason for their low antimycobacterial activity presumably lies in low mycobacterial cell wall penetration or by parallel AdK-independent biosynthesis of AMP,[83] again highlighting the importance of the phenotypic screening approaches from the very beginning of the drug discovery projects.

General structure

41
ADK IC$_{50}$ (human) > 10 µM
ADK IC$_{50}$ (Mtb) = 1.2 nM
MIC (My331/88) = 62.5 µM

42
ADK IC$_{50}$ (human) > 20 µM
ADK IC$_{50}$ (Mtb) = 0.18 µM
MIC (My331/88) = 32 µM
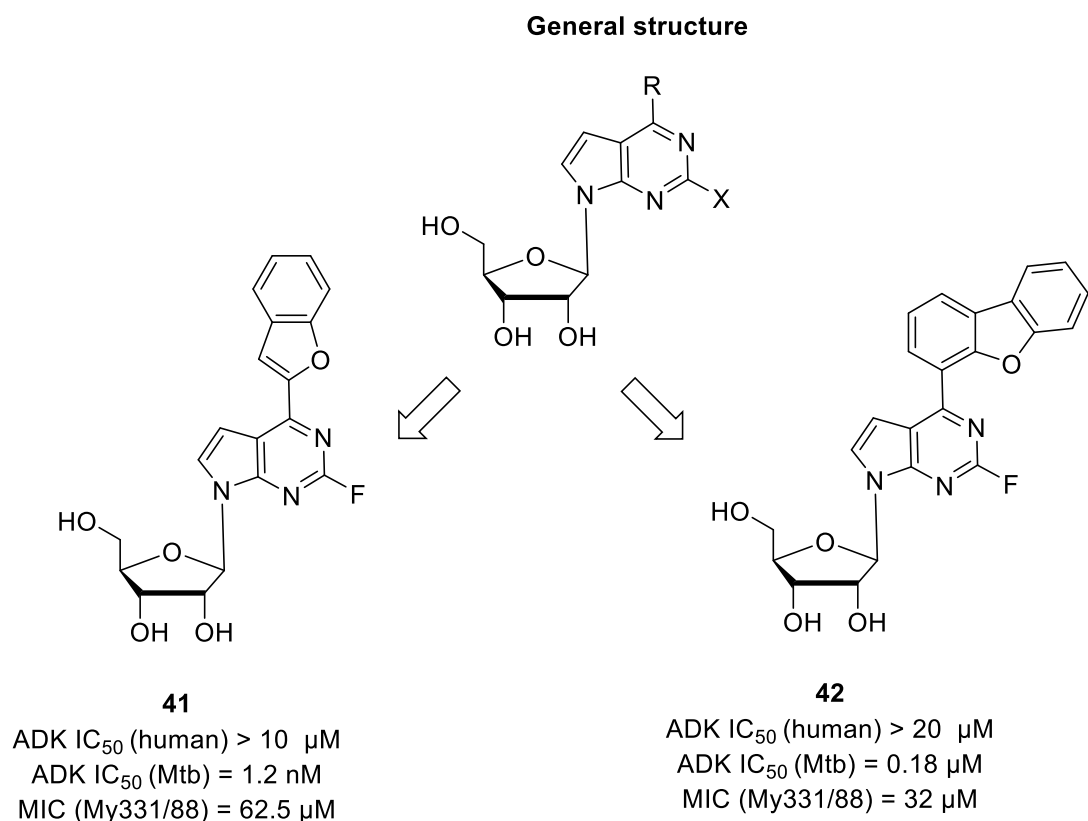
Fig. 19. Potent *Mtb* AdK inhibitors based on deazapurine scaffold developed by Hocek group.[83]

2.1.6 Inhibitors of the tRNA (guanine37-*N*^{1})-methyltransferase

The enzyme tRNA (guanine37-*N*^{1})-methyltransferase (TrmD; EC 2.1.1.228) is essential for the correct reading frame during the process of translation (Fig. 20). The biochemical function of this enzyme is to catalyze methylation of guanine *N*^{1} at nucleotide position 37 in a bacterial tRNA isoacceptors by *S*-adenosyl-*L*-methionine (SAM). SAM is converted into *S*-adenosyl-*L*-homocysteine (SAH).[84–86]
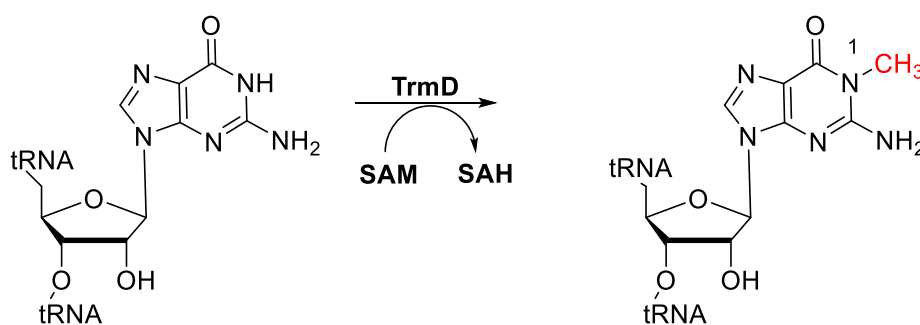
Fig. 20. Methylation of guanine catalyzed by TrmD.

Thienopyrimidinones were firstly reported as potent inhibitors of TrmD in *Haemophilus influenzae* in 2013. The thienopyrimidinone class was discovered by a fragment-based screening approach from a library containing 14,000 compounds from AstraZeneca. The study also confirmed that thienopyrimidinones act as SAM-competitive inhibitors of bacterial TrmD as determined by X-ray analysis. The most active compound **43** (Fig. 21) showed broad range

activity profile, including activity also against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).[87]

The results of this study were pursued by Zhong *et al.,* who tested the inhibitory activity of **43** against TrmD in *Pseudomonas aeruginosa*-PaTrmD (IC$_{50}$ = 180 nM). The binding of **43** to TrmD was further elucidated by surface plasmon resonance (SPR) experiments. Compound **43** showed a higher affinity to the gram-negative bacteria TrmD than mycobacterial and gram-positive bacteria TrmD. This can be explained by the cocrystals of compound **43** with PaTrmD and *Mtb*TrmD (TrmD in *Mycobacterium tuberculosis*), observing that compound **43** induces a unique conformational change of wall-loop residue in PaTrmD. These data led to the design of 33 new compounds with enhanced inhibition activity against PaTrmD. Structural changes were devoted to the replacement of the 4-aminopiperidine moiety and the introduction of a *n*-decylamine group proved to be the most advantageous substitution. The derivative **44** exhibited low antitubercular activity (*Mtb* H$_{37}$R$_v$ MIC$_{90}$ = 43 μM) but excellent inhibitory activity of PaTrmD (IC$_{50}$ = 0.025 μM). [88]

43
IC$_{50}$ (*H.influenzae*) = 0.33 μM
IC$_{50}$ (*E.coli*) = 0.18 μM
IC$_{50}$ (*S.aureus*) = 1.2 μM
IC$_{50}$ (PaTrmD) = 0.18 μM

44
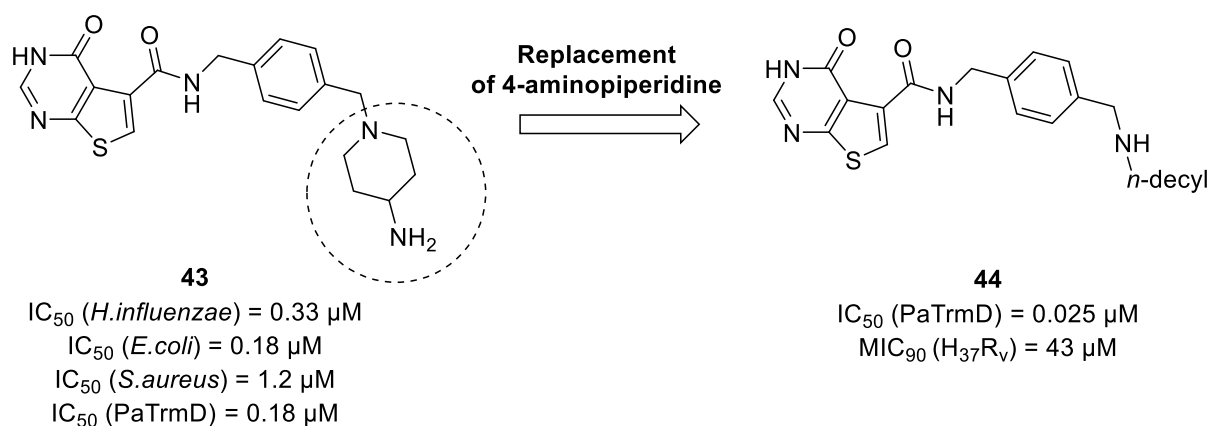IC$_{50}$ (PaTrmD) = 0.025 μM
MIC$_{90}$ (H$_{37}$R$_v$) = 43 μM

Fig. 21. Thienopyrimidines as inhibitors of TrmD. Replacement of 4-aminopiperidine was the most beneficial for antitubercular and PaTrmD inhibitory activity.

2.2 Pyrimidine derivatives active against *Mtb* with unknown mechanism of action

2.2.1 Purine-based and purine-inspired inhibitors of *Mtb*

Gundersen *et al.* have long been concerned with purine anti-TB agents. One of these derivatives, 9-(4-methoxybenzyl)-6-(2-furyl)purine, which has a chlorine at C2 position, is among the most potent anti-TB agents prepared by this research group. This derivative **45** together with other purines variously substituted at C2 served as a template scaffold for further work. [89,89–93] These compounds bearing different substituents at position C2 (CF$_3$, NO$_2$, H, F, Cl, CH$_3$, CH$_2$CH$_3$, OCH$_3$, N(CH$_3$)$_2$), were converted into 5-formylaminopyrimidines by imidazole ring-opening approach (Fig. 22). The rate of this ring-opening reaction is influenced by the effect of the group attached to position C2 of purine scaffold, where the electron-donating groups (Me, Et, OCH$_3$, N(CH$_3$)$_2$) decelerated the speed of reaction, while electron-withdrawing (Cl, NO$_2$, CF$_3$) groups significantly accelerated the rate of reaction. The presence

of fluorine or nitro group in position C2 led to the formation of a small amount of 2-oxopurines as side products. The most active 5-formylaminopyrimidine derivatives were the halogen derivatives **46** (IC$_{90}$ (H$_{37}$R$_v$) < 0.20 µg/mL) and **47** (IC$_{90}$ (H$_{37}$R$_v$) = 0.29 µg/mL) and the methyl-derivative **48** (IC$_{90}$ (H$_{37}$R$_v$) = 0.33 µg/mL). It was also proved that the presence of 5-formylamino group was essential for the anti-TB activity.

Removal of 5-formamido group in 2-chloropyrimidine derivatives **49** and **50** or its replacement with other substituents in pyrimidines **51**, **52**, **53** and **54** led to a significant decrease of antimycobacterial activity. All compounds consistently revealed a good selectivity to *Mtb*, while most of them were inactive against gram-positive bacteria like *S. aureus* and the gram-negative *E. coli*, or displayed a moderate activity only (MIC values > 64 µg/mL). [94]

Another modification of purine **45** led to the synthesis of derivatives **55** and **56** bearing a substituted imidazole group attached directly to the pyrimidine ring. Unfortunately, none of the structural changes enabled activity increment against *Mtb* H$_{37}$R$_v$ (**55**: IC$_{90}$ (H$_{37}$R$_v$) = 82 µg/mL; derivative **56** IC$_{90}$ (H$_{37}$R$_v$) = 14 µg/mL). Finally, a safety profile in the family of 5-formylpyrimidines was predicted by determining cytotoxicity on Vero cells (IC$_{50}$ > 40 µg/mL), suggesting a high selectivity on *Mtb*.[95]

55
IC$_{90}$ (H$_{37}$R$_v$) = 82 µg/mL

56
IC$_{90}$ (H$_{37}$R$_v$) = 14 µg/mL

51: R = MeNH; IC$_{90}$ (H$_{37}$R$_v$) = 15 µg/mL **45**: R = Cl; MIC (H$_{37}$R$_v$) = 0.78 µg/mL **46**: R = F; IC$_{90}$ (H$_{37}$R$_v$) < 0.20 µg/mL
52: R = NO$_2$; IC$_{90}$ (H$_{37}$R$_v$) = 21 µg/mL   **47**: R = Cl; IC$_{90}$ (H$_{37}$R$_v$) = 0.29 µg/mL
53: R = EtNH; IC$_{90}$ (H$_{37}$R$_v$) = 46 µg/mL   **48**: R = CH$_3$; IC$_{90}$ (H$_{37}$R$_v$) = 0.33 µg/mL
54: R = N(CH$_3$)$_2$; IC$_{90}$ (H$_{37}$R$_v$) > 100 µg/mL

49: R = H; IC$_{90}$ (H$_{37}$R$_v$) > 100 µg/mL
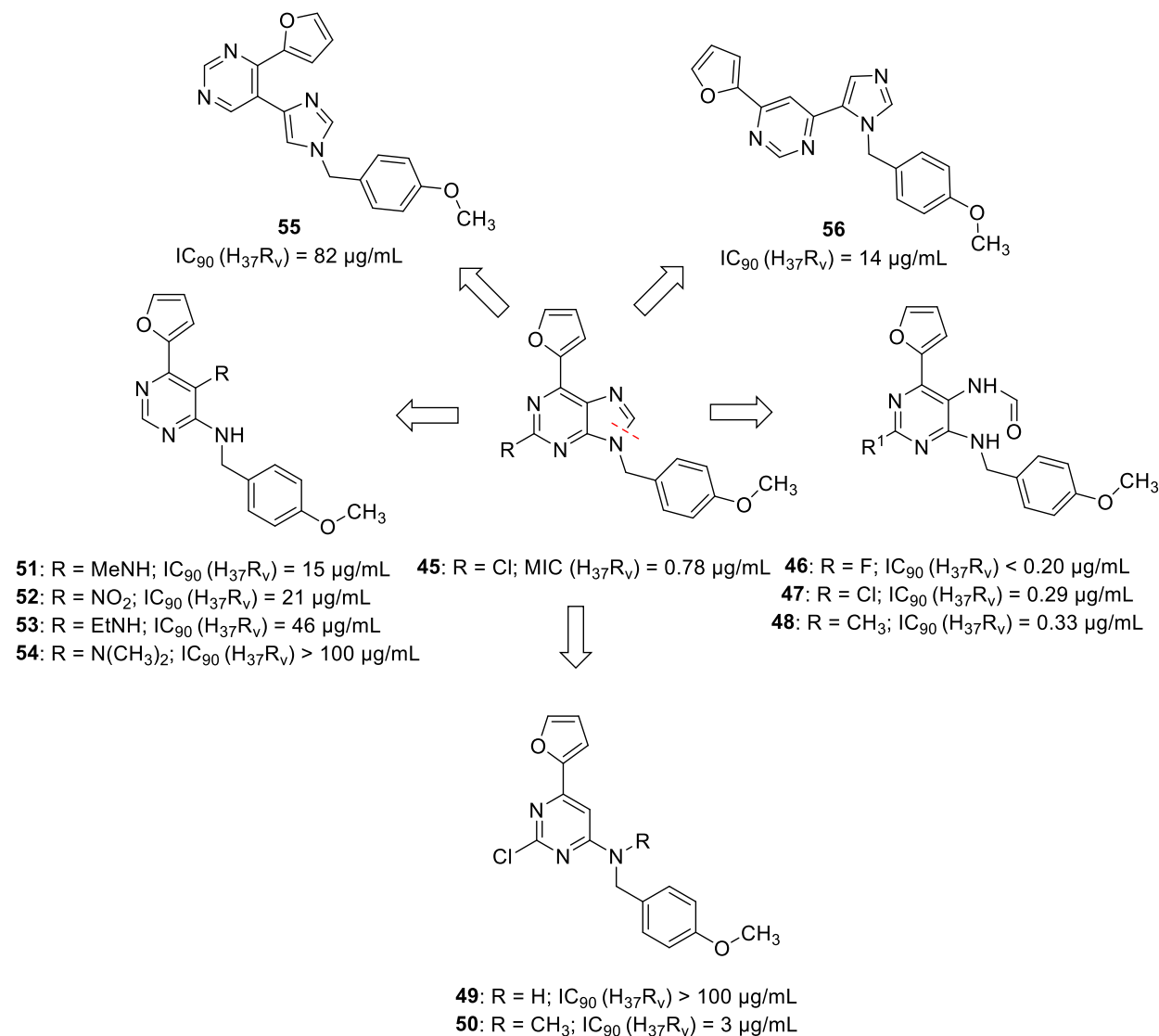**50**: R = CH$_3$; IC$_{90}$ (H$_{37}$R$_v$) = 3 µg/mL

Fig. 22. Pyrimidine derivatives reported by Gundersen *et al.* The red line represents the place where the ring-opening of imidazole core was performed.

2.2.2 Aminopyrimidines inhibitors of *Mtb*

A series of substituted quinoline chalcones were tested against *Mtb* ($H_{37}R_v$) and *Plasmodium falciparium* (*P. falciparium*) NF-H4 responsible for malaria. Only derivatives with 4-amino linkage (Fig. 23) showed antitubercular activity. The two most active compounds were **57** and **58** (MIC = 3.12 µg/mL). Quinoline-chalcones were then converted to 2-aminopyrimidine derivatives **59-62** by the reaction with guanidine (Fig. 23). Compound **59** showed a selectivity pattern to *P. falciparium* (inactive against *Mtb*), whereas derivatives **60** and **61** demonstrated selectivity to *Mtb* (inactive against *P. falciparium*). The most active pyrimidine derivative of the series was **62** exerted moderate activity against *Mtb* $H_{37}R_v$ (MIC = 6.25 µg/mL) and also against *P. falciparium* (MIC = 1 µg/mL).[96]
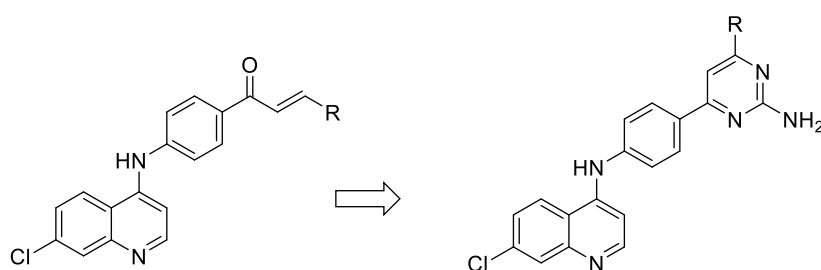
57: R = 2,3-dimethoxyphenyl; MIC ($H_{37}R_v$) = 3.12 µg/mL
58: R = 2,5-dimethoxyphenyl; MIC ($H_{37}R_v$) = 3.12 µg/mL

59: R = 4-methoxyphenyl; MIC (*P. falciparium*) = 2 µg/mL
60: R = 3,4,5-trimethoxyphenyl; MIC ($H_{37}R_v$) = 12.5 µg/mL
61: R = furan-2-yl; MIC ($H_{37}R_v$) = 12.5 µg/mL
62: R = 4-isopropylphenyl; MIC (*P. falciparium*) = 1 µg/mL; MIC ($H_{37}R_v$) = 6.25 µg/mL

Fig. 23. Quinoline-pyrimidines **57**-**62** with antitubercular activity. Where no MIC value for *Mtb* is given, the compound was inactive.

2.2.3 2-Methyl-4-(pyridin-2-yl)-4*H*-pyrimidino[2,1-b]benzothiazole-3-carboxylate inhibitors of *Mtb*

A series of 4*H*-pyrimido[2,1-*b*]benzothiazole derivatives was synthesized via a three-component one-pot reaction.[97] The INH was incorporated into the structure of these compounds with the aim of enhancing the antitubercular activity. In addition to INH, the presence of suitable substituents at positions C5 and C6 was also important for antitubercular activity. The unsubstituted derivative **63** was inactive and only the substituted derivatives **64** and **65** showed moderate activity (Fig. 24). SAR of this class of compounds disclosed that substituents like halogens, alkoxy and alkyl in the positions C5 and C6 improved the potency. ADMET parameters were also calculated *in silico*, suggesting good pharmacokinetic properties of **64**.[98] However, it can be speculated that these compounds act the same way as INH. A detailed study investigating the MoA is needed to uncover benefits in this class over INH.
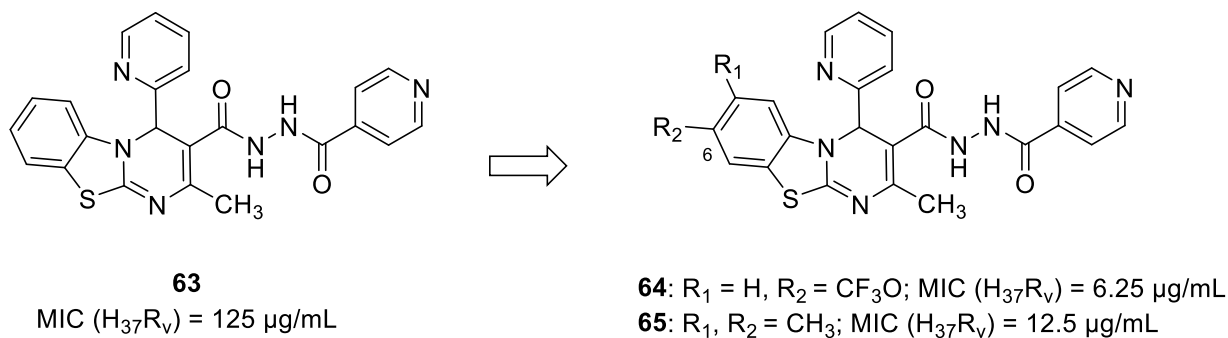
Fig. 24. 2-Methyl-4-(pyridin-2-yl)-4H-pyrimidino[2,1-*b*]benzothiazole-3-carboxylates **63-65** with incorporated INH.

2.2.4 *Ortho*-fused pyrimidine derivatives of *Mtb*

Imidazo[1,2-*c*]pyrimidines based on a structural modification of the recently approved drug pretomanid (PA-824; Fig. 25) were disclosed as potential antitubercular agents. The design considered the replacement of the oxazine ring with a bioisosteric pyrimidine. The structural similarity was checked indirectly by applying molecular modeling studies.[99] Notably, pretomanide has nitro group-dependent MoA, being activated by deazaflavin-dependent nitroreductase, releasing inactive desnitro metabolite. Thus, structural similarity with pretomanide in the absence of nitro group is shrowded with a highly questionable rationale.

A two-step synthetic protocol included a nucleophilic aromatic substitution of chlorine to introduce ethanolamine into the structure and subsequent cyclization to the final derivatives in the presence of POCl₃. All the prepared compounds were tested *in vitro* for their anti-TB activity against the *Mtb* $H_{37}R_v$ strain. Interestingly, MIC values did not change significantly after two and three weeks of incubation, but a significant drop in the activity occurred after 30 days. This behavior was notified for the top-ranked candidate **66,** reaching MIC value 2 µg/mL (comparable activity to amikacin: MIC = 2 µg/mL)[100] after two and three weeks of incubation dropping down to ten times to 20 µg/mL after 30 days.[99]

According to SAR, no substitution at the C5 carbon was tolerated. For all the C5 carbon-unsubstituted derivatives, anti-TB activity was significantly affected by the benzene substitution attached to C7 carbon of the core structure. Indeed, the best activity was asssociated with the presence of electron-withdrawing fluorine atom in the *ortho* and *para* positions or electron-donating group (MeO, Me) in the *para* position.
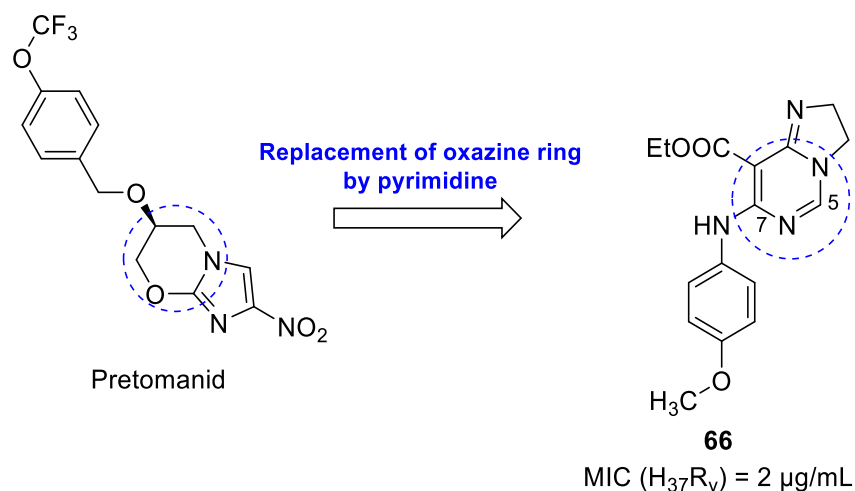
Fig. 25. Imidazo[1,2-c]pyrimidine derivatives as a new class of anti-TB agents inspired by FDA-approved antitubercular drug pretomanide.

2.2.5 Pyrrolo[2,3-*d*]pyrimidine-1,2,3-triazole inhbitors of *Mtb*

Highly potent 1*H*-pyrrolo[2,3-*d*]pyrimidine-1,2,3-triazole derivatives were developed by Shiva Raju et al. via copper-catalyzed intermolecular 1,3-dipolar cycloaddition between alkyne and respective aryl-, heteroaryl-, and alkyl-azides (Fig. 26). Final compounds were screened for the activity against *Mtb* $H_{37}R_v$. Benzyl derivative **67** was inactive (*Mtb* $H_{37}R_v$ MIC > 25 µg/mL) but incorporation of isoxazole into the structure led to the most active compounds **68** and **69** with MIC = 0.78 µg/mL against *Mtb* $H_{37}R_v$. Compound **68** was subjected to *in silico* studies, suggesting DprE1 as a potential target. However, experimental target validation has not been reported yet.[101]

67
MIC ($H_{37}R_v$) > 25 µg/mL

68: R = F; MIC ($H_{37}R_v$) = 0.78 µg/mL
69: R = H; MIC ($H_{37}R_v$) = 0.78 µg/mL
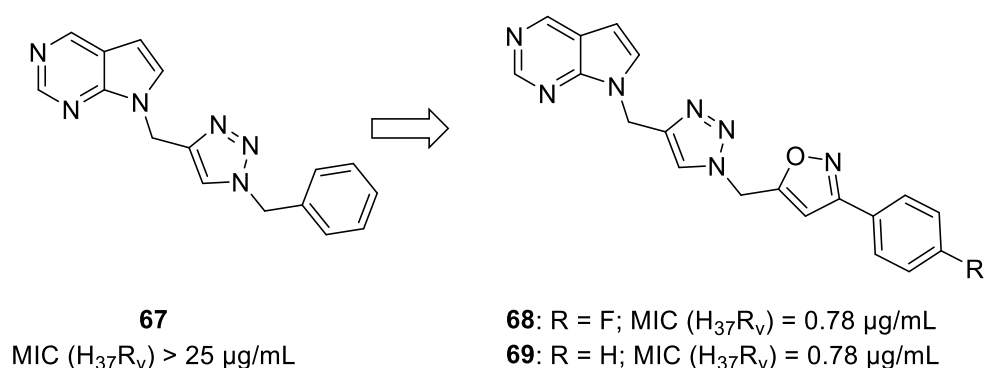
Fig. 26. 1*H*-Pyrrolo[2,3-*d*]pyrimidine-1,2,3-triazoles prepared by copper-catalyzed intermolecular 1,3-dipolar cycloaddition and their anti-TB activities.

2.2.6 Pyrano[2,3-*d*]pyrimidine inhibitors of *Mtb*

A series of pyrano[2,3-*d*]pyrimidine with antitubercular activity were reported by Kamdar *et al.* (Fig. 27).[102] Chromene-3-carbonitrile **70** was obtained via one-pot reaction of malononitrile, 4-methoxybenzaldehyde and variously substituted phenols. The derivative **70**

did not show activity against *Mtb* (MIC ($H_{37}R_v$) > 250 µg/mL) and, for this reason, was subjected to further reactions with different reagents like carbon disulfide forming dithione **71**. The product of the reaction with formic acid was lactam **72** and condensation with thiourea yielded compound **73**. All these derivatives were tested for antibacterial activity against a panel of gram-negative, gram-positive bacteria, and the *Mtb* $H_{37}R_v$ strain. Derivatives **72** and **73** were endowed with very low activity, reaching MIC = 62.5 µg/mL against *Mtb* $H_{37}R_v$, while derivative **71** exhibited activity only against *Steprococcus pyogenes* and *S. aureus* (for both MIC = 62.5 µg/mL).[102]
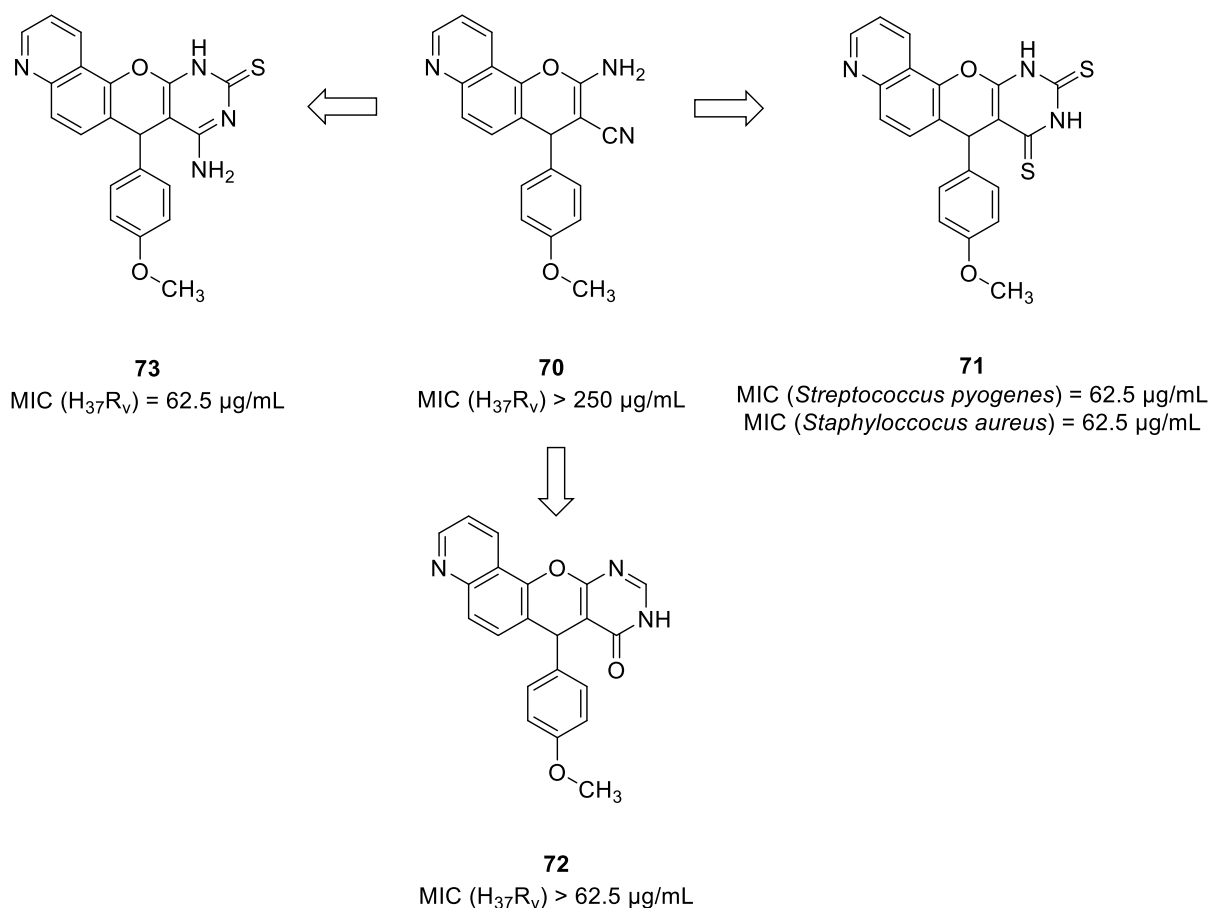

73
MIC ($H_{37}R_v$) = 62.5 µg/mL

70
MIC ($H_{37}R_v$) > 250 µg/mL

71
MIC (*Streptococcus pyogenes*) = 62.5 µg/mL
MIC (*Staphyloccocus aureus*) = 62.5 µg/mL

72
MIC ($H_{37}R_v$) > 62.5 µg/mL

Fig. 27. Pyrano[2,3-*d*]pyrimidines as antitubercular agents.

2.2.7 **5-Cyano-4-chloropyrimidine inhbitors of *Mtb***

In a study by Agarwal *et al.*, novel 4-chloropyrimidine derivatives were synthesized and tested *in vitro* for their antibacterial and anti-TB activities (Fig. 28).[103] The compounds were substituted at positions C2 and C6 by an aryl or alkylthio groups, and at the position C5 by a cyano group. In general, compounds revealed a moderate-to-high antitubercular profile, with compounds **74-77** as the most active against *Mtb* $H_{37}R_v$ strain (MIC = 0.78 µg/mL). The SAR disclosed that the substituents in positions C2 and C6 influenced efficacy the most. The optimal substitution at position C2 was *n*-propylthio appendage. Chain branching had no or negligible impact on the activity. For antitubercular activity, the introduction of an aryl substituent at position C6, particularly a thiophen-2-yl (**74**, **77**), a furan-2-yl (**75**) and a 3-fluorophenyl (**76**) group, was beneficial.[103]
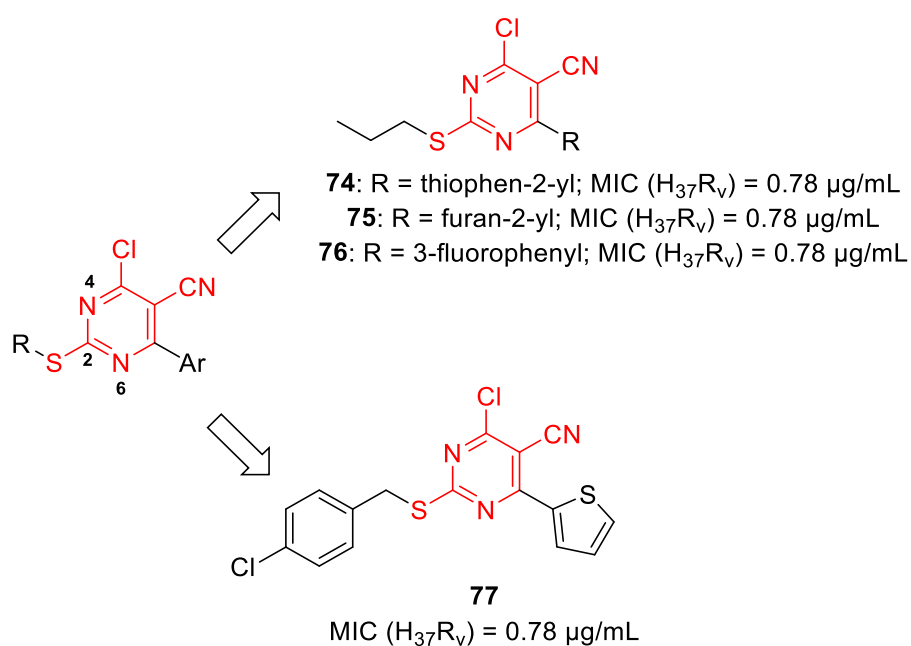
74: R = thiophen-2-yl; MIC (H$_{37}$R$_v$) = 0.78 µg/mL
75: R = furan-2-yl; MIC (H$_{37}$R$_v$) = 0.78 µg/mL
76: R = 3-fluorophenyl; MIC (H$_{37}$R$_v$) = 0.78 µg/mL

77
MIC (H$_{37}$R$_v$) = 0.78 µg/mL

Fig. 28. Synthesis and anti-TB activity of substituted 4-chloropyrimidines. The red part represents part of the molecule necessary for anti-TB activity.

2.2.8 Pyrazolo[1,5-*a*]pyrimidines inhibitors of *Mtb*

Modi *et al.* prepared a series of pyrazolo[1,5-*a*]pyrimidines (Fig. 29) showing promising anti-TB activity. The most potent derivative **78** showed high *in vitro* activities against both the susceptible strain H$_{37}$R$_v$ (MIC = 0.8 µg/mL) and the MDR-TB strain (MIC = 3.12 µg/mL). However, **78** showed a significant decrease in activity against the XDR-TB strain (MIC = 25 µg/mL). Similar observation were made for compounds **79** and **80** when displaying almost identical activity against the H$_{37}$R$_v$ strain (MIC = 3.12 µg/mL) and against MDR-TB (MIC = 6.25 µg/mL). A significant drop in activity was determined for XDR-TB strains, with **79** being completely inactive (MIC > 100 µg/mL), only **80** retained residual activity (MIC = 12.5 µg/mL). Unfortunately, the mechanism of acquired resistance against XDR and MDR-TB strains remained elusive. Cytotoxicity was verified for all three derivatives on Vero cells. Although compound **78** showed higher toxicity (IC$_{50}$ = 13.57 µg/mL) than derivatives **79** (IC$_{50}$ = 20.99 µg/mL) and **80** (IC$_{50}$ = 21.26 µg/mL), derivative **78** had the best selectivity index between MIC and IC$_{50}$(cytotoxicity) values.[104]
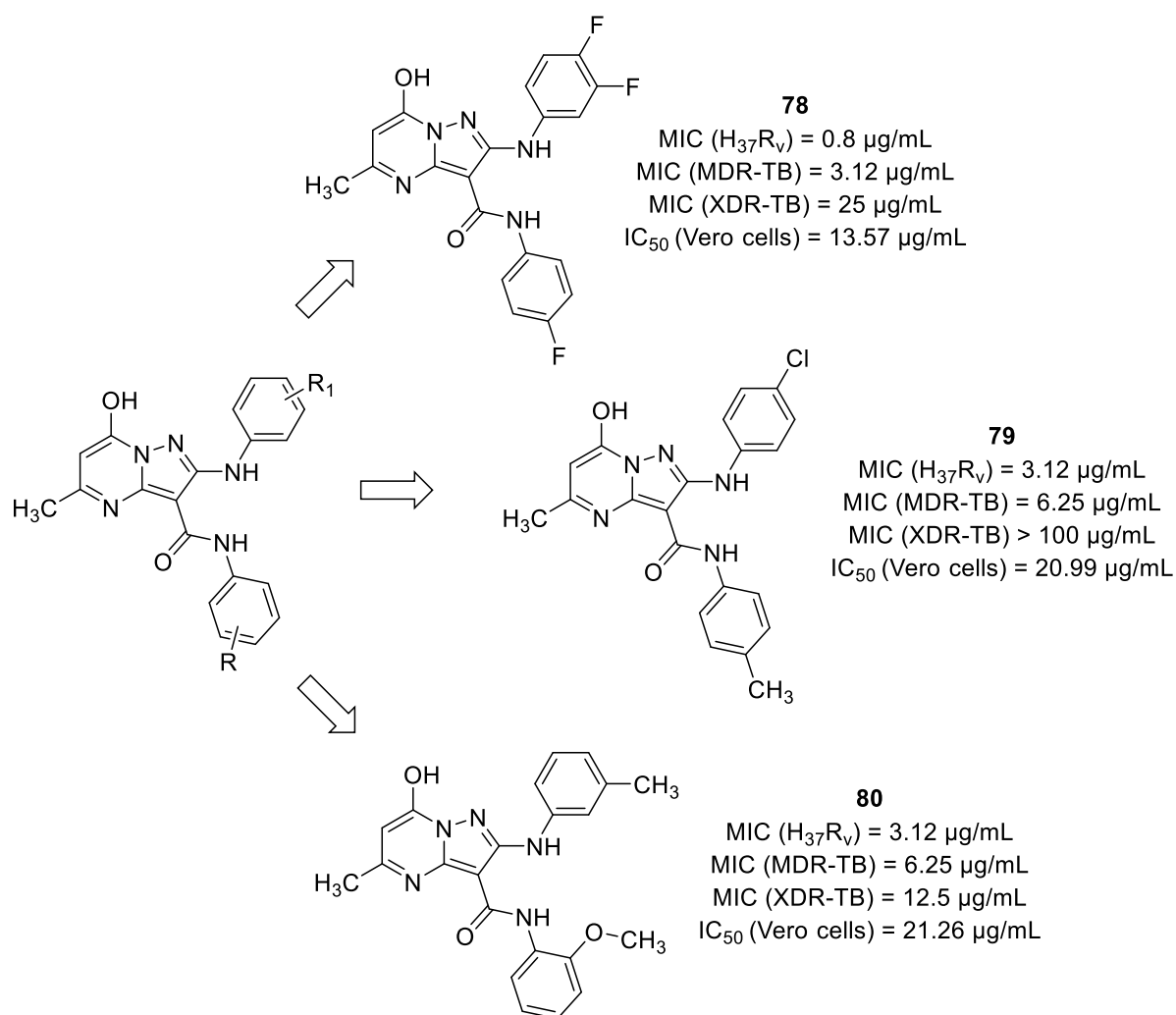
Fig. 29. Pyrazolo[1,5-*a*]pyrimidines with good-to-moderate antitubercular activity against drug-susceptible strain $H_{37}R_v$, MDR-TB and XDR-TB strains.

2.2.9   2-Pyrazolylpyrimidinone inhibitors of *Mtb*

A comprehensive review describing pyrazole-containing antitubercular drugs was published in 2017 highlighting advances in the drug discovery of these agents.[105] Another work identified 2-pyrazolylpyrimidinones with antitubercular activity through a high-throughput screening campaign from a Medicines for Malarie Venture compounds library. From a library counting 530,000 compounds, two hit compounds **81** and **82** were identified (Fig. 30). These compounds had good MIC values in different media, especially in the media containing iron (GAST-Fe, MIC = 0.02 μM), suggesting the iron-chelating properties of this scaffold. The family of 2-pyrazolylpyrimidinones resembles pyrazolopyrimidinone compounds, which are known as intracellular iron chelators useful for the therapy of *Mtb* (see section 2.2.11).[106] In addition, the single-crystal X-ray structure in a similar group of pyrazolylpyridines-Fe complexes was elucidated.[107]

Initial hits **81** and **82** exhibited excellent *in vitro* anti-TB activity, with compound **81** not influencing the activity of potassium channel hERG (IC$_{50}$ > 30 μM). Unfortunately, compounds **81** and **82** suffered from limited water solubility at pH 7.4 (**81** = 10 μM; **82** < 5 μM) and were

cytotoxic against Vero cells (**81**: IC_{50} = 8 µM; **82**: IC_{50} = 1.2 µM). All structural modifications were conducted with an effort to maintain high anti-TB activity, improve solubility and increase the selectivity index between *in vitro* cytotoxicity and antitubercular activity. The most potent derivative **83** (*Mtb* $H_{37}R_v$ MIC = 0.3 µM) was almost insoluble in water (< 5 µM) and hence was excluded from further testing. The second top-ranked derivative in the series was **84** (MIC = 0.9 µM), revealing excellent SI values with moderate water solubility (25 µM). Furthermore, **84** is endowed with metal-chelating properties, forming the critical part of suspected MoA. Iron supplementation using a high concentration of iron salts showed a significant change in MIC values, confirming that 2-pyrazolylpyrimidinones perturbs iron-homeostasis in *Mtb*.
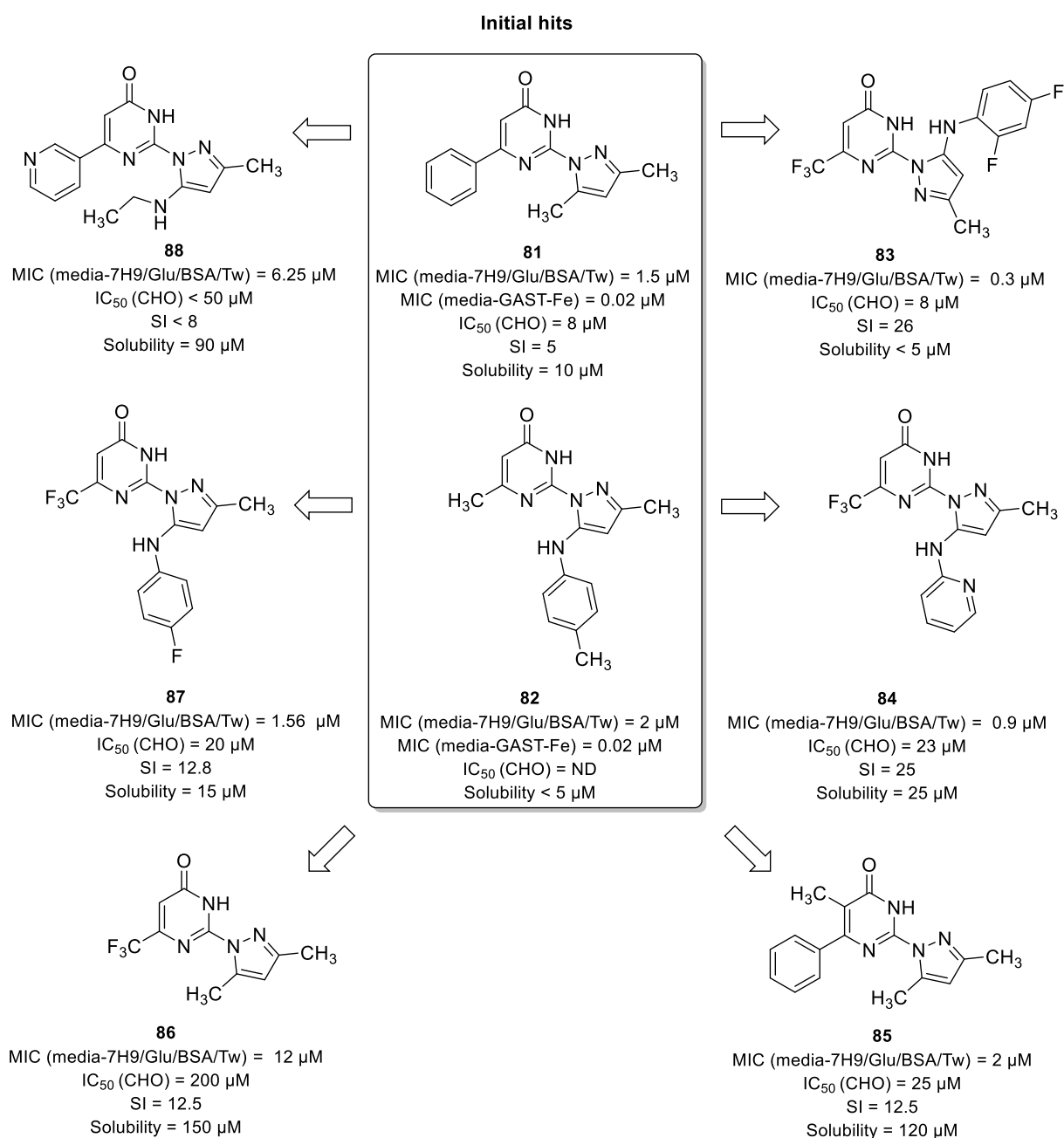

Fig. 30. Initial hits **81** and **82** determined from a high-throughput screening campaign leading to new antitubercular agents **83**-**88** related to 2-pyrazolylpyrimidinones.

The physicochemical and *in vitro* DMPK (drug, metabolism and pharmacokinetics) properties were determined for derivatives **85**-**88**. Compound **85** showed very low microsomal stability, and therefore only compounds **86**-**88** underwent pharmacokinetic determination in mice. Derivatives **86** and **87** showed low values of distribution volume and a low clearance rate with moderate oral bioavailability of approximately 40%. Derivative **88** displayed 33% oral bioavailability, high distribution volume and rapid blood clearance.
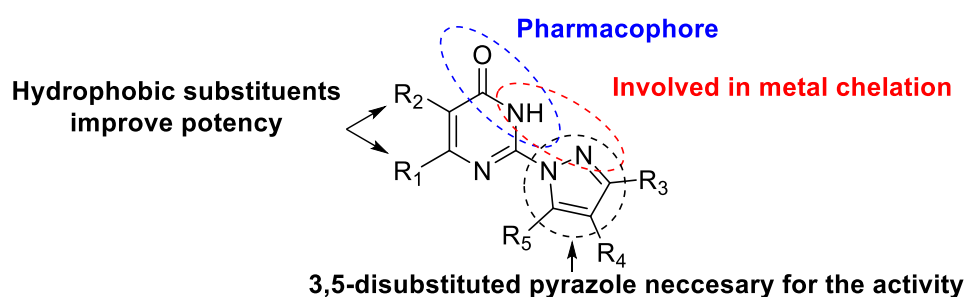
Summary of SAR

Fig. 31. Structural requirements in 2-pyrazolylpyrimidinone family as antitubercular agents.

The MoA of 2-pyrazolylpyrimidinones remains elusive. The compounds retained activity against strains resistant to DprE1 (mutation in C387S) and QcrB (mutation in A396T) inhibitors, suggesting that these are not targets. These derivatives did not display a positive signal in the two bioluminescence assays PiniB-LUX (positive signal confirms that the compound affects mycobacterial cell-wall biosynthesis) and PrecA-LUX (positive signal indicates genotoxicity of the compounds). On the other hand, strains with MmpL3 mutations were cross-resistant to these compounds. Yet using transcriptional silencing of Mmpl3 did not result in a significant change in MIC values, indicating that MmpL3 is not a direct target of these compounds. The authors hypothesized that Mmpl3 is a transporter of these compounds across the cell membrane. The assumption builds on the fact that these compounds can form heme-like-iron complexes, where MmpL3 mediates heme transport. [108]

2.2.10 Ceritinib derivatives as antimycobacterial agents

Ceritinib displaying a good *in vitro* MIC value (9.0 µM) against avirulent strain $H_{37}R_a$ served as a template drug for the synthesis of antitubercular derivatives. The anti-TB activity of ceritinib was found during the screening campaign of MedChemExpress bioactive library of small molecules. According to the SAR in the related series, the presence of 2-isopropoxy-5-methyl-4-(piperidin-4-yl)aniline moiety at position C2 of the ceritinib pyrimidine ring is vital for its antimycobacterial efficiency (Fig. 32). The amine linker in position C4 improved activity and *in vivo* efficacy. The most promising derivative was compound **89** with a naphthalene-2-amine group at position C4 of the pyrimidine core. Unlike other derivatives showing very close *in vitro* efficacy, derivative **89** exhibited good efficacy even under *in vivo* conditions and was non-cytotoxic. Derivative **89** was used for *in vivo* study in BALB/c mouse model infected with autoluminescent $H_{37}R_a$. Mice were treated for six consecutive days with doses 100 mg/kg and 300 mg/kg, respectively. In both treated groups, the total lung and spleen bacterial burden

was reduced by 2.0 and 0.5 $\log_{10}$ relative light unit (RLU), respectively. Compound **89** even exceeded the efficacy of standard ceritinib administered at a dose of 300 mg/kg (1.0 $\log_{10}$ RLU reduction in total lung bacterial burden). The target of these compounds was hypothesized via *in silico* modeling only, pointing out to DHFR.[109]
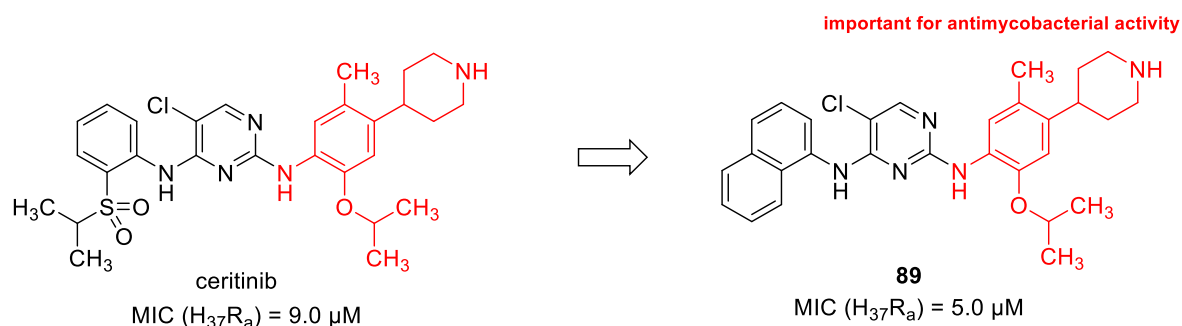

Fig. 32. Structural modifications of ceritinib leading to compound **85** with the highest anti-TB activity in this series.

2.2.11 Pyrazolo[1,5-*a*]pyrimidin-7(4*H*)one as inhibitors of *Mtb*

Oh *et al.* identified pyrazolo[1,5-*a*]pyrimidin-7(4*H*)-one **90**, which exhibited good anti-TB activity (MIC = 3.12 µM).[110] Pyrazolopyrimidines **91**-**93** are known from the literature as anti-TB agents (Fig. 33). Compound **91** was published by the group of Ioerger *et al.* The whole-genome sequencing of resistant mutants to **91** had mutations in *eccB*3 (Rv0283), an essential component of the ESX-3 type VII secretion system of the mycobacterial genome.[111] However, the ongoing study suggested that ESX-3 is not likely the target of **91**, but that the compound interferes with the metabolism of iron, where ESX-3 plays an important role.[106] Resistant mutants to derivative **92** exhibited insertions of a transposon (IS61110) in rv1685c, a transcription factor whose biological role is yet unexplored. Compound **93** was reported as an inhibitor of mycobacterial 1-deoxy-*D*-xylulose-5-phosphate synthase. Interestingly, although all three structures show a high degree of similarity, their MoA is different.[106,112,113]

Pyrazolo[1,5-*a*]pyrimidine-7(4*H*)ones can exist in three tautomeric forms where individual tautomers can interact with different biological targets. Motivated by this fact, the authors resynthesized derivative **93** and performed X-ray structure analysis which showed that pyrazolo[1,5-*a*]pyrimidine-7(4*H*)one is found in the keto-form. According to precA-LUX and piniB-LUX assays, compounds **90** and **92**-**94** do not affect the synthesis of cell wall nor cause DNA damage. It was also estimated that the anti-TB activity of **90** is not mediated by perturbation in iron homeostasis. Derivative **94** was confirmed to have the ability to inhibit the growth of *Mtb* inside macrophages in addition to its high anti-TB activity (MIC = 2.73 µM), low cytotoxicity against HepG2 cells (IC$_{50}$ > 100 µM) and good selectivity index (SI > 36).

The authors also raised *Mtb* mutants against **94** with the aim of determining the target. These resistant strains showed resistance to **90** and **94** and did not reveal any cross-resistance against **91** and **93**, suggesting that **91** and **93** have different MoA. The whole-genome sequencing of resistant mutant strains to compound **94** pointed to Rv1751 encoding FAD-

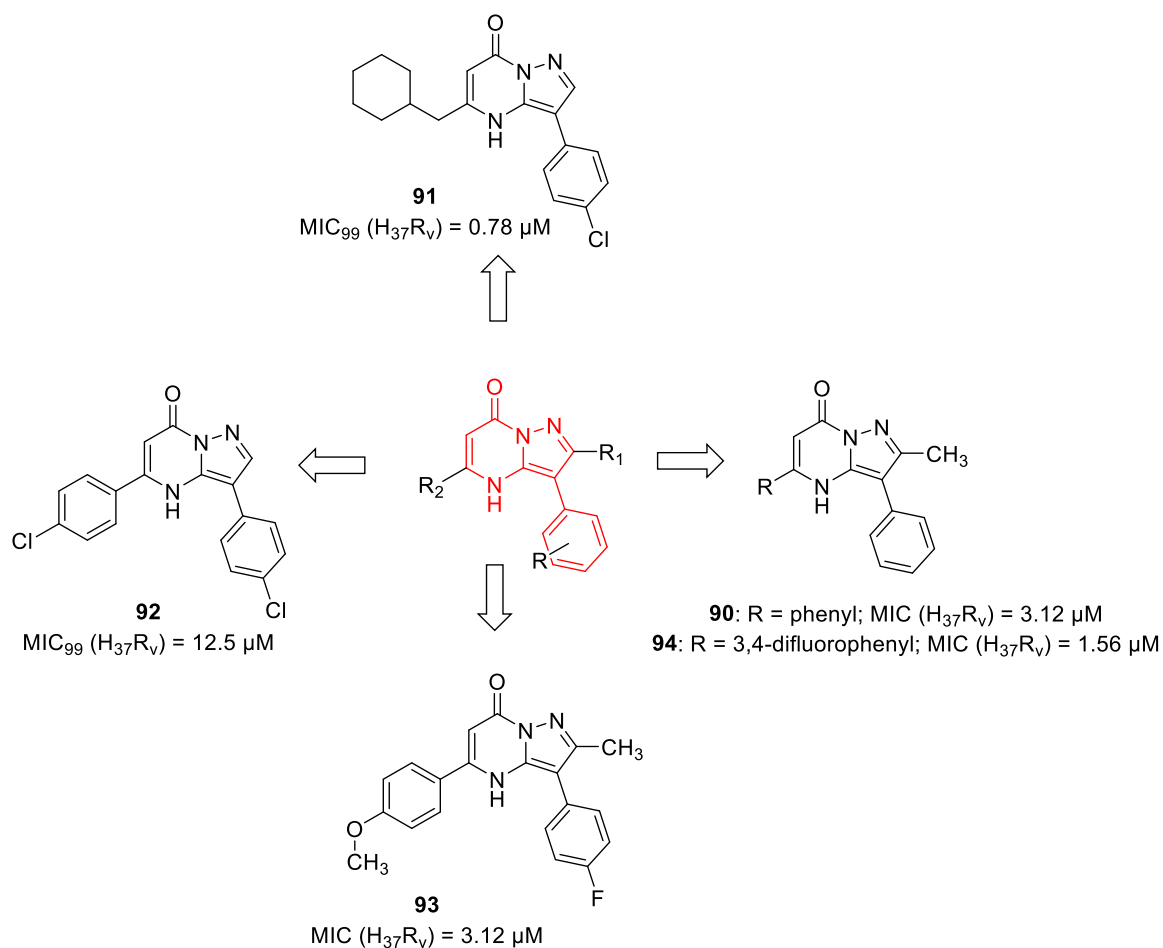dependent hydroxylase that promotes compound catabolism by hydroxylation from molecular oxygen.[110]

Fig. 33. Pyrazolo[1,5-*a*]pyrimidin-7(4*H*)one with anti-TB activity. The red structure highlights previously defined pharmacophore. Adapted from ref.[110]

3. Conclusion

TB is the number one cause of human death from infectious diseases in the world. More strikingly, it is estimated that one-third of the world's population is infected with *Mtb*. These alarming data along with growing numbers in MDR-TB and XDR-TB strains resistant to the currently available drugs call for new drugs with suppressed crossover resistance. TB drug discovery campaigns are chasing new drugs with lower toxicity and shorter treatment times. After decades of low interest and investments into anti-TB research, delamanide, pretomanide and bedaquiline have been approved for TB treatment with a few more drugs in the late stage of clinical trials, all imposing unique MoA compared to standard TB therapy by INH, RIF, EMB and PZA that have been established since 1980s.

The current review highlighted the role of pyrimidine derivatives as a promising class of antitubercular compounds for future drug development. This is corroborated by the fact that there are currently three compounds containing the pyrimidine ring in different phases of clinical trials. Indeed, TBA-7371 and SPR720 are undergoing Phase II clinical trials. These clinical candidates also exert different MoA, with SPR720 inhibiting bacterial DNA gyrase

(GyrB), while TBA-7371 is a noncovalent inhibitor of DprE1.[114] GSK-286, a Phase I drug candidate, is endowed with brand-new MoA associated with cholesterol catabolism. The great advantage of introducing pyrimidine into antitubercular agents is their relatively straightforward synthesis, allowing to attach various substituents to the pyrimidine core for further drug properties improvements. Several classes of pyrimidine-containing compounds have been described to date, revealing different MoA. In this review, we have highlighted the derivatives acting as noncovalent DprE1 inhibitors **32-34** with the promising anti-TB profile. Furthermore, these well water-soluble agents also acted well under *in vivo* conditions using TB-infected mice. Extensive biochemistry and *in vivo* efficacy proof-of-concept in some of the pyrimidine derivatives remain to be performed to enable lead development, and to provide the translational potential for the drug to enter clinical trials. Particular attention is paid to uncovering new MoA from existing TB drugs as it could result in high sensitivity towards TB strains, and it can also provide rational background to develop more potent drugs, e.g. by applying *in silico* techniques. A manageable metabolism profile and good oral availability are other important properties pursued in searching for new anti-TB agents. As TB is a comorbid disease, PK/PD profiles of a new agent should be compatible with HIV treatment and diabetes mellitus management. Most importantly, a new treatment is expected to achieve a stable and relapse-free cure. These aspects are critical to follow in the search for new anti-TB drugs. Finally, we believe that the information presented in this review may inspire drug discovery research of antitubercular agents centered on pyrimidine moiety in the coming years.

Conflict of Interest Statement

Authors declare that they have no competing interest.

Abbreviations

AdK	adenosine kinase
ADMET	absorption, distribution, metabolism, excretion and toxicity
AIDS	Acquired immune deficiency syndrome
AMP	adenosine 5´-monophosphate
ATP	adenosine 5´-triphosphate
ATS/CDC/ERS/IDSA	The American Thoracic Society, U.S. centers for Disease control and Prevention, European Respiratory Society, and Infectious Diseases Society of America
CH$_2$THF	N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate
COVID-19	Coronavirus Disease 2019
DHF	7,8-dihydrofolate

DNA	deoxyribonucleic acid
DOTS	directly observed therapy, short course
DPA	decaprenyl-D-arabinose
DprE1	decaprenylphosphoryl-β-D-ribose-2′-epimerase
DPR	decaprenylphosphoryl-D-ribose
DPX	decaprenylphophoryl-2-keto ribose
DS-TB	drug-susceptible tuberculosis
dTDP	thymidine diphosphate
dTMP	thymidine monophosphate
dUMP	2′-deoxyuridin-5′-monophosphate
EMB	ethambutol
FAD	flavin adenine dinucleotide
hERG	human ether-a-go-go-related gene
HIV	human immunodeficiency virus
INH	isoniazid
LTBI	latent form of tuberculosis infection
MDR-TB	multidrug-resistant tuberculosis
MIC	minimum inhibitory concentration
MoA	mechanism of action
MRC-5	Medical Research Council cell strain 5
Mtb	*Mycobacterium tuberculosis*
NADPH	nicotinamide adenine dinucleotide phosphate
ND	not determined
PanD	aspartate decarboxylase
PBPs	penicillin-binding proteins
PZA	pyrazinamide
QcrB	cytochrome B subunit of the cytochrome bc1 complex
RIF	rifampicin
RpsA	ribosomal protein S1
rRNA	ribosomal ribonucleic acid
SAM	*S*-adenosyl-*L*-methionine
SAR	structure-activity relationships
SDGs	sustainable development goals
TB	tuberculosis
ThyA	thymidylate synthase
ThyX	flavin-dependent thymidylate synthase
TMPK	thymidine monophosphate kinase

TMPKh	human thymidine monophosphate kinase
TMPKm	mycobacterial thymidine monophosphate kinase
TrmD	tRNA (guanine37-N^1)-methyltransferase
XDR-TB	extensively drug-resistant tuberculosis

References

(1) Su, Y.; Garcia Baena, I.; Harle, A. C.; Crosby, S. W.; Micah, A. E.; Siroka, A.; Sahu, M.; Tsakalos, G.; Murray, C. J. L.; Floyd, K.; Dieleman, J. L. Tracking Total Spending on Tuberculosis by Source and Function in 135 Low-Income and Middle-Income Countries, 2000–17: A Financial Modelling Study. *Lancet Infect. Dis.* **2020**, *20* (8), 929–942. https://doi.org/10.1016/S1473-3099(20)30124-9.

(2) *WHO | Global tuberculosis report 2019*. WHO. http://www.who.int/tb/publications/global_report/en/ (accessed 2020-07-08).

(3) World Health Organization. *WHO Consolidated Guidelines on Drug-Resistant Tuberculosis Treatment.*; 2019.

(4) Aubry, A.; Pan, X.-S.; Fisher, L. M.; Jarlier, V.; Cambau, E. Mycobacterium Tuberculosis DNA Gyrase: Interaction with Quinolones and Correlation with Antimycobacterial Drug Activity. *Antimicrob. Agents Chemother.* **2004**, *48* (4), 1281–1288. https://doi.org/10.1128/AAC.48.4.1281-1288.2004.

(5) Diacon, A. H.; Pym, A.; Grobusch, M. P.; de los Rios, J. M.; Gotuzzo, E.; Vasilyeva, I.; Leimane, V.; Andries, K.; Bakare, N.; De Marez, T.; Haxaire-Theeuwes, M.; Lounis, N.; Meyvisch, P.; De Paepe, E.; van Heeswijk, R. P. G.; Dannemann, B. Multidrug-Resistant Tuberculosis and Culture Conversion with Bedaquiline. *N. Engl. J. Med.* **2014**, *371* (8), 723–732. https://doi.org/10.1056/NEJMoa1313865.

(6) Lee, M.; Lee, J.; Carroll, M. W.; Choi, H.; Min, S.; Song, T.; Via, L. E.; Goldfeder, L. C.; Kang, E.; Jin, B.; Park, H.; Kwak, H.; Kim, H.; Jeon, H.-S.; Jeong, I.; Joh, J. S.; Chen, R. Y.; Olivier, K. N.; Shaw, P. A.; Follmann, D.; Song, S. D.; Lee, J.-K.; Lee, D.; Kim, C. T.; Dartois, V.; Park, S.-K.; Cho, S.-N.; Barry, C. E. Linezolid for Treatment of Chronic Extensively Drug-Resistant Tuberculosis. *N. Engl. J. Med.* **2012**, *367* (16), 1508–1518. https://doi.org/10.1056/NEJMoa1201964.

(7) Lechartier, B.; Cole, S. T. Mode of Action of Clofazimine and Combination Therapy with Benzothiazinones against Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **2015**.

(8) Prosser, G. A.; Carvalho, L. P. S. Kinetic Mechanism and Inhibition of *M Ycobacterium Tuberculosis* D -alanine: D -alanine Ligase by the Antibiotic D -cycloserine. *FEBS J.* **2013**, *280* (4), 1150–1166. https://doi.org/10.1111/febs.12108.

(9) *Terizidone - an overview | ScienceDirect Topics*. https://www.sciencedirect.com/topics/medicine-and-dentistry/terizidone (accessed 2021-12-10).

(10) Zimhony, O.; Cox, J. S.; Welch, J. T.; Vilchèze, C.; Jacobs, W. R. Pyrazinamide Inhibits the Eukaryotic-like Fatty Acid Synthetase I (FASI) of Mycobacterium Tuberculosis. *Nat. Med.* **2000**, *6* (9), 1043–1047. https://doi.org/10.1038/79558.

(11) Shi, W.; Zhang, X.; Jiang, X.; Yuan, H.; Lee, J. S.; Barry, C. E.; Wang, H.; Zhang, W.; Zhang, Y. Pyrazinamide Inhibits Trans-Translation in *Mycobacterium Tuberculosis*. *Science* **2011**, *333* (6049), 1630–1632. https://doi.org/10.1126/science.1208813.

(12) Zhang, S.; Chen, J.; Shi, W.; Liu, W.; Zhang, W.; Zhang, Y. Mutations in *PanD* Encoding Aspartate Decarboxylase Are Associated with Pyrazinamide Resistance in *Mycobacterium Tuberculosis*. *Emerg. Microbes Infect.* **2013**, *2* (1), 1–5. https://doi.org/10.1038/emi.2013.38.

(13) Goude, R.; Amin, A. G.; Chatterjee, D.; Parish, T. The Arabinosyltransferase EmbC Is Inhibited by Ethambutol in Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **2009**, *53* (10), 4138–4146. https://doi.org/10.1128/AAC.00162-09.

(14) *Amikin (Amikacin): Uses, Dosage, Side Effects, Interactions, Warning*. RxList. https://www.rxlist.com/amikin-drug.htm (accessed 2021-12-10).

(15) Ruiz, P.; Rodríguez-Cano, F.; Zerolo, F. J.; Casal, M. Investigation of the *In Vitro* Activity of Streptomycin Against *Mycobacterium Tuberculosis*. *Microb. Drug Resist.* **2002**, *8* (2), 147–149. https://doi.org/10.1089/107662902760190707.

(16) Wang, F.; Langley, R.; Gulten, G.; Dover, L. G.; Besra, G. S.; Jacobs, W. R.; Sacchettini, J. C. Mechanism of Thioamide Drug Action against Tuberculosis and Leprosy. *J. Exp. Med.* **2007**, *204* (1), 73–78. https://doi.org/10.1084/jem.20062100.

(17) Chambers, H. F.; Moreau, D.; Yajko, D.; Miick, C.; Wagner, C.; Hackbarth, C.; Kocagoz, S.; Rosenberg, E.; Hadley, W. K.; Nikaido, H. Can Penicillins and Other Beta-Lactam Antibiotics Be Used to Treat Tuberculosis? *Antimicrob. Agents Chemother.* **1995**, *39* (12), 2620–2624. https://doi.org/10.1128/AAC.39.12.2620.

(18) Chakraborty, S.; Gruber, T.; Barry, C. E.; Boshoff, H. I.; Rhee, K. Y. *Para* -Aminosalicylic Acid Acts as an Alternative Substrate of Folate Metabolism in *Mycobacterium Tuberculosis*. *Science* **2013**, *339* (6115), 88–91. https://doi.org/10.1126/science.1228980.

(19) Gler, M. T.; Skripconoka, V.; Sanchez-Garavito, E.; Xiao, H.; Cabrera-Rivero, J. L.; Vargas-Vasquez, D. E.; Gao, M.; Awad, M.; Park, S.-K.; Shim, T. S.; Suh, G. Y.; Danilovits, M.; Ogata, H.; Kurve, A.; Chang, J.; Suzuki, K.; Tupasi, T.; Koh, W.-J.; Seaworth, B.; Geiter, L. J.; Wells, C. D. Delamanid for Multidrug-Resistant Pulmonary Tuberculosis. *N. Engl. J. Med.* **2012**, *366* (23), 2151–2160. https://doi.org/10.1056/NEJMoa1112433.

(20) Parry, G. The Crystal Structure of Uracil. *Acta Crystallogr.* **1954**, *7* (4), 313–320. https://doi.org/10.1107/S0365110X54000904.

(21) Brown, D.; Lyall, J. Fine Structure of Cytosine. *Aust. J. Chem.* **1962**, *15* (4), 851-. https://doi.org/10.1071/CH9620851.

(22) Ozeki, K.; Sakabe, N.; Tanaka, J. The Crystal Structure of Thymine. *Acta Crystallogr. B* **1969**, *25* (6), 1038–1045. https://doi.org/10.1107/S0567740869003505.

(23) Bernal, J. D.; Crowfoot, D. Crystal Structure of Vitamin B1 and of Adenine Hydrochloride. *Nature* **1933**, *131* (3321), 911–912. https://doi.org/10.1038/131911b0.

(24) *Iclaprim FDA Approval Status*. Drugs.com. https://www.drugs.com/history/iclaprim.html (accessed 2022-10-07).

(25) *Stavudine Uses, Side Effects & Warnings*. Drugs.com. https://www.drugs.com/mtm/stavudine.html (accessed 2022-10-07).

(26) *Thonzylamine - brand name list from Drugs.com*. Drugs.com. https://www.drugs.com/ingredient/thonzylamine.html (accessed 2022-10-07).

(27) *Fluorouracil injection Uses, Side Effects & Warnings*. Drugs.com. https://www.drugs.com/mtm/fluorouracil-injection.html (accessed 2022-10-07).

(28) *Buspirone: Uses, Dosage, Side Effects*. Drugs.com. https://www.drugs.com/buspirone.html (accessed 2022-10-07).

(29) Shmalenyuk, E. R.; Kochetkov, S. N.; Alexandrova, L. A. Novel Inhibitors of *Mycobacterium Tuberculosis* Growth Based on Modified Pyrimidine Nucleosides and Their Analogues. *Russ. Chem. Rev.* **2013**, *82* (9), 896–915. https://doi.org/10.1070/RC2013v082n09ABEH004404.

(30) Global Alliance for TB Drug Development. *Phase 1, Partially-Blind, Placebo Controlled Randomized, Combined SAD With Food Effect Cohort and MAD and DDI Study to Evaluate Safety, Tolerability, PK and PK Interaction Between TBA-7371 With Midazolam and Bupropion in Healthy Subjects.*; Clinical trial registration NCT03199339; clinicaltrials.gov, 2018. https://clinicaltrials.gov/ct2/show/NCT03199339 (accessed 2020-07-28).

(31) *GSK-286 | Working Group for New TB Drugs*. https://www.newtbdrugs.org/pipeline/compound/gsk-286 (accessed 2021-08-28).

(32) Nuermberger, E. L.; Martínez-Martínez, M. S.; Sanz, O.; Urones, B.; Esquivias, J.; Soni, H.; Tasneen, R.; Tyagi, S.; Li, S.-Y.; Converse, P. J.; Boshoff, H. I.; Robertson, G. T.; Besra, G. S.; Abrahams, K. A.; Upton, A. M.; Mdluli, K.; Boyle, G. W.; Turner, S.; Fotouhi, N.; Cammack, N. C.; Siles, J. M.; Alonso, M.; Escribano, J.; Lelievre, J.; Pérez-Herrán, E.; Bates, R. H.; Maher-Ewards,

G.; Barros, D.; Ballell, L.; Jiménez, E. GSK2556286 Is a Novel Antitubercular Drug Candidate Effective in Vivo with the Potential to Shorten Tuberculosis Treatment. *bioRxiv* **2022**, 2022.02.04.479214. https://doi.org/10.1101/2022.02.04.479214.

(33) Talley, A. K.; Thurston, A.; Moore, G.; Gupta, V. K.; Satterfield, M.; Manyak, E.; Stokes, S.; Dane, A.; Melnick, D. First-in-Human Evaluation of the Safety, Tolerability, and Pharmacokinetics of SPR720, a Novel Oral Bacterial DNA Gyrase (GyrB) Inhibitor for Mycobacterial Infections. *Antimicrob. Agents Chemother.* 65 (11), e01208-21. https://doi.org/10.1128/AAC.01208-21.

(34) Mori, M.; Villa, S.; Ciceri, S.; Colombo, D.; Ferraboschi, P.; Meneghetti, F. An Outline of the Latest Crystallographic Studies on Inhibitor-Enzyme Complexes for the Design and Development of New Therapeutics against Tuberculosis. *Molecules* **2021**, *26* (23), 7082. https://doi.org/10.3390/molecules26237082.

(35) Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Dugué, L.; Heyerick, A.; De Keukeleire, D.; Pochet, S.; Busson, R.; Herdewijn, P.; Van Calenbergh, S. 3'-*C*-Branched-Chain-Substituted Nucleosides and Nucleotides as Potent Inhibitors of *Mycobacterium t Uberculosis* Thymidine Monophosphate Kinase. *J. Med. Chem.* **2003**, *46* (18), 3811–3821. https://doi.org/10.1021/jm021108n.

(36) Li de la Sierra, I.; Munier-Lehmann, H.; Gilles, A. M.; Bârzu, O.; Delarue, M. X-Ray Structure of TMP Kinase from Mycobacterium Tuberculosis Complexed with TMP at 1.95 Å Resolution11Edited by R. Huber. *J. Mol. Biol.* **2001**, *311* (1), 87–100. https://doi.org/10.1006/jmbi.2001.4843.

(37) Vanheusden, V.; Munier-Lehmann, H.; Froeyen, M.; Busson, R.; Rozenski, J.; Herdewijn, P.; Van Calenbergh, S. Discovery of Bicyclic Thymidine Analogues as Selective and High-Affinity Inhibitors of *Mycobacterium Tuberculosis* Thymidine Monophosphate Kinase. *J. Med. Chem.* **2004**, *47* (25), 6187–6194. https://doi.org/10.1021/jm040847w.

(38) Topliss, J. G. Utilization of Operational Schemes for Analog Synthesis in Drug Design. *J. Med. Chem.* **1972**, *15* (10), 1006–1011. https://doi.org/10.1021/jm00280a002.

(39) Phetsuksiri, B.; Jackson, M.; Scherman, H.; McNeil, M.; Besra, G. S.; Baulard, A. R.; Slayden, R. A.; DeBarber, A. E.; Barry, C. E.; Baird, M. S.; Crick, D. C.; Brennan, P. J. Unique Mechanism of Action of the Thiourea Drug Isoxyl on Mycobacterium Tuberculosis. *J. Biol. Chem.* **2003**, *278* (52), 53123–53130. https://doi.org/10.1074/jbc.M311209200.

(40) Van Daele, I.; Munier-Lehmann, H.; Froeyen, M.; Balzarini, J.; Van Calenbergh, S. Rational Design of 5'-Thiourea-Substituted α-Thymidine Analogues as Thymidine Monophosphate Kinase Inhibitors Capable of Inhibiting Mycobacterial Growth. *J. Med. Chem.* **2007**, *50* (22), 5281–5292. https://doi.org/10.1021/jm0706158.

(41) Van Poecke, S.; Munier-Lehmann, H.; Helynck, O.; Froeyen, M.; Van Calenbergh, S. Synthesis and Inhibitory Activity of Thymidine Analogues Targeting Mycobacterium Tuberculosis Thymidine Monophosphate Kinase. *Bioorg. Med. Chem.* **2011**, *19* (24), 7603–7611. https://doi.org/10.1016/j.bmc.2011.10.021.

(42) Toti, K. S.; Verbeke, F.; Risseeuw, M. D. P.; Frecer, V.; Munier-Lehmann, H.; Van Calenbergh, S. Synthesis and Evaluation of 5'-Modified Thymidines and 5-Hydroxymethyl-2'-Deoxyuridines as Mycobacterium Tuberculosis Thymidylate Kinase Inhibitors. *Bioorg. Med. Chem.* **2013**, *21* (1), 257–268. https://doi.org/10.1016/j.bmc.2012.10.018.

(43) Martínez-Botella, G.; Breen, J. N.; Duffy, J. E. S.; Dumas, J.; Geng, B.; Gowers, I. K.; Green, O. M.; Guler, S.; Hentemann, M. F.; Hernandez-Juan, F. A.; Joseph-McCarthy, D.; Kawatkar, S.; Larsen, N. A.; Lazari, O.; Loch, J. T.; Macritchie, J. A.; McKenzie, A. R.; Newman, J. V.; Olivier, N. B.; Otterson, L. G.; Owens, A. P.; Read, J.; Sheppard, D. W.; Keating, T. A. Discovery of Selective and Potent Inhibitors of Gram-Positive Bacterial Thymidylate Kinase (TMK). *J. Med. Chem.* **2012**, *55* (22), 10010–10021. https://doi.org/10.1021/jm3011806.

(44) Song, L.; Risseeuw, M. D. P.; Froeyen, M.; Karalic, I.; Goeman, J.; Cappoen, D.; Van der Eycken, J.; Cos, P.; Munier-Lehmann, H.; Van Calenbergh, S. Elaboration of a Proprietary Thymidylate Kinase Inhibitor Motif towards Anti-Tuberculosis Agents. *Bioorg. Med. Chem.* **2016**, *24* (21), 5172–5182. https://doi.org/10.1016/j.bmc.2016.08.041.

(45) Song, L.; Merceron, R.; Gracia, B.; Quintana, A. L.; Risseeuw, M. D. P.; Hulpia, F.; Cos, P.; Aínsa, J. A.; Munier-Lehmann, H.; Savvides, S. N.; Van Calenbergh, S. Structure Guided Lead Generation toward Nonchiral *M. Tuberculosis* Thymidylate Kinase Inhibitors. *J. Med. Chem.* **2018**, *61* (7), 2753–2775. https://doi.org/10.1021/acs.jmedchem.7b01570.

(46) Lv, K.; Li, L.; Wang, B.; Liu, M.; Wang, B.; Shen, W.; Guo, H.; Lu, Y. Design, Synthesis and Antimycobacterial Activity of Novel Imidazo[1,2-a]Pyridine-3-Carboxamide Derivatives. *Eur. J. Med. Chem.* **2017**, *137*, 117–125. https://doi.org/10.1016/j.ejmech.2017.05.044.

(47) Wu, Z.; Lu, Y.; Li, L.; Zhao, R.; Wang, B.; Lv, K.; Liu, M.; You, X. Identification of N-(2-Phenoxyethyl)Imidazo[1,2-a]Pyridine-3-Carboxamides as New Antituberculosis Agents. *ACS Med. Chem. Lett.* **2016**, *7* (12), 1130–1133. https://doi.org/10.1021/acsmedchemlett.6b00330.

(48) Christophe, T.; Jackson, M.; Jeon, H. K.; Fenistein, D.; Contreras-Dominguez, M.; Kim, J.; Genovesio, A.; Carralot, J.-P.; Ewann, F.; Kim, E. H.; Lee, S. Y.; Kang, S.; Seo, M. J.; Park, E. J.; Škovierová, H.; Pham, H.; Riccardi, G.; Nam, J. Y.; Marsollier, L.; Kempf, M.; Joly-Guillou, M.-L.; Oh, T.; Shin, W. K.; No, Z.; Nehrbass, U.; Brosch, R.; Cole, S. T.; Brodin, P. High Content Screening Identifies Decaprenyl-Phosphoribose 2′ Epimerase as a Target for Intracellular Antimycobacterial Inhibitors. *PLOS Pathog.* **2009**, *5* (10), e1000645. https://doi.org/10.1371/journal.ppat.1000645.

(49) Li, L.; Lv, K.; Yang, Y.; Sun, J.; Tao, Z.; Wang, A.; Wang, B.; Wang, H.; Geng, Y.; Liu, M.; Guo, H.; Lu, Y. Identification of N-Benzyl 3,5-Dinitrobenzamides Derived from PBTZ169 as Antitubercular Agents. *ACS Med. Chem. Lett.* **2018**, *9* (7), 741–745. https://doi.org/10.1021/acsmedchemlett.8b00177.

(50) Trefzer, C.; Rengifo-Gonzalez, M.; Hinner, M. J.; Schneider, P.; Makarov, V.; Cole, S. T.; Johnsson, K. Benzothiazinones: Prodrugs That Covalently Modify the Decaprenylphosphoryl-β-D-Ribose 2′-Epimerase DprE1 of Mycobacterium Tuberculosis. *J. Am. Chem. Soc.* **2010**, *132* (39), 13663–13665. https://doi.org/10.1021/ja106357w.

(51) Kappes, B.; Neumann, T.; Unger, E.; Dahse, H.-M.; Moellmann, U.; Schlegel, B. Use of 3,5-Dinitrobenzoate Ester, 3,5-Dinitrobenzamide or 3,5-Dinitrophenyl Ketone Derivatives for Therapy and/or Diagnosis of Microbial Infections, Especially Tuuberculosis or Malaria. DE10158057A1, June 12, 2003. https://patents.google.com/patent/DE10158057A1/en (accessed 2022-08-11).

(52) Jian, Y.; Merceron, R.; De Munck, S.; Forbes, H. E.; Hulpia, F.; Risseeuw, Martijn. D. P.; Van Hecke, K.; Savvides, S. N.; Munier-Lehmann, H.; Boshoff, Helena. I. M.; Van Calenbergh, S. Endeavors towards Transformation of M. Tuberculosis Thymidylate Kinase (MtbTMPK) Inhibitors into Potential Antimycobacterial Agents. *Eur. J. Med. Chem.* **2020**, 112659. https://doi.org/10.1016/j.ejmech.2020.112659.

(53) Song, L.; Merceron, R.; Hulpia, F.; Lucía, A.; Gracia, B.; Jian, Y.; Risseeuw, M. D. P.; Verstraelen, T.; Cos, P.; Aínsa, J. A.; Boshoff, H. I.; Munier-Lehmann, H.; Savvides, S. N.; Van Calenbergh, S. Structure-Aided Optimization of Non-Nucleoside M. Tuberculosis Thymidylate Kinase Inhibitors. *Eur. J. Med. Chem.* **2021**, *225*, 113784. https://doi.org/10.1016/j.ejmech.2021.113784.

(54) Gasse, C.; Douguet, D.; Huteau, V.; Marchal, G.; Munier-Lehmann, H.; Pochet, S. Substituted Benzyl-Pyrimidines Targeting Thymidine Monophosphate Kinase of Mycobacterium Tuberculosis: Synthesis and in Vitro Anti-Mycobacterial Activity. *Bioorg. Med. Chem.* **2008**, *16* (11), 6075–6085. https://doi.org/10.1016/j.bmc.2008.04.045.

(55) Familiar, O.; Munier-Lehmann, H.; Negri, A.; Gago, F.; Douguet, D.; Rigouts, L.; Hernández, A.; Camarasa, M.; Pérez-Pérez, M. Exploring Acyclic Nucleoside Analogues as Inhibitors of *Mycobacterium Tuberculosis* Thymidylate Kinase. *ChemMedChem* **2008**, *3* (7), 1083–1093. https://doi.org/10.1002/cmdc.200800060.

(56) Familiar, O.; Munier-Lehmann, H.; Aínsa, J. A.; Camarasa, M.-J.; Pérez-Pérez, M.-J. Design, Synthesis and Inhibitory Activity against Mycobacterium Tuberculosis Thymidine Monophosphate Kinase of Acyclic Nucleoside Analogues with a Distal Imidazoquinolinone. *Eur. J. Med. Chem.* **2010**, *45* (12), 5910–5918. https://doi.org/10.1016/j.ejmech.2010.09.056.

(57) Kögler, M.; Vanderhoydonck, B.; De Jonghe, S.; Rozenski, J.; Van Belle, K.; Herman, J.; Louat, T.; Parchina, A.; Sibley, C.; Lescrinier, E.; Herdewijn, P. Synthesis and Evaluation of 5-Substituted 2'-Deoxyuridine Monophosphate Analogues As Inhibitors of Flavin-Dependent Thymidylate Synthase in *Mycobacterium Tuberculosis*. *J. Med. Chem.* **2011**, *54* (13), 4847–4862. https://doi.org/10.1021/jm2004688.

(58) Carreras, C. W.; Santi, D. V. The Catalytic Mechanism and Structure of Thymidylate Synthase. *Annu. Rev. Biochem.* **1995**, *64* (1), 721–762. https://doi.org/10.1146/annurev.bi.64.070195.003445.

(59) Basta, T.; Boum, Y.; Briffotaux, J.; Becker, H. F.; Lamarre-Jouenne, I.; Lambry, J.-C.; Skouloubris, S.; Liebl, U.; Graille, M.; van Tilbeurgh, H.; Myllykallio, H. Mechanistic and Structural Basis for Inhibition of Thymidylate Synthase ThyX. *Open Biol.* **2012**, *2* (10), 120120. https://doi.org/10.1098/rsob.120120.

(60) Crofts, A. R.; Berry, E. A. Structure and Function of the Cytochrome Bc1 Complex of Mitochondria and Photosynthetic Bacteria. *Curr. Opin. Struct. Biol.* **1998**, *8* (4), 501–509. https://doi.org/10.1016/S0959-440X(98)80129-2.

(61) Crofts, A. R. The Cytochrome *Bc*$_1$ Complex: Function in the Context of Structure. *Annu. Rev. Physiol.* **2004**, *66* (1), 689–733. https://doi.org/10.1146/annurev.physiol.66.032102.150251.

(62) Huszár, S.; Chibale, K.; Singh, V. The Quest for the Holy Grail: New Antitubercular Chemical Entities, Targets and Strategies. *Drug Discov. Today* **2020**, *25* (4), 772–780. https://doi.org/10.1016/j.drudis.2020.02.003.

(63) Pethe, K.; Bifani, P.; Jang, J.; Kang, S.; Park, S.; Ahn, S.; Jiricek, J.; Jung, J.; Jeon, H. K.; Cechetto, J.; Christophe, T.; Lee, H.; Kempf, M.; Jackson, M.; Lenaerts, A. J.; Pham, H.; Jones, V.; Seo, M. J.; Kim, Y. M.; Seo, M.; Seo, J. J.; Park, D.; Ko, Y.; Choi, I.; Kim, R.; Kim, S. Y.; Lim, S.; Yim, S.-A.; Nam, J.; Kang, H.; Kwon, H.; Oh, C.-T.; Cho, Y.; Jang, Y.; Kim, J.; Chua, A.; Tan, B. H.; Nanjundappa, M. B.; Rao, S. P. S.; Barnes, W. S.; Wintjens, R.; Walker, J. R.; Alonso, S.; Lee, S.; Kim, J.; Oh, S.; Oh, T.; Nehrbass, U.; Han, S.-J.; No, Z.; Lee, J.; Brodin, P.; Cho, S.-N.; Nam, K.; Kim, J. Discovery of Q203, a Potent Clinical Candidate for the Treatment of Tuberculosis. *Nat. Med.* **2013**, *19* (9), 1157–1160. https://doi.org/10.1038/nm.3262.

(64) Harrison, G. A.; Mayer Bridwell, A. E.; Singh, M.; Jayaraman, K.; Weiss, L. A.; Kinsella, R. L.; Aneke, J. S.; Flentie, K.; Schene, M. E.; Gaggioli, M.; Solomon, S. D.; Wildman, S. A.; Meyers, M. J.; Stallings, C. L. Identification of 4-Amino-Thieno[2,3-*d*]Pyrimidines as QcrB Inhibitors in *Mycobacterium Tuberculosis*. *mSphere* **2019**, *4* (5), e00606-19, /msphere/4/5/mSphere606-19.atom. https://doi.org/10.1128/mSphere.00606-19.

(65) Mikušová, K.; Huang, H.; Yagi, T.; Holsters, M.; Vereecke, D.; D'Haeze, W.; Scherman, M. S.; Brennan, P. J.; McNeil, M. R.; Crick, D. C. Decaprenylphosphoryl Arabinofuranose, the Donor of the d-Arabinofuranosyl Residues of Mycobacterial Arabinan, Is Formed via a Two-Step Epimerization of Decaprenylphosphoryl Ribose. *J. Bacteriol.* **2005**, *187* (23), 8020–8025. https://doi.org/10.1128/JB.187.23.8020-8025.2005.

(66) Makarov, V.; Manina, G.; Mikusova, K.; Mollmann, U.; Ryabova, O.; Saint-Joanis, B.; Dhar, N.; Pasca, M. R.; Buroni, S.; Lucarelli, A. P.; Milano, A.; De Rossi, E.; Belanova, M.; Bobovska, A.; Dianiskova, P.; Kordulakova, J.; Sala, C.; Fullam, E.; Schneider, P.; McKinney, J. D.; Brodin, P.; Christophe, T.; Waddell, S.; Butcher, P.; Albrethsen, J.; Rosenkrands, I.; Brosch, R.; Nandi, V.; Bharath, S.; Gaonkar, S.; Shandil, R. K.; Balasubramanian, V.; Balganesh, T.; Tyagi, S.; Grosset, J.; Riccardi, G.; Cole, S. T. Benzothiazinones Kill Mycobacterium Tuberculosis by Blocking Arabinan Synthesis. *Science* **2009**, *324* (5928), 801–804. https://doi.org/10.1126/science.1171583.

(67) Trefzer, C.; Škovierová, H.; Buroni, S.; Bobovská, A.; Nenci, S.; Molteni, E.; Pojer, F.; Pasca, M. R.; Makarov, V.; Cole, S. T.; Riccardi, G.; Mikušová, K.; Johnsson, K. Benzothiazinones Are Suicide Inhibitors of Mycobacterial Decaprenylphosphoryl-β-d-Ribofuranose 2'-Oxidase DprE1. *J. Am. Chem. Soc.* **2012**, *134* (2), 912–915. https://doi.org/10.1021/ja211042r.

(68) Chikhale, R. V.; Barmade, M. A.; Murumkar, P. R.; Yadav, M. R. Overview of the Development of DprE1 Inhibitors for Combating the Menace of Tuberculosis. *J. Med. Chem.* **2018**, *61* (19), 8563–8593. https://doi.org/10.1021/acs.jmedchem.8b00281.

(69) Chikhale, R.; Menghani, S.; Babu, R.; Bansode, R.; Bhargavi, G.; Karodia, N.; Rajasekharan, M. V.; Paradkar, A.; Khedekar, P. Development of Selective DprE1 Inhibitors: Design, Synthesis, Crystal Structure and Antitubercular Activity of Benzothiazolylpyrimidine-5-Carboxamides. *Eur. J. Med. Chem.* **2015**, *96*, 30–46. https://doi.org/10.1016/j.ejmech.2015.04.011.

(70) Crellin, P. K.; Brammananth, R.; Coppel, R. L. Decaprenylphosphoryl-β-D-Ribose 2'-Epimerase, the Target of Benzothiazinones and Dinitrobenzamides, Is an Essential Enzyme in Mycobacterium Smegmatis. *PLoS ONE* **2011**, *6* (2), e16869. https://doi.org/10.1371/journal.pone.0016869.

(71) Manina, G.; R. Pasca, M.; Buroni, S.; De Rossi, E.; Riccardi, G. Decaprenylphosphoryl-β-D-Ribose 2-Epimerase from Mycobacterium Tuberculosis Is a Magic Drug Target. *Curr. Med. Chem.* **2010**, *17* (27), 3099–3108. https://doi.org/10.2174/092986710791959693.

(72) Makarov, V.; Lechartier, B.; Zhang, M.; Neres, J.; Sar, A. M.; Raadsen, S. A.; Hartkoorn, R. C.; Ryabova, O. B.; Vocat, A.; Decosterd, L. A.; Widmer, N.; Buclin, T.; Bitter, W.; Andries, K.; Pojer, F.; Dyson, P. J.; Cole, S. T. Towards a New Combination Therapy for Tuberculosis with next Generation Benzothiazinones. *EMBO Mol. Med.* **2014**, *6* (3), 372–383. https://doi.org/10.1002/emmm.201303575.

(73) Shirude, P. S.; Shandil, R.; Sadler, C.; Naik, M.; Hosagrahara, V.; Hameed, S.; Shinde, V.; Bathula, C.; Humnabadkar, V.; Kumar, N.; Reddy, J.; Panduga, V.; Sharma, S.; Ambady, A.; Hegde, N.; Whiteaker, J.; McLaughlin, R. E.; Gardner, H.; Madhavapeddi, P.; Ramachandran, V.; Kaur, P.; Narayan, A.; Guptha, S.; Awasthy, D.; Narayan, C.; Mahadevaswamy, J.; Vishwas, K.; Ahuja, V.; Srivastava, A.; Prabhakar, K.; Bharath, S.; Kale, R.; Ramaiah, M.; Choudhury, N. R.; Sambandamurthy, V. K.; Solapure, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Azaindoles: Noncovalent DprE1 Inhibitors from Scaffold Morphing Efforts, Kill Mycobacterium Tuberculosis and Are Efficacious *in Vivo*. *J. Med. Chem.* **2013**, *56* (23), 9701–9708. https://doi.org/10.1021/jm401382v.

(74) Hariguchi, N.; Chen, X.; Hayashi, Y.; Kawano, Y.; Fujiwara, M.; Matsuba, M.; Shimizu, H.; Ohba, Y.; Nakamura, I.; Kitamoto, R.; Shinohara, T.; Uematsu, Y.; Ishikawa, S.; Itotani, M.; Haraguchi, Y.; Takemura, I.; Matsumoto, M. OPC-167832, a Novel Carbostyril Derivative with Potent Antituberculosis Activity as a DprE1 Inhibitor. *Antimicrob. Agents Chemother.* **2020**, *64* (6), e02020-19, /aac/64/6/AAC.02020-19.atom. https://doi.org/10.1128/AAC.02020-19.

(75) Ballell, L.; Bates, R. H.; Young, R. J.; Alvarez-Gomez, D.; Alvarez-Ruiz, E.; Barroso, V.; Blanco, D.; Crespo, B.; Escribano, J.; González, R.; Lozano, S.; Huss, S.; Santos-Villarejo, A.; Martín-Plaza, J. J.; Mendoza, A.; Rebollo-Lopez, M. J.; Remuiñan-Blanco, M.; Lavandera, J. L.; Pérez-Herran, E.; Gamo-Benito, F. J.; García-Bustos, J. F.; Barros, D.; Castro, J. P.; Cammack, N. Fueling Open-Source Drug Discovery: 177 Small-Molecule Leads against Tuberculosis. *ChemMedChem* **2013**, *8* (2), 313–321. https://doi.org/10.1002/cmdc.201200428.

(76) Batt, S. M.; Cacho Izquierdo, M.; Castro Pichel, J.; Stubbs, C. J.; Vela-Glez Del Peral, L.; Pérez-Herrán, E.; Dhar, N.; Mouzon, B.; Rees, M.; Hutchinson, J. P.; Young, R. J.; McKinney, J. D.; Barros Aguirre, D.; Ballell, L.; Besra, G. S.; Argyrou, A. Whole Cell Target Engagement Identifies Novel Inhibitors of *Mycobacterium Tuberculosis* Decaprenylphosphoryl-β- D -Ribose Oxidase. *ACS Infect. Dis.* **2015**, *1* (12), 615–626. https://doi.org/10.1021/acsinfecdis.5b00065.

(77) Borthwick, J. A.; Alemparte, C.; Wall, I.; Whitehurst, B. C.; Argyrou, A.; Burley, G.; de Dios-Anton, P.; Guijarro, L.; Monteiro, M. C.; Ortega, F.; Suckling, C. J.; Pichel, J. C.; Cacho, M.; Young, R. J. Mycobacterium Tuberculosis Decaprenylphosphoryl-β-d-Ribose Oxidase Inhibitors: Expeditious Reconstruction of Suboptimal Hits into a Series with Potent in Vivo Activity. *J. Med. Chem.* **2020**, *63* (5), 2557–2576. https://doi.org/10.1021/acs.jmedchem.9b01561.

(78) Rullas, J.; García, J. I.; Beltrán, M.; Cardona, P.-J.; Cáceres, N.; García-Bustos, J. F.; Angulo-Barturen, I. Fast Standardized Therapeutic-Efficacy Assay for Drug Discovery against Tuberculosis. *Antimicrob. Agents Chemother.* **2010**, *54* (5), 2262–2264. https://doi.org/10.1128/AAC.01423-09.

(79) Boyd, V. A.; Mason, J.; Hanumesh, P.; Price, J.; Russell, C. J.; Webb, T. R. 2-Substituted-4,5-Dihydroxypyrimidine-6-Carboxamide Antiviral Targeted Libraries. *J. Comb. Chem.* **2009**, *11* (6), 1100–1104. https://doi.org/10.1021/cc900111u.

(80) Evering, T. H.; Markowitz, M. Raltegravir: An Integrase Inhibitor for HIV-1. *Expert Opin. Investig. Drugs* **2008**, *17* (3), 413–422. https://doi.org/10.1517/13543784.17.3.413.

(81) Mouscadet, J.-F.; Tchertanov, L. Raltegravir: Molecular Basis of Its Mechanism of Action. *Eur. J. Med. Res.* **2009**, *14* (Suppl 3), 5. https://doi.org/10.1186/2047-783X-14-S3-5.

(82) Oh, S.; Park, Y.; Engelhart, C. A.; Wallach, J. B.; Schnappinger, D.; Arora, K.; Manikkam, M.; Gac, B.; Wang, H.; Murgolo, N.; Olsen, D. B.; Goodwin, M.; Sutphin, M.; Weiner, D. M.; Via, L. E.; Boshoff, H. I. M.; Barry, C. E. Discovery and Structure–Activity-Relationship Study of *N* -Alkyl-5-Hydroxypyrimidinone Carboxamides as Novel Antitubercular Agents Targeting Decaprenylphosphoryl-β- D -Ribose 2'-Oxidase. *J. Med. Chem.* **2018**, *61* (22), 9952–9965. https://doi.org/10.1021/acs.jmedchem.8b00883.

(83) Malnuit, V.; Slavětínská, L. P.; Nauš, P.; Džubák, P.; Hajdúch, M.; Stolaříková, J.; Snášel, J.; Pichová, I.; Hocek, M. 2-Substituted 6-(Het)Aryl-7-Deazapurine Ribonucleosides: Synthesis, Inhibition of Adenosine Kinases, and Antimycobacterial Activity. *ChemMedChem* **2015**, *10* (6), 1079–1093. https://doi.org/10.1002/cmdc.201500081.

(84) Kobayashi, K.; Ehrlich, S. D.; Albertini, A.; Amati, G.; Andersen, K. K.; Arnaud, M.; Asai, K.; Ashikaga, S.; Aymerich, S.; Bessieres, P.; Boland, F.; Brignell, S. C.; Bron, S.; Bunai, K.; Chapuis, J.; Christiansen, L. C.; Danchin, A.; Debarbouille, M.; Dervyn, E.; Deuerling, E.; Devine, K.; Devine, S. K.; Dreesen, O.; Errington, J.; Fillinger, S.; Foster, S. J.; Fujita, Y.; Galizzi, A.; Gardan, R.; Eschevins, C.; Fukushima, T.; Haga, K.; Harwood, C. R.; Hecker, M.; Hosoya, D.; Hullo, M. F.; Kakeshita, H.; Karamata, D.; Kasahara, Y.; Kawamura, F.; Koga, K.; Koski, P.; Kuwana, R.; Imamura, D.; Ishimaru, M.; Ishikawa, S.; Ishio, I.; Le Coq, D.; Masson, A.; Mauel, C.; Meima, R.; Mellado, R. P.; Moir, A.; Moriya, S.; Nagakawa, E.; Nanamiya, H.; Nakai, S.; Nygaard, P.; Ogura, M.; Ohanan, T.; O'Reilly, M.; O'Rourke, M.; Pragai, Z.; Pooley, H. M.; Rapoport, G.; Rawlins, J. P.; Rivas, L. A.; Rivolta, C.; Sadaie, A.; Sadaie, Y.; Sarvas, M.; Sato, T.; Saxild, H. H.; Scanlan, E.; Schumann, W.; Seegers, J. F. M. L.; Sekiguchi, J.; Sekowska, A.; Seror, S. J.; Simon, M.; Stragier, P.; Studer, R.; Takamatsu, H.; Tanaka, T.; Takeuchi, M.; Thomaides, H. B.; Vagner, V.; van Dijl, J. M.; Watabe, K.; Wipat, A.; Yamamoto, H.; Yamamoto, M.; Yamamoto, Y.; Yamane, K.; Yata, K.; Yoshida, K.; Yoshikawa, H.; Zuber, U.; Ogasawara, N. Essential Bacillus Subtilis Genes. *Proc. Natl. Acad. Sci.* **2003**, *100* (8), 4678–4683. https://doi.org/10.1073/pnas.0730515100.

(85) Masuda, I.; Sakaguchi, R.; Liu, C.; Gamper, H.; Hou, Y.-M. The Temperature Sensitivity of a Mutation in the Essential TRNA Modification Enzyme TRNA Methyltransferase D (TrmD). *J. Biol. Chem.* **2013**, *288* (40), 28987–28996. https://doi.org/10.1074/jbc.M113.485797.

(86) O'Dwyer, K.; Watts, J. M.; Biswas, S.; Ambrad, J.; Barber, M.; Brulé, H.; Petit, C.; Holmes, D. J.; Zalacain, M.; Holmes, W. M. Characterization of Streptococcus Pneumoniae TrmD, a TRNA Methyltransferase Essential for Growth. *J. Bacteriol.* **2004**, *186* (8), 2346–2354. https://doi.org/10.1128/JB.186.8.2346-2354.2004.

(87) Hill, P. J.; Abibi, A.; Albert, R.; Andrews, B.; Gagnon, M. M.; Gao, N.; Grebe, T.; Hajec, L. I.; Huang, J.; Livchak, S.; Lahiri, S. D.; McKinney, D. C.; Thresher, J.; Wang, H.; Olivier, N.; Buurman, E. T. Selective Inhibitors of Bacterial T-RNA-(N [1] G37) Methyltransferase (TrmD) That Demonstrate Novel Ordering of the Lid Domain. *J. Med. Chem.* **2013**, *56* (18), 7278–7288. https://doi.org/10.1021/jm400718n.

(88) Zhong, W.; Pasunooti, K. K.; Balamkundu, S.; Wong, Y. H.; Nah, Q.; Gadi, V.; Gnanakalai, S.; Chionh, Y. H.; McBee, M. E.; Gopal, P.; Lim, S. H.; Olivier, N.; Buurman, E. T.; Dick, T.; Liu, C. F.; Lescar, J.; Dedon, P. C. Thienopyrimidinone Derivatives That Inhibit Bacterial TRNA (Guanine37-*N* [1])-Methyltransferase (TrmD) by Restructuring the Active Site with a Tyrosine-Flipping Mechanism. *J. Med. Chem.* **2019**, *62* (17), 7788–7805. https://doi.org/10.1021/acs.jmedchem.9b00582.

(89) Bakkestuen, A. K.; Gundersen, L.-L.; Utenova, B. T. Synthesis, Biological Activity, and SAR of Antimycobacterial 9-Aryl-, 9-Arylsulfonyl-, and 9-Benzyl-6-(2-Furyl)Purines. *J. Med. Chem.* **2005**, *48* (7), 2710–2723. https://doi.org/10.1021/jm0408924.

(90) Bakkestuen, A. K.; Gundersen, L.-L.; Langli, G.; Liu, F.; Nolsøe, J. M. J. 9-Benzylpurines with Inhibitory Activity against Mycobacterium Tuberculosis. *Bioorg. Med. Chem. Lett.* **2000**, *10* (11), 1207–1210. https://doi.org/10.1016/S0960-894X(00)00188-8.

(91) Andresen, G.; Gundersen, L.-L.; Nissen-Meyer, J.; Rise, F.; Spilsberg, B. Cytotoxic and Antibacterial Activity of 2-Oxopurine Derivatives. *Bioorg. Med. Chem. Lett.* **2002**, *12* (4), 567–569. https://doi.org/10.1016/S0960-894X(01)00803-4.

(92) Gundersen, L.-L.; Nissen-Meyer, J.; Spilsberg, B. Synthesis and Antimycobacterial Activity of 6-Arylpurines: The Requirements for the N-9 Substituent in Active Antimycobacterial Purines. *J. Med. Chem.* **2002**, *45* (6), 1383–1386. https://doi.org/10.1021/jm0110284.

(93) Brændvang, M.; Gundersen, L.-L. Selective Anti-Tubercular Purines: Synthesis and Chemotherapeutic Properties of 6-Aryl- and 6-Heteroaryl-9-Benzylpurines. *Bioorg. Med. Chem.* **2005**, *13* (23), 6360–6373. https://doi.org/10.1016/j.bmc.2005.06.054.

(94) Brændvang, M.; Charnock, C.; Gundersen, L.-L. Synthesis and Antimycobacterial Activity of 5-Formylaminopyrimidines; Analogs of Antibacterial Purines. *Bioorg. Med. Chem. Lett.* **2009**, *19* (12), 3297–3299. https://doi.org/10.1016/j.bmcl.2009.04.082.

(95) Read, M. L.; Brændvang, M.; Miranda, P. O.; Gundersen, L.-L. Synthesis and Biological Evaluation of Pyrimidine Analogs of Antimycobacterial Purines. *Bioorg. Med. Chem.* **2010**, *18* (11), 3885–3897. https://doi.org/10.1016/j.bmc.2010.04.035.

(96) Sharma, M.; Chaturvedi, V.; Manju, Y. K.; Bhatnagar, S.; Srivastava, K.; Puri, S. K.; Chauhan, P. M. S. Substituted Quinolinyl Chalcones and Quinolinyl Pyrimidines as a New Class of Anti-Infective Agents. *Eur. J. Med. Chem.* **2009**, *44* (5), 2081–2091. https://doi.org/10.1016/j.ejmech.2008.10.011.

(97) Bhoi, M. N.; Borad, M. A.; Pithawala, E. A.; Patel, H. D. Novel Benzothiazole Containing 4H-Pyrimido[2,1-b]Benzothiazoles Derivatives: One Pot, Solvent-Free Microwave Assisted Synthesis and Their Biological Evaluation. *Arab. J. Chem.* **2019**, *12* (8), 3799–3813. https://doi.org/10.1016/j.arabjc.2016.01.012.

(98) Bhoi, M. N.; Borad, M. A.; Jethava, D. J.; Acharya, P. T.; Pithawala, E. A.; Patel, C. N.; Pandya, H. A.; Patel, H. D. Synthesis, Biological Evaluation and Computational Study of Novel Isoniazid Containing 4H-Pyrimido[2,1-b]Benzothiazoles Derivatives. *Eur. J. Med. Chem.* **2019**, *177*, 12–31. https://doi.org/10.1016/j.ejmech.2019.05.028.

(99) Chhabria, M. T.; Jani, M. H. Design, Synthesis and Antimycobacterial Activity of Some Novel Imidazo[1,2-c]Pyrimidines. *Eur. J. Med. Chem.* **2009**, *44* (10), 3837–3844. https://doi.org/10.1016/j.ejmech.2009.04.002.

(100) Dijkstra, J. A.; van der Laan, T.; Akkerman, O. W.; Bolhuis, M. S.; de Lange, W. C. M.; Kosterink, J. G. W.; van der Werf, T. S.; Alffenaar, J. W. C.; van Soolingen, D. In Vitro Susceptibility of Mycobacterium Tuberculosis to Amikacin, Kanamycin, and Capreomycin. *Antimicrob. Agents Chemother.* **2018**, *62* (3), e01724-17. https://doi.org/10.1128/AAC.01724-17.

(101) Shiva Raju, K.; AnkiReddy, S.; Sabitha, G.; Siva Krishna, V.; Sriram, D.; Bharathi Reddy, K.; Rao Sagurthi, S. Synthesis and Biological Evaluation of 1H-Pyrrolo[2,3-d]Pyrimidine-1,2,3-Triazole Derivatives as Novel Anti-Tubercular Agents. *Bioorg. Med. Chem. Lett.* **2019**, *29* (2), 284–290. https://doi.org/10.1016/j.bmcl.2018.11.036.

(102) Kamdar, N. R.; Haveliwala, D. D.; Mistry, P. T.; Patel, S. K. Design, Synthesis and in Vitro Evaluation of Antitubercular and Antimicrobial Activity of Some Novel Pyranopyrimidines. *Eur. J. Med. Chem.* **2010**, *45* (11), 5056–5063. https://doi.org/10.1016/j.ejmech.2010.08.014.

(103) Agarwal, N.; Srivastava, P.; Raghuwanshi, S. K.; Upadhyay, D. N.; Sinha, S.; Shukla, P. K.; Ji Ram, V. Chloropyrimidines as a New Class of Antimicrobial Agents. *Bioorg. Med. Chem.* **2002**, *10* (4), 869–874. https://doi.org/10.1016/S0968-0896(01)00374-1.

(104) Modi, P.; Patel, S.; Chhabria, M. Structure-Based Design, Synthesis and Biological Evaluation of a Newer Series of Pyrazolo[1,5-a]Pyrimidine Analogues as Potential Anti-Tubercular Agents. *Bioorganic Chem.* **2019**, *87*, 240–251. https://doi.org/10.1016/j.bioorg.2019.02.044.

(105) Xu, Z.; Gao, C.; Ren, Q.-C.; Song, X.-F.; Feng, L.-S.; Lv, Z.-S. Recent Advances of Pyrazole-Containing Derivatives as Anti-Tubercular Agents. *Eur. J. Med. Chem.* **2017**, *139*, 429–440. https://doi.org/10.1016/j.ejmech.2017.07.059.

(106) Dragset, M. S.; Poce, G.; Alfonso, S.; Padilla-Benavides, T.; Ioerger, T. R.; Kaneko, T.; Sacchettini, J. C.; Biava, M.; Parish, T.; Argüello, J. M.; Steigedal, M.; Rubin, E. J. A Novel Antimycobacterial Compound Acts as an Intracellular Iron Chelator. *Antimicrob. Agents Chemother.* **2015**, *59* (4), 2256–2264. https://doi.org/10.1128/AAC.05114-14.

(107) Rajnák, C.; Schäfer, B.; Šalitroš, I.; Fuhr, O.; Ruben, M.; Boča, R. Influence of the Charge of the Complex Unit on the SCO Properties in Pyrazolyl-Pyridinyl-Benzimidazole Based Fe(II) Complexes. *Polyhedron* **2017**, *135*, 189–194. https://doi.org/10.1016/j.poly.2017.06.035.

(108) Soares de Melo, C.; Singh, V.; Myrick, A.; Simelane, S. B.; Taylor, D.; Brunschwig, C.; Lawrence, N.; Schnappinger, D.; Engelhart, C. A.; Kumar, A.; Parish, T.; Su, Q.; Myers, T. G.; Boshoff, H. I. M.; Barry, C. E.; Sirgel, F. A.; van Helden, P. D.; Buchanan, K. I.; Bayliss, T.; Green, S. R.; Ray, P. C.; Wyatt, P. G.; Basarab, G. S.; Eyermann, C. J.; Chibale, K.; Ghorpade, S. R. Antitubercular 2-Pyrazolylpyrimidinones: Structure–Activity Relationship and Mode-of-Action Studies. *J. Med. Chem.* **2021**, *64* (1), 719–740. https://doi.org/10.1021/acs.jmedchem.0c01727.

(109) Liu, P.; Yang, Y.; Tang, Y.; Yang, T.; Sang, Z.; Liu, Z.; Zhang, T.; Luo, Y. Design and Synthesis of Novel Pyrimidine Derivatives as Potent Antitubercular Agents. *Eur. J. Med. Chem.* **2019**, *163*, 169–182. https://doi.org/10.1016/j.ejmech.2018.11.054.

(110) Oh, S.; Libardo, M. D. J.; Azeeza, S.; Pauly, G. T.; Roma, J. S. O.; Sajid, A.; Tateishi, Y.; Duncombe, C.; Goodwin, M.; Ioerger, T. R.; Wyatt, P. G.; Ray, P. C.; Gray, D. W.; Boshoff, H. I. M.; Barry, C. E. Structure–Activity Relationships of Pyrazolo[1,5-a]Pyrimidin-7(4H)-Ones as Antitubercular Agents. *ACS Infect. Dis.* **2021**, *7* (2), 479–492. https://doi.org/10.1021/acsinfecdis.0c00851.

(111) Ioerger, T. R.; O'Malley, T.; Liao, R.; Guinn, K. M.; Hickey, M. J.; Mohaideen, N.; Murphy, K. C.; Boshoff, H. I. M.; Mizrahi, V.; Rubin, E. J.; Sassetti, C. M.; Iii, C. E. B.; Sherman, D. R.; Parish, T.; Sacchettini, J. C. Identification of New Drug Targets and Resistance Mechanisms in Mycobacterium Tuberculosis. *PLOS ONE* **2013**, *8* (9), e75245. https://doi.org/10.1371/journal.pone.0075245.

(112) Mao, J.; Eoh, H.; He, R.; Wang, Y.; Wan, B.; Franzblau, S. G.; Crick, D. C.; Kozikowski, A. P. Structure-Activity Relationships of Compounds Targeting Mycobacterium Tuberculosis 1-Deoxy-D-Xylulose 5-Phosphate Synthase. *Bioorg. Med. Chem. Lett.* **2008**, *18* (19), 5320–5323. https://doi.org/10.1016/j.bmcl.2008.08.034.

(113) Masini, T.; Lacy, B.; Monjas, L.; Hawksley, D.; Voogd, A. R. de; Illarionov, B.; Iqbal, A.; Leeper, F. J.; Fischer, M.; Kontoyianni, M.; Hirsch, A. K. H. Validation of a Homology Model of Mycobacterium Tuberculosis DXS: Rationalization of Observed Activities of Thiamine Derivatives as Potent Inhibitors of Two Orthologues of DXS. *Org. Biomol. Chem.* **2015**, *13* (46), 11263–11277. https://doi.org/10.1039/C5OB01666E.

(114) Robertson, G. T.; Ramey, M. E.; Massoudi, L. M.; Carter, C. L.; Zimmerman, M.; Kaya, F.; Graham, B. G.; Gruppo, V.; Hastings, C.; Woolhiser, L. K.; Scott, D. W. L.; Asay, B. C.; Eshun-Wilson, F.; Maidj, E.; Podell, B. K.; Vásquez, J. J.; Lyons, M. A.; Dartois, V.; Lenaerts, A. J. Comparative Analysis of Pharmacodynamics in the C3HeB/FeJ Mouse Tuberculosis Model for DprE1 Inhibitors TBA-7371, PBTZ169, and OPC-167832. *Antimicrob. Agents Chemother.* *65* (11), e00583-21. https://doi.org/10.1128/AAC.00583-21.