Endeavors towards transformation of *M. tuberculosis* thymidylate kinase (*Mtb*TMPK) inhibitors into potential antimycobacterial agents

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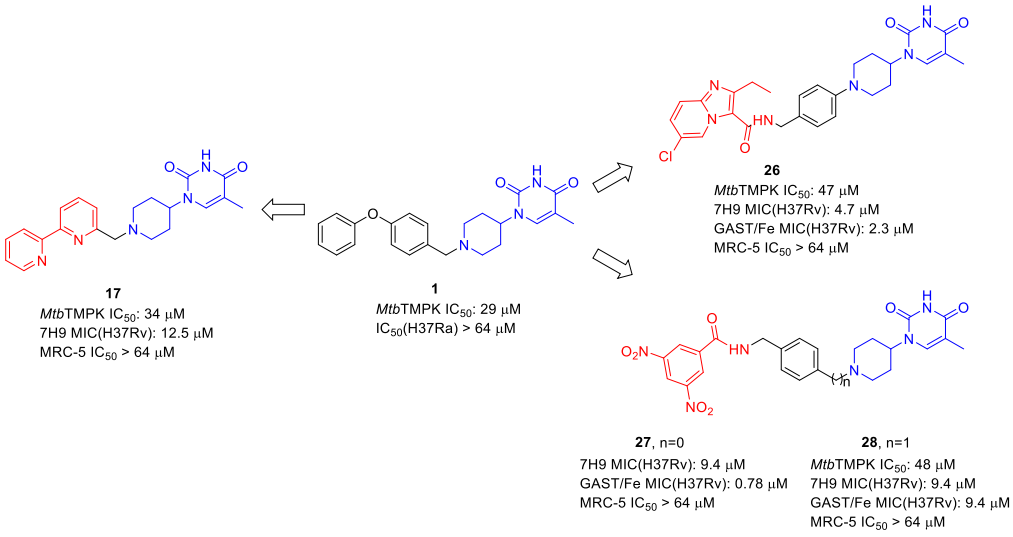
Abstract

As the last enzyme in nucleotide synthesis as precursors for DNA replication, thymidylate kinase of *M. tuberculosis* (*Mtb*TMPK) attracts significant interest as a target in the discovery of new anti-tuberculosis agents. Earlier, we discovered potent *Mtb*TMPK inhibitors, but these generally suffered from poor antimycobacterial activity, which we hypothesize is due to poor bacterial uptake. To address this, we herein describe our efforts to equip previously reported *Mtb*TMPK inhibitors with targeting moieties to increase the whole cell activity of the hybrid analogues. Introduction of a simplified Fe-chelating siderophore motif gave rise to analogue **17** that combined favorable enzyme inhibitory activity with significant activity against *M. tuberculosis* (MIC of 12.5 μM). Conjugation of *Mtb*TMPK inhibitors with an imidazo[1,2-*a*]pyridine or 3,5-dinitrobenzamide scaffold afforded analogues **26**, **27** and **28**, with moderate *Mtb*TMPK enzyme inhibitory potency, but sub-micromolar activity against mycobacteria without significant cytotoxicity. These results indicate that conjugation with structural motifs known to favor mycobacterial uptake may be a valid approach for discovering new antimycobacterial agents.

Keywords: *Mycobacteria tuberculosis*, thymidine monophosphate kinase (TMPK), antimycobacterial, siderophore, uptake

Graphical abstract



Introduction

Tuberculosis, caused by the infectious agent *Mycobacteria tuberculosis* (*M. tuberculosis*), leads to a high death rate with more than 1.2 million patients succumbing to the infection every year [1]. Over the past decade, the overall incidence of tuberculosis has declined, although regions in sub-Saharan Africa and Asia show alarmingly high incidence rates. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains threatens any achieved progress in combatting this disease [2]. The increasing number of drug-resistant tuberculosis places an unsustainable burden on health-care systems, in addition to affecting economical as well as societal growth.

The approval of bedaquiline and delamanid by the EMA and FDA has provided new hope for afflicted patients, but the first *M. tuberculosis* isolate resistant to bedaquiline was reported shortly after clinical introduction of this drug [3]. Very recently pretomanid has been approved as a combination partner for bedaquiline and linezolid in treating highly resistant forms of TB [4]. Nonetheless, this regimen is contra-indicated in patients with hypersensitivity to bedaquiline or linezolid, effectively narrowing its use. Taken together, research efforts toward the discovery and development of new antitubercular agents, particularly those with a novel mechanism of action, should be actively pursued to keep pace with the high incidence of tuberculosis [5].

M. tuberculosis thymidine monophosphate kinase (*Mtb*TMPK), the last specific enzyme in the biosynthesis of thymidine triphosphate, is indispensable for mycobacterial growth and survival [6]. A crystal structure [7] of the protein has been reported, showcasing a unique configuration of its active site [6], making *Mtb*TMPK a promising target to pursue for the discovery of new bio-active molecules to treat tuberculosis.

Previous studies have described nucleoside [8-12] and non-nucleoside [13] *Mtb*TMPK inhibitors. Even though these campaigns resulted in potent *Mtb*TMPK inhibitors [8-12,14-17], these mostly suffered from low to moderate whole-cell activity. For a *Mtb*TMPK inhibitor to show an antimycobacterial effect, it must cross both the inner and outer membranes to reach cytosolic concentrations to allow sufficient target enzyme inhibition to effect mycobacterial growth inhibition. While the required physicochemical properties for intracellular accumulation has been studied in some detail for Gram-negative bacteria [18-20], this remains speculative

for mycobacteria.

Mycobacteria rely on the secretion of low molecular weight siderophores to chelate and subsequently capture iron (Fe^{3+}), which constitutes an essential nutrient for bacterial growth [21]. The receptor-mediated active import of siderophores turns these into possible antibiotic delivery vectors [22-24]. Typically, a siderophore-mediated drug delivery system is comprised of an Fe-targeting siderophore moiety, an antibiotic and a connecting linker (Figure 1). Based on the stability of the linker, these conjugates can be further divided into non-releasable (stable) and releasable (liable) ones (Figure 1) [25]. In response to low-iron conditions present in the host, *M. tuberculosis* secrets two salicylate-derived siderophores, namely carboxymycobactin and mycobactin [26]. To date, only the latter has been successfully used as drug delivery vector [27]. In order to reduce the inherent complexity, simplified single bidentate ligands such as catechol or hydroxypyridone might be considered as surrogates, since their utility has already been demonstrated in Gram-negative bacteria [28-31]. In this study we explore the use of simplified siderophores to facilitate TMPK inhibitor uptake in *M. tuberculosis*.

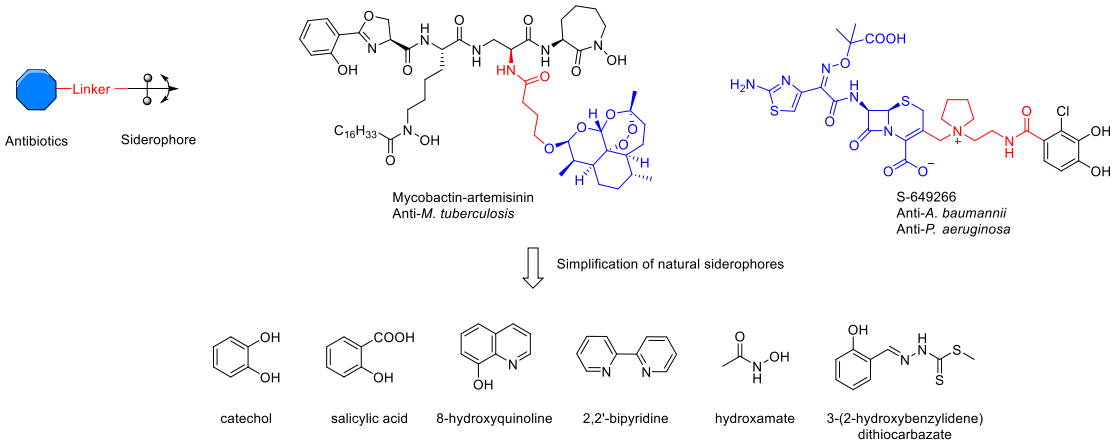


Figure 1. Generalized structure of a siderophore mediated drug delivery system and two successful examples of siderophore conjugation: the mycobactin-artemisinin conjugate and the single bidentate catechol-cephalosporin conjugate S-649266 [27,28].

In the past decade new chemical entities emerged with promising whole-cell activity against *M. tuberculosis*. Imidazo[1,2-*a*]pyridine analogue Q203 [32] was reported as a promising antimycobacterial agent targeting the respiratory cytochrome bc1 complex (Figure 2). Nitroaromatic compounds such as 3,5-dinitrobenzamides [33] were also found to exhibit excellent antimycobacterial activity. The fact that both compounds share a *N*-substituted 4-

arylpiperidine scaffold with our previously reported *Mtb*TMPK inhibitor **1** [12] (Figure 3), inspired us to synthesize hybrid compounds of the latter that comprise the imidazo[1,2-*a*]pyridine and 3,5-dinitrobenzamide moieties anticipated to be favourable for mycobacterial uptake.

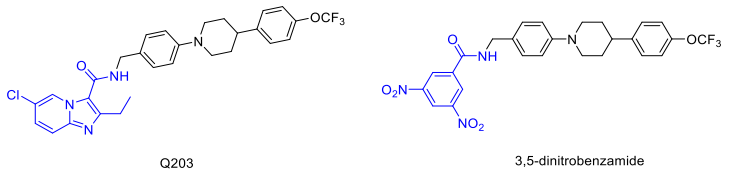


Figure 2. Structures of anti-TB scaffold of Q203 and 3,5-dinitrobenzamide.

In this study we thus sought to combine the scaffold of previously reported *Mtb*TMPK inhibitors with either simplified Fe-chelating motifs [26,34-36] or motifs from compounds with known whole-cell antimycobacterial activity, e.g. imidazo[1,2-*a*]pyridine and 3,5-dinitrobenzamide, and evaluate the effect of the resulting hybrid compounds on *Mtb*TMPK inhibitory activity and *in vitro* anti-TB activity. An overview of the target analogues is presented in Figure 3.

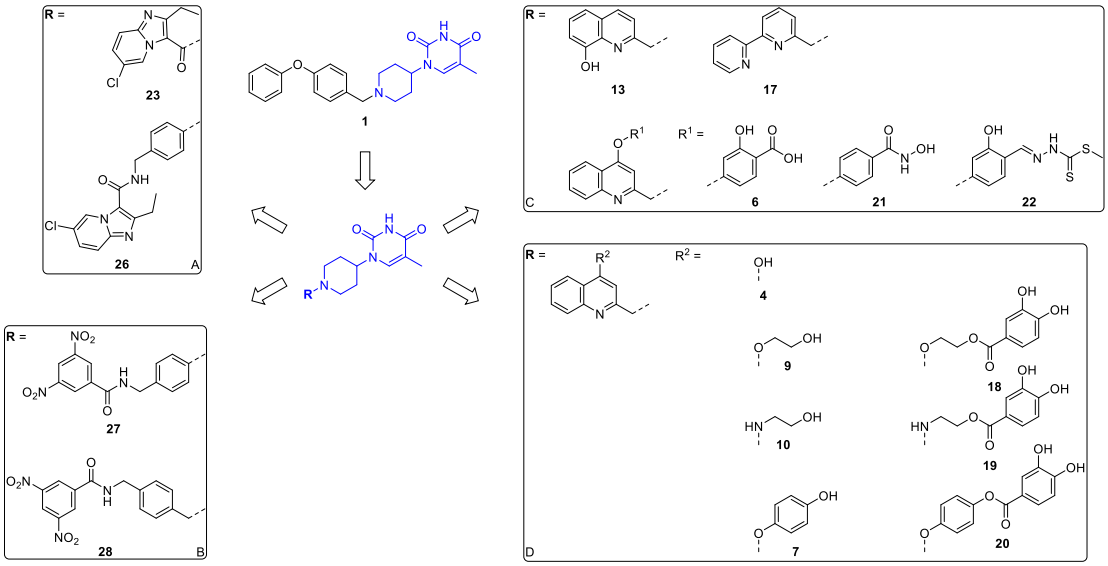


Figure 3. Compounds synthesized and evaluated in this study. A: Q203-based hybrids; B. 3,5-dinitrobenzamide hybrids; C: non-cleavable siderophore hybrids; D. cleavable siderophore hybrids and their precursors.

Results and discussion

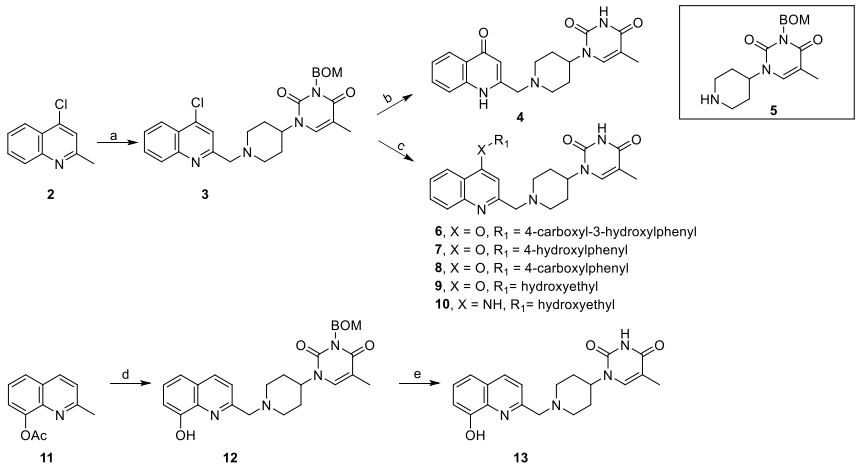
Chemistry

Generally, the envisioned analogues were obtained through either reductive amination of N^3-BOM-protected 1-(piperidin-4-yl)thymine with an appropriate aldehyde [12], or via alkylation of

the same intermediate with a suitable benzyl bromide derivative [37], followed by TFA-mediated BOM deprotection (Scheme 1) [11,12,38]. Preparation of the 4-quinolone derivative **4** was achieved by reductive amination of in situ generated aldehyde from 4-chloro-2-methylquinoline, and treatment of the resulting 4-chloroquinoline intermediate **3** with acetic acid at 125 °C [39], after multiple attempts to achieve substitution under basic conditions were met with failure (data not shown). 4-Substituted quinoline derivatives **6**-**10** were prepared by nucleophilic aromatic substitution [40] of **3** with different nucleophiles at 180 °C followed by BOM-deprotection.

The 8-hydroxyquinoline derivative **13**, on the other hand, was prepared via alkylation of **5** with an in situ generated benzyl bromide reagent derived from 2-methylquinolin-8-ylacetate, followed by global deprotection under acidic conditions.

Scheme 1.



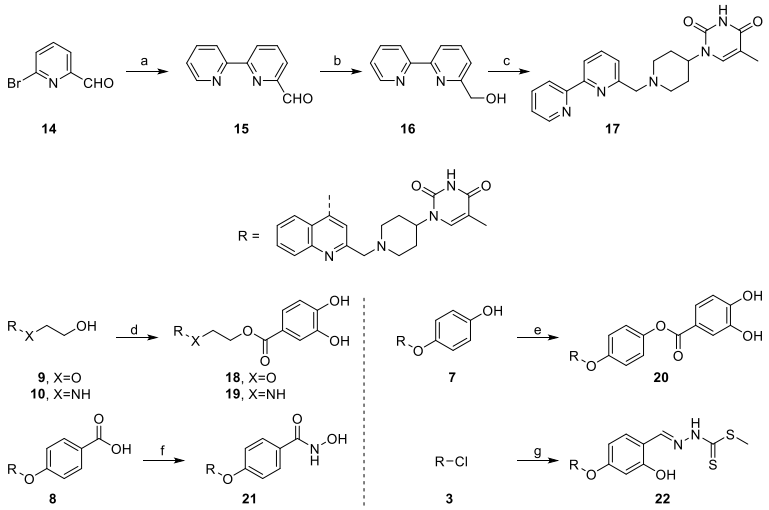
Reagents and conditions: (a) (i) SeO_2, 1,4-dioxane, reflux; (ii) **5**, sodium triacetoxyborohydride, 1,2-dichloroethane, 32%; (b) (i) acetic acid, 125 ℃; (ii) neat TFA, 73 ℃, 58%; (c) (i) RXH, EA, 180 ℃; (ii) neat TFA, 73 ℃, 10-50%; (d) (i) NBS, AIBN, CCl_4, 80 ℃; (ii) **5**, K_2CO_3, ethanol, reflux, 20%; (e) neat TFA, 73 ℃, 58%.

The synthesis of bipyridyl analogue **17** commenced with a Stille coupling between 6-bromopicolinaldehyde and 2-(tributylstannyl)pyridine to deliver aldehyde **15** [41], which was subsequently reduced (Scheme 2). The resulting alcohol was transformed into its mesylate and immediately substituted with **5** and BOM-deprotected to afford the desired **17**.

Several intermediates described above (**3**, **7**-**10**) were employed for the quinolone-based iron chelating ester/amide analogues **18**-**21** or hydrazine derivative **22**. Compounds **18** and **19** were obtained by esterification of **9** and **10** with 3,4-bis(benzyloxy)benzoylchloride, followed

hydrogenolysis. A similar reaction sequence failed to produce **20**, since the hydrogenolysis conditions (H_2/Pd) led to benzoyl ester hydrolysis. To circumvent this issue, **7** was coupled with 3,4-bis((tert-butyldimethylsilyl)oxy)benzoic acid, from which the tert-butyldimethylsilyl group could be easily removed with HF·pyridine. Hydroxamate **21** was obtained by coupling **8** with $BnONH_2$·HCl and subsequent hydrogenolysis. Nucleophilic aromatic substitution of **3** with 2,4-dihydroxybenzaldehyde at 180 °C, followed by reaction with methyl hydrazinecarbodithioate [42] furnished the desired hydrazine **22**

Scheme 2.

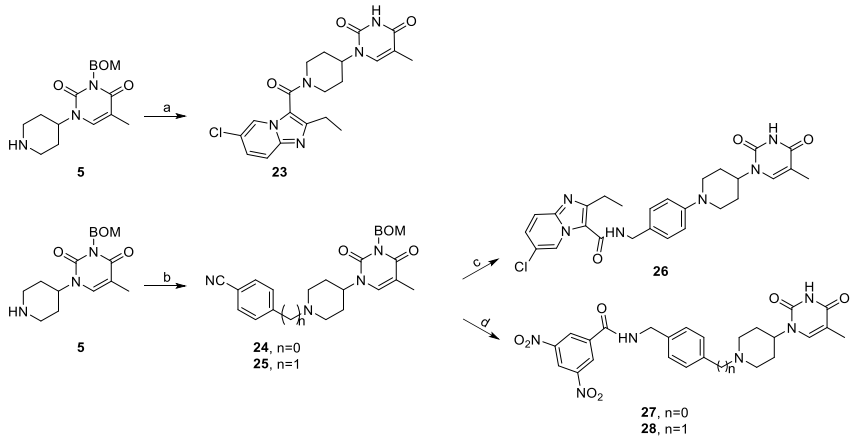


Reagents and conditions: (a) $Pd(PPh_3)_4$, 2-(tributylstannyl)pyridine, toluene, reflux, 40%; (b) sodium triacetoxyborohydride, 1,2-dichloroethane, 49%; (c) (i) methanesulfonyl chloride, Et_3N, CH_2Cl_2; (ii) **5**, K_2CO_3, ethanol, 80 ℃; (iii) neat TFA, 73 ℃, 26%; (d) (i) 3,4-bis(benzyloxy)benzoyl chloride , *N*-methylmorpholine, CH_2Cl_2; (ii) Pd/C, H_2, EtOH. 21%; (e) (i) EDC·HCl, DMAP, 3,4-bis((tert-butyldimethylsilyl)oxy)benzoic acid, CH_2Cl_2; (ii) HF·pyridine, pyridine, THF, 0 ℃, 16%; (f) (i) EDC·HCl, DMAP, $BnONH_2$·HCl, CH_2Cl_2; (ii) Pd/C, H_2, EtOH, 20%; (g) (i) 2,4-dihydroxybenzaldehyde, EA, 180 ℃; (ii) neat TFA, 73 ℃; (iii) methyl hydrazinecarbodithioate, MeOH, reflux, 6%.

To access compounds featuring imidazo[1,2-*a*]pyridine amide or 3,5-dinitrobenzamide motifs, intermediate **5** was directly coupled to 6-chloro-2-ethylimidazo[1,2-*a*]pyridine-3-carboxylic acid to give amide **23** after BOM- deprotection. Alternatively, **5** was first reacted with 4-fluorobenzonitrile or reductively alkylated with 4-cyanobenzaldehyde to yield **24** and **25**, respectively (Scheme 3). BOM-deprotection and subsequent reduction of the nitrile with

$NaBH_4/CoCl_2$ gave rise to the benzylamine intermediates, which were amidated to the desired final compounds **26-28**.

Scheme 3.



Reagents and conditions: (a) (i) EDC·HCl, HOBt, Et_3N, 6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylic acid, DMF, 70 ℃; (ii) neat TFA, 73 ℃, 21%; (b) n = 0, K_2CO_3, 4-fluorobenzonitrile, DMSO, 120 ℃; n = 1, *p*-cyanobenzaldehyde, sodium triacetoxyborohydride, 1,2-dichloroethane, 41%; (c) (i) neat TFA, 73 ℃; (ii) $NaBH_4$, $CoCl_2$, THF/H_2O; (iii) 6-chloro-2-ethylimidazo[1,2-*a*]pyridine-3-carboxylic acid, EDC·HCl, HOBt, Et_3N, DMF, 70 ℃, 28%; (d) (i) neat TFA, 73 ℃; (ii) $NaBH_4$, $CoCl_2$, THF/H_2O; (iii) 3,5-dinitrobenzoic acid, EDC·HCl, HOBt, Et_3N, DMF, 70 ℃, 10-20%.

Biological activity

Enzyme inhibition

All target compounds were tested for *Mtb*TMPK inhibitory activity, including the precursors/potential degradation products of cleavable iron chelator *Mtb*TMPK inhibitors, i.e. **4**, **7**, **9** and **10** (Table1). Unexpectedly, all these precursors exhibited better enzyme inhibitory activity than compound **1** [12]. It is noteworthy that the hydroxyethyl amine **10** displays almost 5-fold better activity than its hydroxyethyl ether analogue **9**.

Table 1. The *Mtb*TMPK enzymatic activity of compounds.

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	29 ± 3	**19**	0.24 ± 0.02
4	7.8 ± 0.4	**20**	0.18 ± 0.02
6	1.3 ± 0.1	**21**	1.0 ± 0.1
7	0.83 ± 0.05	**22**	0.31 ± 0.02
9	2.4 ± 0.3	**23**	879 ± 122

10	0.53 ± 0.02	**26**	47 ± 7
13	23 ± 2	**27**	ND[a]
17	34 ± 5	**28**	48 ± 2
18	0.14 ± 0.01		

[a]ND: Not determined, due to precipitation.

Interestingly, the inhibitory potency generally increased upon introduction of catechol (pairs **9**/**18**, **10**/**19**, **7**/**20**). To gain insight in their putative binding mode, **9** and **18** were docked into the enzyme (Figure 4, PDB: 5NR7 [12]). These studies suggest that hydroxyethyl ether in **9** forms a weak electrostatic interaction with Tyr39. Yet, the additional catechol ester of **18** protrudes outward the active site and may form hydrogen bonds with Asp9, Lys13 and Arg95, thereby stabilizing a bent conformation and resulting in an improved inhibitory activity.

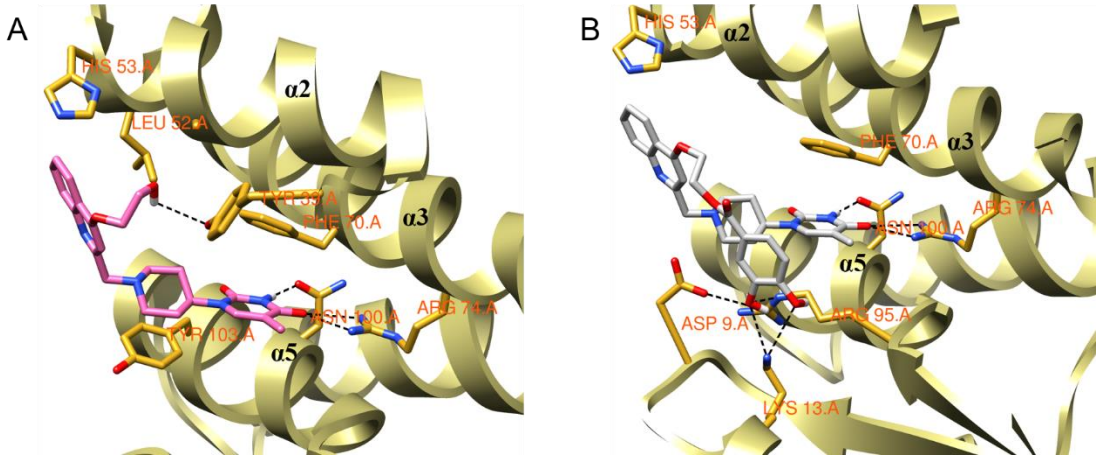


Figure 4. A. Structure of **9** docked in the active site of *Mtb*TMPK (PDB: 5NR7 [12]). *Mtb*TMPK is shown in a pale yellow cartoon representation with selected side chains labeled and shown as sticks with carbon atoms colored orange. Inhibitor **9** is drawn in stick representation with carbon atoms in pink, hydrogen-bonding interactions are shown as black dotted lines. B. Docking pose of inhibitor **18** in *Mtb*TMPK (PDB: 5NR7 [12]) catalytic pocket. The protein is depicted as pale yellow cartoon representation with selected side chain labeled (orange). Inhibitor **18** is drawn in stick representation with carbon atoms in white.

Except for bipyridyl analogue **17**, the non-cleavable chelator hybrid analogues **21** and especially **22** displayed promising inhibitory potency.

In line with earlier observations for other piperidine amide analogues [11,43], amide **23** showed very poor inhibitory potency. However, the insertion of a phenylmethylene group between the piperidyl and amide group as in **26** resulted in a 19-fold better potency. Since **26** structurally

deviates from all previous analogues, its co-crystal structure with *Mtb*TMPK was determined at 1.9 Å resolution (Table S2, PDB code: 6YT1). The crystal structure shows that **26** binds into the catalytic pocket contacting the same residues as analogous compounds (Figure 5A&B). Compound **26** is mainly accommodated by π−π electron stacking interactions above and below the plane of the heterocyclic system mediated by Tyr39, Tyr103, and Phe70, and is further stabilized by polar interactions mediated by Arg74 and Asn100 (Figure 5B). Surprisingly, compound **26** has the ability to engage residues of a symmetry-related TMPK protomer including Tyr39 and His53. This binding mode brings two antiparallel-oriented molecules of **26** in close proximity to each other (Figure 5A). To investigate whether this unusual phenomenon might be due to a particular tendency of compound **26** to pre-organize, the X-ray structure of compound **26** alone was also determined (Figure 5C and 5D). In this structure the relative organization of two inhibitor molecules does not resemble the antiparallel orientation observed in the complex with the protein. However, similar π - π stacking between the two central phenyl rings of two neighboring compounds was observed in both structures, which may account for the unusual phenomenon seen in the structure with the protein. In the catalytic pocket, **26** adopts an L-shaped (torsion angle 96º) conformation with the amide tail toward the α7- helix, which is distinct from the docked pose of **9** in which the hydroxyethyl tail protrudes to the α2-helix. While similar to **9**, the thymine ring adopts an identical pose in the catalytic pocket of the enzyme (Figure 5B) and a π-alkyl interaction between a 4-piperidine ring and Leu52 was observed. The amide tail fails to show specific interactions, possibly accounting for the moderate inhibitory activity of **26**. Encouraged by this finding, the 3,5-dinitrobenzamide analogues **27** and **28** were also prepared. Whereas the inhibitory potency of **27** could not be determined due to solubility problems in the *Mtb*TMPK assay, **28** showed similar inhibitory potency as **26**.

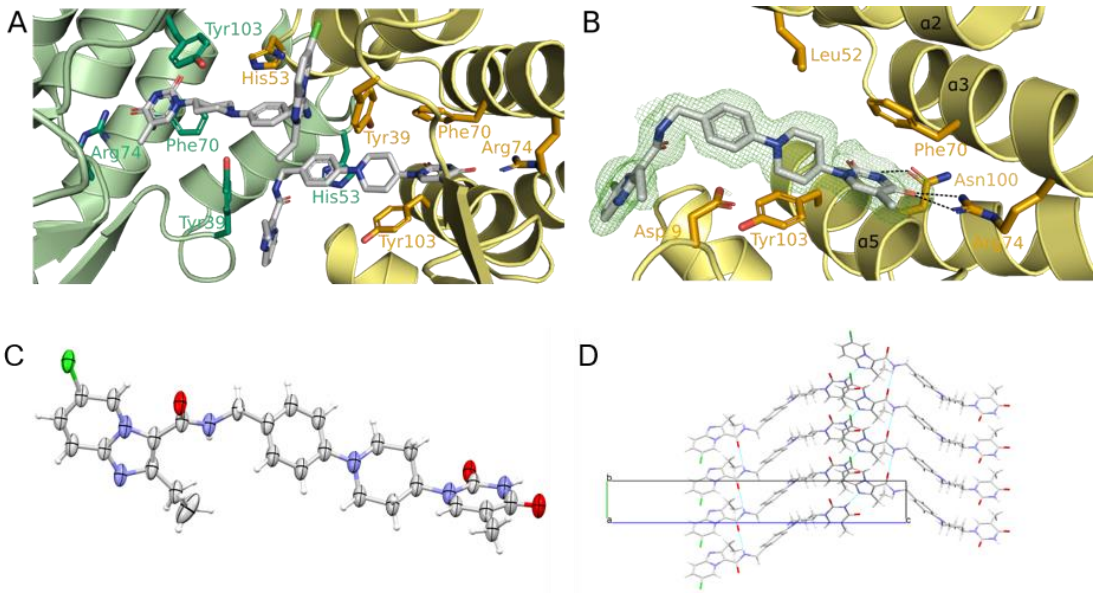


Figure 5. A. Co-crystal structure of compound **26** in complex with *Mtb*TMPK at 1.9 Å resolution. One TMPK protomer in the asymmetric unit (pale yellow) is shown in cartoon representation together with a symmetry related protomer (pale green). Two symmetry-related molecules of compound **26** are shown in stick representation with carbon atoms in white. B. View of binding mode and interaction of compound **26** in the *Mtb*TMPK active site. *Mtb*TMPK is shown in a pale yellow cartoon representation. Compound **26** (carbon atoms in white) and the side chains of *Mtb*TMPK interacting residues (carbon atoms in orange) are depicted as sticks. Hydrogen bonds are depicted as black dotted lines. The unbiased Fo-Fc difference electron density (contoured to +3 sigma) calculated before adding the ligand in the refinement process is shown as a green mesh. C. Crystal structure of compound **26**. D. Packing in the crystal structure and unit cell of compound **26**.

Stability of cleavable *Mtb*TMPK inhibitors

The stability of selected compounds **19**, **20** and **22** in ammonium bicarbonate-formic acid buffer solution (pH 6.6) was analyzed by LC-MS over the course of 24 h, and the results are summarized in Figure 6. Less than 10% degradation was found for inhibitor **20** and **22** up to 6 h. At 24 h, the percentage of compounds remaining in buffer were above 70% for all three inhibitors. These data reflect that although ester (**19**) and hydrazine (**22**) derivatives are hydrolyzed, it is not instantaneous, which is the desired profile. Analogue **20** did not show significant hydrolysis over the entire time course of the experiment.

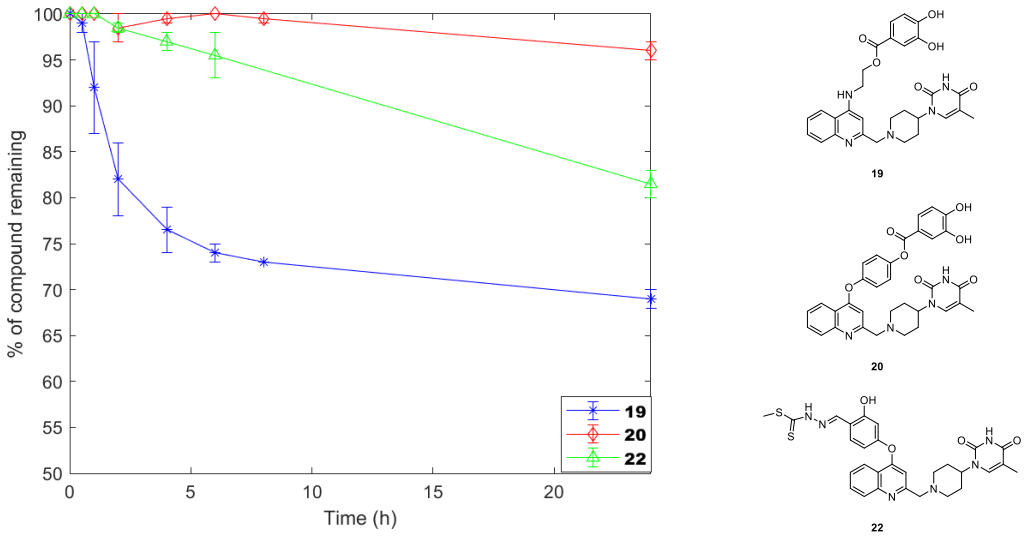


Figure 6. 24 h stability of inhibitors **19** (blue line), **20** (red line) and **22** (green line) in ammonium bicarbonate-formic acid buffer (pH 6.6). Error bar represents standard error between two replicates.

Antimicrobial activity

All the target compounds were also evaluated their antitubercular activity (Table 2). The precursor and most iron chelator containing compounds failed to show antimycobacterial activity. However, the (2,2'-bipyridin)-6-ylmethyl derivative **17** displayed a MIC value of 12.5 μM in 7H9/glucose medium, despite its moderate *Mtb*TMPK inhibitory potency. Only **22** displayed better activity in GAST (Fe (-)) medium compared to GAST (Fe (+)). In line with previous piperidine amide analogues [11,12] and its very weak on-target activity, compound **23** lacked antimycobacterial activity. Interestingly, compound **26**, which also features an imidazo[1,2-*a*]pyridine motif but more remote from the piperidine ring, displayed a MIC of 4.7 μM in standard medium**.** Likewise, the dinitrobenzamide derivatives **27** and **28** exhibit low micromolar whole-cell activity, and remain active in BSA containing medium (Table S3). Additionally, none of the analogues with promising antitubercular activity displayed cytotoxicity against MRC-5 fibroblasts.

Table 2. Antimycobacterial activity (H37Rv) of compounds in this study.

	Mtb H37Rv MIC[a] (μM)			
Compound	7H9/glucose	GAST (Fe(+))	GAST (Fe(-))	MRC-5 CC_{50}(μM)
1	>64[b]	-	-	-
4	>100	>100	>100	>64
6	>100	100	≥100	>64
7	≥100	>100	≥100	>64
9	>100	>100	>100	>64
10	>100	>100	>100	>64
13	75	>100	>100	53.98
17	12.5	>100	>100	>64
18	≥100	>100	>100	6.39
19	>100	>100	100	>64
20	>50	37	-	-
21	>50	50	-	32.22
22	>100	100	25	1.45
23	>100	>100	>100	>64
26	4.7	2.3	-	>64
27	9.4	0.78	1.2	>64
28	9.4	9.4	6.25	>64
Isoniazid	0.15	0.15	0.15	-

[a]Minimum inhibitory concentration (MIC) is the minimum concentration required to inhibit >99% growth of *M. tuberculosis* H37Rv in liquid culture. [b]Reported IC_{50}, which was tested on H37Ra strain.

Conclusion

The overall goal of this study was to synthesize hybrid compounds fusing or merging potential iron chelating moieties or motifs of compounds with known whole-cell antitubercular activity with earlier identified *Mtb*TMPK inhibitors, as to increase the bacterial uptake. Unexpectedly, the subset of iron chelating hybrids generally showed increased *Mtb*TMPK inhibitory potency, but only for **22** this resulted in moderate whole-cell antitubercular activity in iron-deficient GAST medium. A possible explanantion for the lack of whole-cell activity in this subset is that natural siderophores like mycobactin consist of multiple bidentate ligands for stoichiometric binding of iron(III), which is not the case for the simplified chelating moieties used in this study. Further analysis of their uptake by *M. tuberculosis* would assist active anti-tubercular agents identification, which will be done in future reserch.

However, we were more succesfull with hybrids containing imidazo[1,2-*a*]pyridine and

dinitrobenzamide motifs. While merging these motifs with a known *Mtb*TMPK inhibitor did not improve activity against the target, it afforded several non-toxic compounds (e.g. **26**, **27** and **28**) with promising antimycobacterial activity in all tested media. While at this stage we cannot exclude that this whole-cell activity is due to modulation of additional targets. Nevertheless, this study suggests that scaffold merging may be a fruitful approach for the discovery of new of antimycobacterial agents.

Experimental section

Expression and purification of *Mtb*TMPK

*Mtb*TMPK was expressed and purified as previously described [44]. In short, the pHL50 vector containing the coding sequence of wild-type *Mtb*TMPK was used to transform *E. coli* BLi5 competent cells. Transformed cells used to inoculate 1 L of 2XYT medium supplemented with antibiotics. The cells were allowed to grow at 37 °C, and protein production was induced with 1 mM isopropyl-1-thio-β-D-thiogalactoside when the culture reached an absorbance of 1.5 at 600 nm. After 3 h of incubation at 37 °C, cells were harvested by centrifugation at 8000g at 4 °C and stored at −80 °C. Cells from 1 L of culture were resuspended in 50 mL of cold lysis buffer containing 50 mM Tris HCl pH 8.0 supplemented with an antiprotease cocktail (Roche) and disrupted by sonication. After centrifugation at 20000g for 30 min at 4 °C, the filtered bacterial lysate was loaded on a 5 mL Blue-Sepharose column pre-equilibrated with lysis buffer. The column was washed with lysis buffer and most of the protein was eluted with elution buffer containing 50 mM Tris HCl pH 8.0, 1 M NaCl. A second elution was performed using elution buffer containing 50 mM Tris HCl pH 8.0, 2 M NaCl. Fractions containing *Mtb*TMPK were pooled and injected on a HiLoad Superdex 75 16/600 column equilibrated with 20 mM Tris HCl pH 7.4, 1 mM EDTA as a further polishing and buffer exchange step. Fractions containing pure *Mtb*TMPK were pooled and Tris(2-carboxyethyl)-phosphine (TCEP) was added to 1 mM final concentration before flash-freezing the protein samples in liquid nitrogen.

Crystal structure of *Mtb*TMPK in complex with compound 26

Before each cocrystallization experiment, *Mtb*TMPK samples were thawed and concentrated to 8 mg/mL. Compound **26** was initially dissolved in 100% DMSO at a concentration of 100 mM and diluted to 50 mM with pure isopropanol. Diluted compound was added to the concentrated

protein stock and incubated for 1 h at room temperature. Cocrystallization conditions of *Mtb*TMPK-inhibitor complexes were screened at 20 °C by the sitting-drop vapor-diffusion method using a 1:1 protein/solution volume ratio and available commercial screens.

Crystals of *Mtb*TMPK in complex with compound **26** were obtained in condition H2 of commercial PEG/ION screen containing 0.05 M citric acid, 0.05 M BIS-TRIS propane pH 5.0 and 16% w/v polyethylene glycol 3,350. Crystals were cryoprotected in mother liquor supplemented with ethylene glycol at a final concentration of 15%. Data collection on crystals of *Mtb*TMPK in complex with compound **26** was achieved at the Proxima1 beamline at the Soleil synchrotron (Paris, France) at a wavelength of 0.97857 Å and a temperature of 100 K on a Dectris Pilatus 6 M pixel detector.

Processing, indexing and scaling of the dataset was performed using XDS/ XSCALE [45], followed by mtz conversion with XDSCONV.

The structure was solved by molecular replacement using the pdb of 5NQ5 as initial model using PHASER (Table S2) [12]. The structure was further refined by one cycle of rigid-body refinement in BUSTER (https://www.globalphasing.com/buster/) followed by positional and individual isotropic B-factor refinement in BUSTER and PHENIX [46]. During the course of refinement the model was manually improved in COOT [47]. NCS torsion restraints were used during the refinement. The grade Web Server (http://grade.globalphasing.org) was used to generate ligand coordinates as well as energy minimization and restraint generation.

Ligand molecules were modeled in sigma-weighted Fo-Fc difference electron density maps in the course of the refinement. TLS refinement with TLS group definition defined by the TLSMD server was applied in the latest stages of refinement.

The quality of the final crystal structures was assessed with MOLPROBITY [48] prior to deposition in the PDB database (www.rcsb.org) with accession code 6YT1.

Enzymatic assay

After expression and purification of *Mtb*TMPK as described by Munier-Lehmann *et al.* [44], the enzymatic assay was conducted as previously reported [12]. Briefly, at fixed concentration of ATP (0.5 mM) and dTMP (0.05 mM), compounds were evaluated at different concentrations using the spectrophotometric assay described by Blondin et al [49]. The reaction medium consists of 50 mM Tris-HCl pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol

pyruvate, and 2 units each of coupling enzymes (lactate dehydrogenase, pyruvate kinase and nucleoside diphosphate kinase). IC_{50} value was calculated using Kaleida Graph to plot and fit the experimental data points.

Computational studies.

AutoDock vina and AutodockTools-1.5.6. [50] were used for the molecular modeling, with publicly available X-ray structure of the *Mtb*TMPK (PDB entry 5NR7 [12]). After being minimized the energy (minimum RMS gradient: 0.001), the PDB files of all ligands were generated in ChemDraw 3D 16.0. The PDBQT files of the ligands and receptor were prepared by AutodockTools-1.5.6, including atom types, atomic partial charges and the information on the ligand torsional degrees. Centered on *Mtb*TMPK active site PHE70 CE2 (the coordinates x, y, z were -0.997, 26.240, -4.528 correspondingly), the prepared PDBQT files of ligands and receptors were docked using a grid spacing of 0.375 and 60 x 60 x 60 number of grid points. Through Lamarckian 4.2 method, each ligand was docked in autodock vina for 3 times, which generated total 60 possible conformations. To analyze the results, Chimera was initially used to view the binding energy, in combination with analysis and validation of possible interactions in LigPlus.

***In vitro* antituberculosis activity**

The MIC values of all compounds were determined as previously decribed [51,52]. In brief, *M. tuberculosis* H37Rv (ATCC 27294) was grown to OD_{650nm} 0.2 in the respective medium prior to further 1000-fold dilution in fresh medium. Drugs were 2-fold serially diluted in duplicate in the medium of choice (50 μL/well) in a concentration range spanning 100-0.049 μM in sterile 96-well U-bottom clear polysteyrene microtiter plates. Isoniazid and DMSO as postive and negative controls, respectively. An equal volume (50 μL) of diluted cells was added to the plates with the serial drug dilution. Plates were sealed in ziplock bags and incubated at 37 ℃. After 7-14 days, plates were read with enlarging inverted mirror plate reader. The MIC was recorded as the concentration that fully inhibited all visible growth.

***In vitro* cytotoxicity assay**

The cytotoxicity of compounds on MRC-5 fibroblasts was performed exactly as previously reported [12].

Stability assay

Preparation of pH 6.6 bicarbonate-formic acid buffer: 158.12 mg ammonium bicarbonate was dissolved in 10 ml H_2O to prepara 0.2 M ammonium bicarbonate. 0.2 M formic acid solution was prepared by dissolving 150.8 ul formic acid in 20 ml H_2O. To obtain ammonium pH 6.6 bicarbonate-formic acid buffer, 10 ml 0.2 M ammonium bicarbonate solution was titrated with 0.2 M formic acid solution under monitoration of pH meter. Preparation of sample: the tested compound was solubilized in DMSO (Sigma - Aldrich) at stock concentration of 8 mM. Serial dilutions were made in bicarbonate-formic acid buffer. Volumes of 20 µL of the serial dilutions were injected into LC-MS system to build the standard curve. Volumes of 3 mL solutions at concentration 80 µM were used as working solution. Volumes of 20 µL of the working solutions were measured with LC-MC at time piont 0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h. Area of the curve was collected and analysed by excel and MatLab.

Chemistry

Regeants were purchased and used without any further purification. All synthetic compounds presented in this study were checked with precoated Alugram Silica TLC F254 plates (Machhery-Nagel), visualized under UV light at 254 nm or stained by potassium permanganate, and purified by column chromatography on a Reveleris X2 (Grace) automated flash unit. ^{1}H and ^{13}C NMR spectral data were obtained on a Varian Mercury 300/75 MHz spectrometer at 300 K using TMS as an internal standard. Structural assignment was confirmed with the assistance of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC. High resolution mass spectrometry (HRMS) was performed at 1ng/ml on a Waters LCT Premier XE™ time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray™ interface, using 0.1% HCOOH in MeCN/H_2O (1:1) as mobile phase. Purity of final compounds was determined by LC-MS analysis on a Phenomenex Kinetex EVO C18 5 µm 100 mm × 2.1 mm column at flow rate of 1.4 mL/min (Waters Alliance 2695 XE separation module), using HCOOH in H_2O (0.1%, v/v)/ MeCN as gradient system.

General procedure 1: synthsis of 3-((benzyloxy)methyl)-5-methy-1-(1-(substituted quinolin-2-ylmethyl)-4-yl)pyrimidine-2,4(1H,3H)-dione.

Method A To a solution of substituted quinoline buiding blocks (1.0 eq.) in dioxane was added selenium dioxide (1.3 eq). The resulting mixture was refluxed overnight. Then, the reaction mixture was cooled to room temperature and appropriate volume of EA was added for dilution,

followed by filtration through celite. The collected filtrate was extracted with water and the organic phase was dried and concentrated *in vacuo*. The resulting residue and 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione [11] (**5**, 0.67 eq.) were dissolved in 1,2-dichloroethane under N_2 and the mixture was stirred for 0.5 h, followed by addition of sodium triacetoxyborohydride (2.0 eq.). After stirring overnight, the reaction mixture was diluted with CH_2Cl_2, washed with sat. $NaHCO_3$ and brine, and dried over Na_2SO_4. The combined organic layers were filtered, concentrated and purified by silica chromatography to give the title compound.

Method B To a solution of substituted quinoline buiding blocks (1.0 eq.) in CCl_4 were added N-bromosuccinimide (0.9 eq.) and 2,2-azobisisobutyronitrile (0.2 eq.). The resulting mixture was refluxed overnight. Then the reaction mixture was cooled to room temperature and filtered to remove the solid. After being extracted with sat. $NaHCO_3$ and brine, the organic layer was dried and concentrated. The resulting residue, **5** (1.0 eq.) and K_2CO_3 (3 eq.) were dissolved in ethanol and the mixture was refluxed for 3 h. The reaction mixture was cooled to room temperature and filtered. The concentrated filtrate was purified by silica chromatography to give the title compound.

General procedure 2: synthesis of 5-methyl-1-(1-(4-substituted quinolin-2-ylmethyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione

To a solution of 3-((benzyloxy)methyl)-1-(1-((substituted 4-chloroquinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (1.0 eq.) in EA (2.0 mL) was added the phenol derivatives (1.5 eq.) and the resulting mixture was heated at 180 ℃ for 0.5 h. After cooled to room temperature, the reaction mixture was purified by silica chromatography to give BOM-protected intermediate, which was heated at 73 ℃ in TFA to yield the title compound after purification.

*3-((Benzyloxy)methyl)-1-(1-((4-chloroquinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**3**)* Following method A in *General procedure 1*, 4-chloro-2-methyl quinoline (0.20 g, 1.1 mmol) and selenium dioxide (0.16 g, 1.5 mmol) in dioxane (20 mL) afforded the aldehyde intermediate, which was dissolved in 1,2-dichloroethane together with **5** (0.25 g, 0.75 mmol) synthesized according to previously reported procedure [11], followed by addition of sodium triacrtoxyborohydride (0.48 g, 2.3 mmol) to yield **3** (eluent system: 55% EA in petroleum

ether, 0.18 g, 32%). ^{1}H NMR (300 MHz, *CDCl$_3$*) *δ* ppm 1.77 - 1.96 (m, 7 H, 5-CH_3, piperidin-3-yl, piperidin-5-yl), 2.27 - 2.40 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.02 (d, *J* = 12.0 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.81 (s, 2 H, $N_{piperidyl}CH_2$), 4.47 - 4.62 (m, 1 H, piperidin-4-yl), 4.70 (s, 2 H, (Ph)CH_2), 5.50 (s, 2 H, N^3CH_2), 7.06 (d, *J* = 1.2 Hz, 1 H, H-6), 7.17 - 7.40 (m, 5 H, Ph, quinolin-3-yl), 7.60 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1 H, Ph), 7.68 - 7.80 (m, 2 H, Ph), 8.06 (dd, *J* = 8.5, 0.6 Hz, 1 H, Ph), 8.19 (dd, *J* = 8.4, 1.3 Hz, 1 H, Ph). ^{13}C NMR (75 MHz, *DMSO-d$_6$*) *δ* ppm 13.16 (1 C, 5-CH_3), 30.72 (2 C, piperidin-3-yl, piperidin-5-yl), 52.96 (2 C, piperidin-2-yl, piperidin-6-yl), 53.13 (1 C, piperidin-4-yl), 64.28 (1 C, $N_{piperidyl}CH_2$), 70.73 (1 C, N^3CH_2), 72.12 (1 C, (Ph)CH_2), 109.93 (1 C, C-5), 120.69 (1 C, quinolin-3-yl), 123.85 (1 C, Ph), 125.47 (1 C, Ph), 127.15 (1 C, Ph), 217.44 (1 C, Ph), 127.47 (2 C, Ph), 128.11 (2 C, Ph), 129.28 (1 C, Ph), 130.31 (1 C, Ph), 134.88 (1 C, C-6), 137.96 (1 C, Ph), 142.93 (1 C, quinolin-4-yl), 148.33 (1 C, Ph), 151.42 (1 C, C-2), 159.37 (1 C, quibolin-2-yl), 163.12 (1 C, C-4). HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{28}H_{29}ClN_4O_3$ + H]$^+$ 505.2001, found 505.1318.

*5-Methyl-1-(1-((4-oxo-1,4-dihydroquinolin-2-yl)methyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (**4**)* To a 25 mL flask were added **3** (0.10 g, 0.20 mmol) in acetic acid (10 mL) and the mixture was heated at 125 ℃ for 2 h. After total consumption of **3** checked with TLC, solvent was removed *in vacuo*. The resulting residue was dissolved in TFA (5.0 mL) and heated at 73 ℃ for 4 h, followed by removing the volatile composition, adjusting the pH to 6-7 with sat. $NaHCO_3$ and the concentrated residue was purified with silica chromatography to give **4** (eluent system: 5% methanol in CH_2Cl_2, 42 mg, 58%). ^{1}H NMR (300 MHz, *DMSO-d$_6$*) *δ* ppm 1.61-1.72 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 1.76 (s, 3 H, 5-CH_3), 1.81 - 2.01 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.20 (t, *J* = 11.0 Hz, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.97 (d, *J* = 11.1 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.50 (s, 2 H, $N_{piperidyl}CH_2$), 4.20 - 4.33 (m, 1 H, piperidin-4-yl), 6.07 (s, 1 H, quinolin-3-yl), 7.26 (dt, *J* = 7.8, 4.1 Hz, 1 H, Ph), 7.50 - 7.67 (m, 3 H, H-6, Ph), 8.03 (d, *J* = 8.2 Hz, 1 H, Ph), 11.19 (br. s., 1 H,), 11.41 (br. s., 1 H). ^{13}C NMR (75 MHz, *DMSO-d$_6$*) *δ* ppm 11.74 (1 C, 5-CH_3), 29.51 (2 C, piperidin-3-yl, piperidin-5-yl), 52.03 (1 C, piperidin-4-yl), 52.21 (2 C, piperidin-2-yl, piperidin-6-yl), 58.64 (1 C, $N_{piperidyl}CH_2$), 107.85 (1 C, quinolin-3-yl), 108.66 (1 C, C-5), 117.91 (1 C, Ph), 122.57 (1 C, Ph), 124.49 (1 C, Ph), 124.70 (1 C, Ph), 131.24 (1 C, Ph), 137.33 (1 C, Ph), 139.85 (1 C, C-6), 149.57 (1 C, quinolin-2-yl), 150.52 (1 C,

C-2), 163.38 (1 C, C-4), 176.60 (1 C, quinolin-4-yl). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{20}H_{22}N_4O_3 + H]^+$ 367.1765, found 367.1714.

*2-Hydroxy-4-((2-((4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)oxy)benzoic acid (**6**)* Following General procedure 2, **3** (0.20 g, 0.40 mmol) and 2,4-dihydroxybenzoic acid (92 mg, 0.60 mmol) in EA (2.0 mL) afforded the protected intermediate, which was heated in TFA (5.0 mL) at 73 ℃ for 4 h to yield **6** (eluent system: 10% methanol in CH_2Cl_2, 76 mg, 38%). ^{1}H NMR (300 MHz, *DMSO-d*$_6$) *δ* ppm 1.76 (s, 3 H, 5-CH_3), 1.80 - 1.97 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 2.01 - 2.27 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.97 - 3.15 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.43 (d, *J* = 11.4 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.24 - 4.60 (m, 3 H, piperidin-4-yl, $N_{piperidyl}CH_2$), 6.62 - 6.76 (m, 2 H, H-6, Ph), 7.09 (s, 1 H, quinolin-3-yl), 7.46 (s, 1 H, Ph), 7.68 (t, *J* = 7.6 Hz, 1 H, Ph), 7.79 - 7.92 (m, 2 H, Ph), 8.07 (d, *J* = 8.5 Hz, 1 H, Ph), 8.24 (d, *J* = 7.9 Hz, 1 H, Ph), 11.26 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d*$_6$) *δ* ppm 12.14 (1 C, 5-CH_3), 26.95 (2 C, piperidin-3-yl, piperidin-5-yl), 50.09 (1 C, piperidin-4-yl), 51.52 (2 C, piperidin-2-yl, piperidin-2-yl), 59.81 (1 C, $N_{piperidyl}CH_2$), 106.02 (1 C, quinolin-3-yl), 107.90 (1 C, Ph), 109.25 (1 C, Ph), 110.05 (1 C, C-5), 115.30 (1 C, Ph), 120.32 (1 C, Ph), 121.57 (1 C, Ph), 127.10 (1 C, Ph), 128.60 (1 C, Ph), 130.98 (1 C, Ph), 132.38 (1 C, Ph), 137.14 (1 C, C-6), 148.52 (1 C, Ph), 150.71 (1 C, C-2), 154.61 (1 C, quinolin-2-yl), 158.15 (t, *J* = 31.5 Hz, 1 C, Ph) , 160.64 (1 C, quinolin-4-yl), 163.61 (1 C, C-4), 163.77 (1 C, C-OH), 171.37 (1 C, COOH). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{27}H_{26}N_4O_6 + H]^+$ 503.1925, found 503.1951.

*1-(1-((4-(4-Hydroxyphenoxy)quinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**7**)* Following General procedure 2, **3** (0.40 g, 0.79 mmol) and hydroquinone (0.30 g, 2.7 mmol) in EA (2.0 mL) afforded the protected intermediate, which was heated in TFA (5.0 mL) at 73 ℃ for 4 h to yield **7** (eluent system: 5% methanol in CH_2Cl_2, 0.17 g, 46%). ^{1}H NMR (300 MHz, *DMSO-d*$_6$) *δ* ppm 1.46 - 1.86 (m, 7 H, 5-CH_3, piperidin-3-yl, piperidin-5-yl), 2.08 (t, *J* = 10.7 Hz, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.78 - 2.88 (m, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.61 (s, 2 H, $N_{piperidyl}CH_2$), 4.14 - 4.26 (m, 1 H, piperidin-4-yl), 6.67 (s, 1 H, quinolin-3-yl), 6.87 (d, *J* = 9.1 Hz, 2 H, Ph), 7.08 (d, *J* = 8.8 Hz, 2 H, Ph), 7.47 (s, 1 H, H-6), 7.53 - 7.61 (m, 1 H, Ph), 7.71 - 7.80 (m, 1 H, Ph), 7.93 (d, *J* = 8.5 Hz, 1 H, Ph), 8.23 (d, *J* = 7.9 Hz, 1 H, Ph), 9.66 (br.s., 1 H, OH), 11.12 (br.s., 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d*$_6$) *δ* ppm

11.82 (1 C, 5-CH_3), 29.63 (2 C, piperidin-3-yl, piperidin-5-yl), 52.10 (1 C, piperidin-4-yl), 52.32 (2 C, piperidin-2-yl, piperidin-6-yl), 63.84 (1 C, $N_{piperidyl}CH_2$), 102.66 (1 C, quinolin-3-yl), 108.77 (1 C, C-5), 116.38 (2 C, Ph), 119.71 (1 C, Ph), 121.15 (1 C, Ph), 121.80 (2 C, Ph), 125.68 (1 C, Ph), 128.15 (1 C, Ph), 130.00 (1 C, Ph), 137.25 (1 C, C-6), 145.56 (1 C, Ph), 148.23 (1 C, Ph), 150.56 (1 C, C-2), 154.87 (1 C, Ph), 160.38 (1 C, quinolin-2-yl), 161.90 (1 C, quinolin-4-yl), 163.48 (1 C, C-4). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{26}H_{26}N_4O_4 + H]^+$ 459.2027, found 459.2043.

*4-((2-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)oxy)benzoic acid (**8**)* Following General procedure 2, **3** (0.45 g, 0.89 mmol) and methyl 4-hydroxybenzoate (0.20 g, 1.3 mmol) in EA (2.0 mL) afforded the protected intermediate, which was hydrolysis in 2 N aq. NaOH solution (20 mL) at 50 ℃ for 4h, and acidified with 2 M aq. HCl (25 mL) to give the acid intermediate. The resulting residue was heated in TFA (5.0 mL) at 73 ℃ for 4 h to yield **8** (eluent system: 10% methanol in CH_2Cl_2, 0.18 g, 41%). ^{1}H NMR (300 MHz, *DMSO-d_6*) *δ* ppm 1.77 (s, 3 H, 5-CH_3), 1.87 - 1.95 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 2.23 - 2.32 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 3.21 - 3.28 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.55 - 3.62 (m, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.49 - 4.66 (m, 3 H, $N_{piperidyl}CH_2$, piperidin-4-yl), 7.04 (s, 1 H, quinolin-3-yl), 7.43 (d, *J* = 8.8 Hz, 3 H, H-6, Ph), 7.68 - 7.77 (m, 1 H, Ph), 7.85 - 7.96 (m, 1 H, Ph), 8.08 (d, *J* = 8.8 Hz, 3 H, Ph), 8.30 (d, *J* = 8.8 Hz ,1 H, Ph), 11.29 (s, 1 H, NH), H (COOH) could not be observed. ^{13}C NMR (75 MHz, *DMSO-d_6*) *δ* ppm 12.22 (1 C, 5-CH_3), 26.17 (2 C, piperidin-3-yl, piperidin-5-yl), 49.68 (1 C, piperidin-4-yl), 51.26 (2 C, piperidin-2-yl, piperidin-6-yl), 59.75 (1 C, $N_{piperidyl}CH_2$), 106.25 (1 C, quinolin-3-yl), 109.35 (1 C, C-5), 120.32 (1 C, Ph), 120.55 (3 C, Ph), 121.63 (1 C, Ph), 127.44 (1 C, Ph), 128.09 (1 C, Ph), 128.61 (1 C, Ph), 131.27 (1 C, Ph), 131.98 (2 C, Ph), 137.05 (1 C, C-6), 150.68 (1 C, C-2), 157.55 (1 C, Ph), 160.60 (1 C, quinolin-4-yl), 163.60 (1 C, C-4), 166.56 (1 C, COOH), C (quinolin-2-yl) could not be observed. HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{27}H_{26}N_4O_5 + H]^+$ 487.1976, found 487.2204.

*1-(1-((4-(2-Hydroxyethoxy)quinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**9**)* Following General procedure 2, **3** (0.20 g, 0.40 mmol) and ethylene glycol (37 mg, 0.60 mmol) in EA (2.0 mL) afforded the protected intermediate, which was heated in TFA (5.0 mL) at 73 ℃ for 4 h to yield **9** (eluent system: 10% methanol in CH_2Cl_2, 66 mg, 41%). ^{1}H NMR

(300 MHz, *DMSO-d*$_6$) *δ* ppm 1.79 (s, 3 H, 5-CH_3), 1.85 - 2.01 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 2.16 - 2.36 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 3.18 - 3.30 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.5 - 3.67 (m, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.88 (t, *J* = 4.7 Hz, 2 H, O$\underline{CH_2}$), 4.29 (t, *J* = 4.7 Hz, 2 H, O$\underline{CH_2}$), 4.45 - 4.71 (m, 3 H, piperidin-4-yl, $N_{piperidyl}CH_2$), 7.18 (s, 1 H, quinolin-3-yl), 7.46 (s, 1 H, H-6), 7.62 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1 H, Ph), 7.81 (ddd, *J* = 8.4, 7.0, 1.3 Hz, 1 H, Ph), 8.00 (d, *J* = 8.2 Hz, 1 H, Ph), 8.26 (dd, *J* = 8.5, 0.9 Hz, 1 H, Ph), 11.30 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d*$_6$) *δ* ppm 12.67 (1 C, 5-CH_3), 26.99 (2 C, piperidin-3-yl, piperidin-5-yl), 50.42 (1 C, piperidin-4-yl), 52.03 (2 C, piperidin-2-yl, piperidin-6-yl), 59.65 (2 C, $\underline{CH_2}$OH, $N_{piperidyl}CH_2$), 71.25 (1 C, O$\underline{CH_2}$), 102.01 (1 C, quinolin-3-yl), 109.77 (1 C, C-5), 120.65 (1 C, Ph), 122.51 (1 C, Ph), 126.79 (1 C, Ph), 128.42 (1 C, Ph), 131.12 (1 C, Ph), 137.61 (1 C, C-6), 147.99 (1 C, Ph), 151.16 (1 C, C-2), 162.64 (1 C, quinolin-4-yl), 164.08 (1 C, C-4), C (quinolin-2-yl) could not be observed. HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{22}H_{26}N_4O_4$ + H]$^+$ 411.2027, found 411.2037.

*1-(1-((4-((2-Hydroxyethyl)amino)quinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**10**)* Following General procedure 2, **3** (0.20 g, 0.40 mmol) and ethanolamine (36 mg, 0.60 mmol) in EA (2.0 mL) afforded the protected intermediate, which was heated in TFA (5.0 mL) at 73 ℃ for 4 h to yield **10** (eluent system: 10% methanol in CH_2Cl_2, 55 mg, 34%). ^{1}H NMR (300 MHz, *DMSO-d*$_6$) *δ* ppm 1.57 - 1.83 (m, 5 H, 5-CH_3, piperidin-3a-yl, piperidin-5a-yl), 1.87 - 2.06 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.32 - 2.44 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.07 (d, *J* = 11.1 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.58 - 3.66 (m, 2 H, N$\underline{CH_2}$), 3.71 (t, *J* = 5.6 Hz, 2 H, O$\underline{CH_2}$), 3.91 (br. s., 2 H, $N_{piperidyl}CH_2$), 4.23 - 4.41 (m, 1 H, piperidin-4-yl), 6.93 (s, 1 H, quinolin-3-yl), 7.55 (s, 1 H, H-6), 7.61 - 7.73 (m, 1 H, Ph), 7.84 - 8.03 (m, 2 H, Ph), 8.48 (d, *J* = 8.5 Hz, 1 H, Ph), 9.17 (t, *J* = 6.2 Hz, 1 H, (Ph)NH), 11.22 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d*$_6$) *δ* ppm 12.37 (1 C, 5-CH_3), 29.60 (2 C, piperidin-3-yl, piperidin-5-yl), 46.10 (1 C, N$\underline{CH_2}$), 52.32 (1 C, piperidin-4-yl), 52.59 (2 C, piperidin-2-yl, piperidin-6-yl), 58.47 (1 C, $N_{piperidyl}CH_2$), 59.06 (1 C, $\underline{CH_2}$OH), 98.68 (1 C, quinolin-3-yl), 109.27 (1 C, C-5), 116.52 (1 C, Ph), 120.54 (1 C, Ph), 123.28 (1 C, Ph), 126.54 (1 C, Ph), 133.63 (1 C, Ph), 137.79 (1 C, C-6), 138.55 (1 C, Ph), 151.07 (1 C, C-2), 156.04 (1 C, quinolin-4-yl), 158.44 (1 C, quinolin-2-yl), 163.93 (1 C, C-4). HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{22}H_{27}N_5O_3$ + H]$^+$ 410.2187, found 410.2199.

*3-((Benzyloxy)methyl)-1-(1-((8-hydroxyquinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**12**)* Following method B in General procedure 1, 2-methylquinolin-8-yl acetate (0.55 g, 2.7 mmol), N-bromosuccinimide (0.44 g, 2.5 mmol) and 2,2-azobisisobutyronitrile (90 mg, 0.55 mmol) were refluxed in CCl_4 (25 mL) to give the bromo substituted intermediate, which was further treated with **5** (0.90 g, 2.7 mmol) and K_2CO_3 (1.1 g, 8.2 mmol) in ethanol to afford **12** (eluent system: 55% EA in petroleum ether, 0.25 g, 20%). ^{1}H NMR (300 MHz, *CDCl$_3$*) *δ* ppm 1.85 - 2.00 (m, 7 H, 5-CH_3, piperidin-3-yl, piperidin-5-yl), 2.21 - 2.39 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.06 (d, *J* = 11.7 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.82 - 3.88 (m, 2 H, $N_{piperidyl}CH_2$), 4.48 - 4.64 (m, 1 H, piperidin-4-yl), 4.69 (s, 2 H, (Ph)CH_2), 5.49 - 5.51 (m, 2 H, N^3CH_2), 7.09 (d, *J* = 0.9 Hz, 1 H, H-6), 7.15 (dd, *J* = 7.5, 1.3 Hz, 1 H, quinolin-3-yl), 7.19 - 7.32 (m, 4 H, Ph), 7.33 - 7.45 (m, 3 H, Ph), 7.52 (d, *J* = 8.2 Hz, 1 H, Ph), 8.11 (d, *J* = 8.5 Hz, 1 H, Ph). ^{13}C NMR (75 MHz, *CDCl$_3$*) *δ* ppm 13.15 (1 C, 5-CH_3), 29.92 (2 C, piperidin-3-yl, piperidin-5-yl), 52.82 (2 C, piperidin-2-yl, piperidin-6-yl), 53.14 (1 C, piperidin-4-yl), 63.53 (1 C, $N_{piperidyl}CH_2$), 70.80 (1 C, N^3CH_2), 72.16 (1 C, (Ph)CH_2), 110.16 (1 C, C-5), 110.38 (1 C, Ph), 117.50 (1 C, Ph), 121.47 (1 C, quinolin-3-yl), 127.51 (4 C, Ph), 127.62 (1 C, Ph), 128.15 (2 C, Ph), 134.43 (1 C, C-6), 135.01 (1 C, quinolin-4-yl), 136.70 (1 C, Ph), 137.97 (1 C, Ph), 151.46 (1 C, C-2), 152.15 (1 C, C-OH), 163.17 (1 C, C-4), 168.89 (1 C, quinolin-2-yl). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{28}H_{30}N_4O_4 + H]^+$ 487.2340, found 487.2344.

*1-(1-((8-Hydroxyquinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**13**)* A solution of **12** (0.20 g, 0.41 mmol) in TFA (4.0 mL) was heated at 73 ℃ for 4 h to yield **13** (eluent system: 5% methanol in CH_2Cl_2, 87 mg, 58%). ^{1}H NMR (300 MHz, *DMSO-d$_6$*) ppm *δ* 1.62 - 1.71 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 1.76 (s, 3 H, 5-CH_3), 1.82 - 1.99 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.14 - 2.27 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.95 (d, *J* = 12.0 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.81 (s, 2 H, $N_{piperidyl}CH_2$), 4.22 - 4.37 (m, 1 H, piperidin-4-yl), 7.06 (dd, *J* = 6.7, 2.3 Hz, 1 H, Ph), 7.31 - 7.42 (m, 2 H, Ph), 7.61 - 7.69 (m, 2 H, H-6, quinolin-3-yl), 8.27 (d, *J* = 8.8 Hz, 1 H, quinolin-4-yl), 9.48 (s, 1 H, OH), 11.18 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d$_6$*) *δ* ppm 12.00 (1 C, 5-CH_3), 30.08 (2 C, piperidin-3-yl, piperidin-5-yl), 52.27 (1 C, piperidin-4-yl), 52.75 (2 C, piperidin-2-yl, piperidin-6-yl), 63.86 (1 C, $N_{piperidyl}CH_2$), 108.95 (1 C, C-5), 111.25 (1 C, Ph), 117.59 (1 C, Ph), 121.33 (1 C, quinolin-3-yl), 126.96 (1 C, Ph), 127.80 (1 C, Ph), 136.35 (1 C, Ph), 137.69 (1 C, C-6), 150.82 (1 C, C-2),

152.84 (1 C, C-OH), 157.55 (2 C, quinolin-2-yl, quinolin-4-yl), 163.67 (1 C, C-4). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{20}H_{22}N_4O_3 + H]^+$ 367.1765, found 367.1778.

*[2,2'-Bipyridine]-6-carbaldehyde (**15**)* According to a literature procedure [53], a solution of 6-bromo-2-pyridinecarboxaldehyde (0.20 g, 1.1 mmol), 2-(tributylstannyl)pyridine (0.37 g, 1.0 mmol) and tetrakis(triphenylphosphine)palladium (41 mg, 0.035 mmol) in degased tolune (20 mL) was refluxed overnight under nitrogen. The volatile in the resulting brown mixture was removed *in vacuo*. The crude aldehyde was purified by column chromatography to afford **15** (eluent system: 25% EA in petroleum, 74 mg, 40%). ^{1}H NMR (300 MHz, *CDCl$_3$*) *δ* ppm 7.30 - 7.39 (m, 1 H, Py), 7.80 - 7.88 (m, 1 H, Py), 7.91 - 7.98 (m, 2 H, Py), 8.49 - 8.55 (m, 1 H, Py), 8.59 - 8.65 (m, 1 H, Py), 8.66 - 8.71 (m, 1 H, Py), 10.15 (s, 1 H, CHO). ^{13}C NMR (75 MHz, *CDCl$_3$*) *δ* ppm 121.18 (1 C, Py), 121.30 (1 C, Py), 124.23 (1 C, Py), 125.05 (1 C, Py), 136.99 (1 C, Py), 137.83 (1 C, Py), 149.22 (1 C, Py), 152.20 (1 C, Py), 154.85 (1 C, Py), 156.55 (1 C, Py), 193.56 (1 C, CHO). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{11}H_8N_2O + H]^+$ 185.0710, found 185.0696.

*[2,2'-Bipyridin]-6-ylmethanol (**16**)* To a solution of **15** (0.10 g, 0.54 mmol) in 1,2-dichloroethane was added sodium triacetylborohydride (0.23 g, 1.1 mmol) and the resulting mixture was stirred at room temperature overnight. After completion of starting material checked with TLC, the mixture was washed with sat. $NaHCO_3$ (25 mL), brine (25 mL) and dried with Na_2SO_4. The crude alcohol was concentrated and purified by column chromatography to afford pure **16** (eluent system: 50% EA in petroleum, 50 mg, 49%). ^{1}H NMR (300 MHz, *CDCl$_3$*) *δ* ppm 4.54 (br. s., 1 H, OH), 4.78 (s, 2 H, CH_2), 7.18 - 7.29 (m, 2 H, Py), 7.66 - 7.78 (m, 2 H, Py), 8.21 (d, *J* = 7.9 Hz, 1 H, Py), 8.27 - 8.34 (m, 1 H, Py), 8.57 - 8.65 (m, 1 H, Py). ^{13}C NMR (75 MHz, *CDCl$_3$*) *δ* ppm 64.12 (1 C, CH_2), 119.48 (1 C, Py), 120.32 (1 C, Py), 120.96 (1 C, Py), 123.65 (1 C, Py), 136.79 (1 C, Py), 137.45 (1 C, Py), 148.99 (1 C, Py), 154.62 (1 C, Py), 155.51 (1 C, Py), 158.65 (1 C, Py). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{11}H_{10}N_2O + H]^+$ 187.0866, found 187.0855.

*1-(1-([2,2'-Bipyridin]-6-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**17**)* To a solution of **16** (50 mg, 0.27 mmol) in CH_2Cl_2 were added methanesulfonyl chloride (25 µl, 0.32 mmol) and triethylamine (58 µl, 0.42 mmol) and the resulting solution was stirred at room temperature for 1 h. The reaction mixture was washed with 1 M aq. HCl (25 mL), sat. $NaHCO_3$ (25 mL) and brine (25 mL), and dried over Na_2SO_4. The concentrated residue, **5** (0.11 g, 0.34

mmol) and K_2CO_3 (63 mg, 0.46 mmol) were dissolved in ethanol (20 mL) and the solution was refluxed for 2 h. The reaction mixture was concentrated *in vacuo*, and the residue was washed with 1 M aq. HCl (25 mL), sat. $NaHCO_3$ (25 mL) and brine (25 mL), and dried over Na_2SO_4. After solvent evaporation *in vacuo*, the BOM protected intermediate was treated with TFA (4.0 mL) at 73 °C for 4 h to yield **17** (eluent system: 7% methanol in CH_2Cl_2, 26 mg, 26%). ^{1}H NMR (300 MHz, *DMSO-d_6*) δ ppm 1.60 - 1.71 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 1.75 (s, 3 H, 5-CH_3), 1.88 (qd, *J* = 12.1, 3.4 Hz, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.19 (t, *J* = 10.8 Hz, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.98 (d, *J* = 11.7 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.71 (s, 2 H, $N_{piperidyl}CH_2$), 4.20 - 4.34 (m, 1 H, piperidin-4-yl), 7.42 (ddd, *J* = 7.6, 4.7, 1.2 Hz, 1 H, Py), 7.50 (dd, *J* = 7.8, 1.0 Hz, 1 H, Py), 7.64 (d, *J* = 1.2 Hz, 1 H, H-6), 7.86 - 7.97 (m, 2 H, Py), 8.25 (dd, *J* = 7.9, 0.9 Hz, 1 H, Py), 8.36 (dt, *J* = 8.0, 1.1 Hz, 1 H, Py), 8.62 - 8.68 (m, 1 H, Py), 11.17 (br. s., 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d_6*) δ ppm 12.3 (1 C, 5-CH_3), 30.3 (2 C, piperidin-3-yl, piperidin-5-yl), 52.6 (1 C, piperidin-4-yl), 52.9 (2 C, piperidin-2-yl, piperidin-6-yl), 63.8 (1 C, $N_{piperidyl}CH_2$), 109.2 (1 C, C-5), 119.0 (1 C, Py), 120.7 (1 C, Py), 123.3 (1 C, Py), 124.4 (1 C, Py), 137.5 (1 C, Py), 137.9 (1 C, Py), 138.0 (1 C, C-6), 149.5 (1 C, Py), 151.1 (1 C, C-2), 154.8 (1 C, Py), 155.5 (1 C, Py), 158.7 (1 C, Py), 164.0 (1 C, C-4). HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{21}H_{23}N_5O_2$ + H]$^+$ 378.1925, found 378.1385.

*2-((2-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)oxy)ethyl 3,4-dihydroxybenzoate (**18**)* 3,4-Bis(benzyloxy)benzoyl chloride was synthesized according to the literature [54]. To a solution of **9** (0.10 g, 0.24 mmol) and 4-methylatemorpholine (32 µl, 0.29 mmol) in CH_2Cl_2 (10 mL) was added 3,4-bis(benzyloxy)benzoyl chloride (90 mg, 0.26 mmol) and the resulting solution was stirred for 1 h. The reaction mixture was quenched with water and washed with 1 M aq. HCl (25 mL), sat. $NaHCO_3$ (25 mL) and brine (25 mL), dried with Na_2SO_4, followed by concentration to give the intermediate. The BOM protected intermediated and Pd/C were dissolved in ethanol (10 mL) and the mixture was stirred at room temperature under a hydrogen balloon overnight to give **18** (eluent system: 7% methanol in CH_2Cl_2, 28 mg, 21%). ^{1}H NMR (300 MHz, *METHANOL-d_4*) δ ppm 1.89 (s, 3 H, 5-CH_3), 1.97 - 2.10 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 2.25 - 2.47 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 3.11 (t, *J* = 11.9 Hz, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.54 - 3.68 (m, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.43 (br. s., 2 H, $N_{piperidyl}CH_2$), 4.53 - 4.64

(m, 1 H, piperidin-4-yl), 4.68 (br. s., 2 H, O$\underline{CH_2}$), 4.79 (s, 2 H, O$\underline{CH_2}$), 6.76 (d, *J* = 8.2 Hz, 1 H, Ph), 7.19 (s, 1 H, quinolin-3-yl), 7.35 - 7.44 (m, 2 H, Ph), 7.49 (s, 1 H, H-6), 7.59 (t, *J* = 8.2 Hz, 1 H, Ph), 7.79 (t, *J* = 7.6 Hz, 1 H, Ph), 8.02 (d, *J* = 8.5 Hz, 1 H, Ph), 8.24 (d, *J* = 8.2 Hz, 1 H, Ph). ^{13}C NMR (75 MHz, *DMSO-d$_6$*) *δ* ppm 12.49 (1 C, 5-CH_3), 27.07 (2 C, piperidin-3-yl, piperidin-5-yl), 50.65 (1 C, piperidin-4-yl), 52.01 (2 C, piperidin-2-yl, piperidin-6-yl), 62.45 (2 C, $N_{piperidyl}\underline{CH_2}$, O$\underline{CH_2}$), 67.54 (1 C, O$\underline{CH_2}$), 101.99 (1 C, quinolin-3-yl), 109.62 (1 C, C-5), 115.64 (1 C, Ph), 116.66 (1 C, Ph), 120.34 (1 C, Ph), 120.54 (1 C, Ph), 121.88 (1 C, Ph), 122.23 (1 C, Ph), 126.81 (1 C, Ph), 128.50 (1 C, Ph), 130.92 (1 C, Ph), 137.53 (1 C, C-6), 145.42 (1 C, Ph), 147.98 (1 C, Ph), 150.97 (1 C, Ph), 151.05 (1 C, C-2), 161.84 (1 C, quinolin-4-yl), 163.98 (1 C, C-4), 165.96 (1 C, $\underline{CO}$(Ph)), C(quinolin-2-yl) could not be observed. HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{29}H_{30}N_4O_7 + H]^+$ 547.2187, found 547.2191.

*2-((2-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)amino)ethyl 3,4-dihydroxybenzoate (**19**)* Following the same procedure as decribed for **18**, using **10** (0.10 g, 0.24 mmol), 4-methylatemorpholine (32 µl, 0.29 mmol) and 3,4-bis(benzyloxy)benzoyl chloride (90 mg, 0.26 mmol) as material yielded the intermediate. The BOM protected intermediated and Pd/C were dissolved in ethanol (10 mL) and the resulting solution was stirred at room temperature under a hydrogen balloon overnight to give **19** (eluent system: 7% methanol in CH_2Cl_2, 29 mg, 21%). ^{1}H NMR (300 MHz, *DMSO-d$_6$*) *δ* ppm 1.59 - 1.70 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 1.74 (s, 3 H, 5-CH_3), 1.82 - 2.01 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.16 - 2.30 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.97 (d, *J* = 11.4 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.69 (br. s., 2 H, $N_{piperidyl}\underline{CH_2}$), 3.74 - 3.86 (m, 2 H, N$\underline{CH_2}$), 4.20 - 4.34 (m, 1 H, piperidin-4-yl), 4.47 (t, *J* = 5.0 Hz, 2 H, O$\underline{CH_2}$), 6.76 (d, *J* = 8.2 Hz, 1 H, Ph), 6.84 (s, 1 H, quinolin-3-yl), 7.22 - 7.35 (m, 2 H, Ph), 7.47 - 7.59 (m, 2 H, H-6, Ph), 7.72 (t, *J* = 6.7 Hz, 1 H, Ph), 7.88 (d, *J* = 8.8 Hz, 1 H, Ph), 8.31 (d, *J* = 7.9 Hz, 1 H, Ph), 9.32 (s, 1 H, NH), 9.85 (s, 2 H, OH), 11.20 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d$_6$*) *δ* ppm 12.38 (1 C, 5-CH_3), 29.54 (2 C, piperidin-3-yl, piperidin-5-yl), 42.47 (1 C, N$\underline{CH_2}$), 52.23 (1 C, piperidin-4-yl), 52.62 (2 C, piperidin-2-yl, piperidin-6-yl), 58.55 (1 C, $N_{piperidyl}\underline{CH_2}$), 62.15 (1 C, O$\underline{CH_2}$), 98.52 (1 C, quinolin-3-yl), 109.30 (1 C, C-5), 115.47 (1 C, Ph), 116.58 (1 C, Ph), 116.73 (1 C, Ph), 120.47 (1 C, Ph), 120.92 (1 C, Ph), 122.22 (1 C, Ph), 123.09 (1 C, Ph), 126.70 (1 C, Ph), 133.60 (1 C, Ph), 137.68 (1 C, C-6), 138.82 (1 C, Ph), 145.33 (1 C, Ph), 150.90 (1 C, Ph), 151.05 (1 C, C-

2), 155.84 (1 C, quinolin-4-yl), 163.93 (1 C, C-4), 165.95 (1 C, COPh), C (quinolin-2-yl) could not be observed. HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{29}H_{31}N_5O_6$ + H]$^+$ 546.2347, found 546.2248.

*4-((2-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)oxy)phenyl 3,4-dihydroxybenzoate (**20**)* 3,4-Bis((tert-butyldimethylsilyl)oxy)benzoic acid was synthesized accoding to the literature [55]. To a solution of **7** (0.24 g, 0.52 mmol) and 3,4-bis((tert-butyldimethylsilyl)oxy)benzoic acid (0.21 g, 0.55 mmol) in CH_2Cl_2 (50 mL) were added EDC·HCl (0.20 g, 1.0 mmol) and DMAP (6.4 mg, 0.052 mmol) and the resulting solution was stirred at room temperature overnight to yield the imtermediate (0.14 g, 0.17 mmol), which was treated with HF·Pyridine (0.33 mL, 0.96 mL/mmol TBDMS) in pyridine (0.33 mL, 0.96 mL/mmol TBDMS)/THF (0.68 mL, 2.0 mL/mmol TBDMS) to give **20** (eluent system: 5% methanol in CH_2Cl_2, 48 mg, 16%). ^{1}H NMR (300 MHz, *DMSO-d_6*) *δ* ppm 1.55 - 1.81 (m, 7 H, 5-CH_3, piperidin-3-yl, piperidin-5-yl), 2.07 - 2.19 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.83 - 2.93 (m, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.68 (s, 2 H, $N_{piperidyl}CH_2$), 4.18 - 4.31 (m, 1 H, piperidin-4-yl), 6.84 (s, 1 H, quinolin-3-yl), 6.86 - 6.91 (m, 1 H, Ph), 7.32 - 7.43 (m, 4 H, Ph), 7.45 - 7.53 (m, 3 H, H-6, Ph), 7.61 (ddd, *J* = 8.2, 6.9, 1.0 Hz, 1 H, Ph), 7.79 (ddd, *J* = 8.3, 6.9, 1.5 Hz, 1 H, Ph), 7.98 (d, *J* = 8.2 Hz, 1 H, Ph), 8.26 (dd, *J* = 8.2, 0.9 Hz, 1 H, Ph), 9.75 (s, 2 H, OH), 11.17 (br. s., 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d_6*) *δ* ppm 12.03 (1 C, 5-CH_3), 29.97 (2 C, piperidin-3-yl, piperidin-5-yl), 52.15 (1 C, piperidin-4-yl), 52.56 (2 C, piperidin-2-yl, piperidin-6-yl), 63.93 (1 C, $N_{piperidyl}CH_2$), 103.88 (1 C, quinolin-3-yl), 108.93 (1 C, C-5), 115.51 (1 C, Ph), 116.78 (1 C, Ph), 119.28 (1 C, Ph), 120.06 (1 C, Ph), 121.38 (1 C, Ph), 121.65 (2 C, Ph), 122.75 (1 C, Ph), 124.00 (2 C, Ph), 126.11 (1 C, Ph), 128.50 (1 C, Ph), 130.35 (1 C, Ph), 137.36 (1 C, C-6), 145.34 (1 C, Ph), 147.95 (1 C, Ph), 148.62 (1 C, Ph), 150.79 (1 C, Ph), 151.40 (1 C, C-2), 151.57 (1 C, Ph), 160.88 (1 C, quinolin-2-yl), 161.25 (1 C, quinolin-4-yl), 163.63 (1 C, COPh), 164.54 (1 C, C-4). HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{33}H_{30}N_4O_7$ + H]$^+$ 595.2187, found 595.2189.

*N-hydroxy-4-((2-((4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)oxy)benzamide (**21**)* To a solution of **8** (0.10 g, 0.21 mmol) and O-benzylhydroxylamine hydrochloride (34 mg, 0.21 mmol) in CH_2Cl_2 (50 mL) were added EDC·HCl (79 mg, 0.41 mmol) and DMAP (2.5 mg, 0.020 mmol) and the resulting solution was

stirred at room temperature overnight to yield the intermediate (60 mg, 0.10 mmol). The intermediate and Pd/C were dissolved in ethanol and the mixture was stirred under hydrogen to give **21** (eluent system: 8% methanol in CH_2Cl_2, 21 mg, 20%). ^{1}H NMR (300 MHz, *DMSO-d_6*) *δ* ppm 1.47 - 1.92 (m, 7 H, 5-CH_3, piperidin-3-yl, piperidin-5-yl), 2.01 - 2.21 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.89 (d, *J* = 10.8 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.70 (s, 2 H, $N_{piperidyl}CH_2$), 4.16 - 4.30 (m, 1 H, piperidin-4-yl), 6.91 (s, 1 H, quinolin-3-yl), 7.27 - 7.37 (m, 2 H, Ph), 7.54 (s, 1 H, H-6), 7.61 (ddd, *J* = 8.2, 6.9, 1.0 Hz, 1 H, Ph), 7.80 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1 H, Ph), 7.86 - 7.92 (m, 2 H, Ph), 8.00 (d, *J* = 7.9 Hz, 1 H, Ph), 8.17 (d, *J* = 8.2 Hz, 1 H, Ph), 9.06 (s, 1 H, NHOH), 11.16 (s, 1 H, NH), 11.28 (br. s., 1 H, NHOH). ^{13}C NMR (75 MHz, *DMSO-d_6*) *δ* ppm 11.97 (1 C, 5-CH_3), 29.85 (2 C, piperidin-3-yl, piperidin-5-yl), 52.26 (1 C, piperidin-4-yl), 52.59 (2 C, piperidin-2-yl, piperidin-6-yl), 63.99 (1 C, $N_{piperidyl}CH_2$), 105.47 (1 C, quinolin-3-yl), 108.95 (1 C, C-5), 115.04 (1 C, Ph), 119.71 (2 C, Ph), 120.29 (1 C, Ph), 121.33 (1 C, Ph), 126.32 (1 C, Ph), 128.61 (1 C, Ph), 129.37 (1 C, Ph), 129.50 (1 C, Ph), 130.44 (1 C, Ph), 137.56 (1 C, C-6), 148.73 (1 C, Ph), 150.78 (1 C, C-2), 156.93 (1 C, Ph), 160.13 (1 C, CONH), 160.87 (1 C, quinolin-2-yl), 163.64 (1 C, C-4), C (quinolin-4-yl) could not be observed. HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{27}H_{27}N_5O_5 + H]^+$ 502.2085, found 502.2238.

*Methyl (E)-2-(2-hydroxy-4-((2-((4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)quinolin-4-yl)oxy)benzylidene)hydrazine-1-carbodithioate (**22**)* Following General procedure 2, **3** (0.14 g, 0.28 mmol) and 2,4-dihydroxybenzaldehyde (57 mg, 0.42 mmol) in EA (2.0 mL) afforded the protected intermediate. The intermediate (0.10 g, 0.16 mmol) was heated in TFA (2.0 mL) at 73 ℃ for 4 h, followed by concentration *in vacuo* to give the deprotected compound, which was further refluxed with methyl hydrazinecarbodithioate (5.0 mg, 0.041 mmol, synthesized according to literature [42]) in methanol (20 mL) to yield **22** (eluent system: 5% methanol in CH_2Cl_2, 9.4 mg, 6%). ^{1}H NMR (300 MHz, *DMSO-d_6*) *δ* ppm 1.68 - 1.94 (m, 5 H, 5-CH_3, piperidin-3a-yl, piperidin-5a-yl), 2.03 - 2.25 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.53 (s, 3 H, SCH_3), 4.32 - 4.65 (m, 3 H, piperidin-4-yl, $N_{piperidyl}CH_2$), 6.79 - 6.91 (m, 2 H, Ph), 7.01 (s, 1 H, quinolin-3-yl), 7.46 (s, 1 H, H-6), 7.71 (t, *J* = 7.5 Hz, 1 H, Ph), 7.80 - 7.97 (m, 2 H, Ph), 8.10 (d, *J* = 8.5 Hz, 1 H, Ph), 8.28 (d, *J* = 8.2 Hz, 1 H, Ph), 8.57 (s, 1 H, CHN), 10.86 (s, 1 H, OH), 11.28 (s, 1 H, CONH), 13.36 (br. s., 1 H, NHCS). H (piperidin-2-yl, piperidin-6-yl) were hidden in the broad solvent peak. ^{13}C NMR (75 MHz, *DMSO-d_6*) *δ* ppm 12.15 (1 C, 5-CH_3),

16.76 (1 C, SCH_3), 26.95 (2 C, piperidin-3-yl, piperidin-5-yl), 50.18 (1 C, piperidin-4-yl), 51.62 (2 C, piperidin-2-yl, piperidin-6-yl), 59.23 (1 C, $N_{piperidyl}\underline{C}H_2$), 105.57 (1 C, quinolin-3-yl), 108.08 (1 C, Ph), 109.23 (1 C, C-5), 111.97 (1 C, Ph), 117.16 (1 C, Ph), 120.19 (1 C, Ph), 121.57 (1 C, Ph),127.12 (1 C, Ph), 128.53 (1 C, Ph), 128.83 (1 C, Ph), 131.05 (1 C, Ph), 137.20 (1 C, C-6), 143.27 (1 C, CHN), 148.51 (1 C, Ph), 150.71 (1 C, C-2), 156.93 (1 C, Ph), 158.94 (1 C, COH), 160.88 (1 C, quinolin-2-yl), 163.61 (1 C, C-4), 197.34 (1 C, CS), C (quinolin-4-yl) could not be observed. HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{29}H_{30}N_6O_4S_2 + H]^+$ 591.1843, found 591.1840.

*1-(1-(6-Chloro-2-ethylimidazo[1,2-a]pyridine-3-carbonyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**23**)* 6-Chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylic acid was synthesied accordign to a literature procedure [32]. To a solution of 6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylic acid (0.30 g, 1.3 mmol) and **5** (0.29 g, 0.89 mmol) in DMF (10 mL) were added EDC·HCl (0.34 g, 1.78 mmol), hydroxybenzotriazole (60 mg, 0.44 mmol) and triethylamine (0.25 mL, 1.8 mmol) and the resulting solution was heated at 70 ℃ for 4 h to give the BOM protected amide, which was heated at 73 ℃ in TFA (4.0 mL) for 4 h to yield **23** (eluent system: 5% methanol in CH_2Cl_2, 79 mg, 21%). ^{1}H NMR (300 MHz, *DMSO-d_6*) *δ* ppm 1.28 (t, *J* = 7.5 Hz, 3 H, $CH_2\underline{CH_3}$), 1.73 - 1.98 (m, 7 H, 5-CH_3, piperidin-3-yl, piperidin-5-yl), 2.77 (q, *J* = 7.5 Hz, 2 H, $\underline{CH_2}CH_3$), 3.09 - 3.23 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 4.18 (d, *J* = 12.6 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.50 - 4.61 (m, 1 H, piperidin-4-yl), 7.46 (dd, *J* = 9.7, 2.1 Hz, 1 H, Py), 7.50 (s, 1 H, H-6), 7.66 (d, *J* = 9.4 Hz, 1 H, Py), 8.59 (s, 1 H, Py), 10.92 (br. s., 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d_6*) *δ* ppm 12.09 (1 C, 5-CH_3), 12.78 (1 C, $CH_2\underline{CH_3}$), 20.44 (1 C, $\underline{CH_2}CH_3$), 30.0 (2 C, piperidin-3-yl, piperidin-5-yl), 43.97 (2 C, piperidin-2-yl, piperidin-6-yl), 51.78 (1 C, piperidin-4-yl), 108.99 (1 C, C-5), 116.12 (1 C, Py), 120.67 (1 C, (Cl)Py), 124.90 (1 C, Py), 128.71 (1 C, Py), 137.47 (1 C, C-6), 142.21 (1 C, Py), 146.31 (1 C, $(CH_2)\underline{C}_{imidazole}$), 150.74 (1 C, C-2), 159.79 (1 C, CO), 163.64 (1 C, C-4), C ($\underline{C}_{imidazole}$(CO)) could not be observed. HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{20}H_{22}ClN_5O_3 + H]^+$ 416.1484, found 416.1474.

*4-(4-(3-((Benzyloxy)methyl)-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)benzonitrile (**24**)* **24** was synthesized according to a literature reported procedure [32]. To a solution of **5** (0.50 g, 1.5 mmol) and 4-fluorobenzonitrile (0.20 g, 1.7 mmol) in DMSO was added K_2CO_3 (0.42 g, 3.0 mmol) and the resulting solution was heated at 120 ℃ overnight. After cooling down to room temperature, water (20 mL) was addded to the reaction mixture, followed

by filtration. The cake was collected and purified to yield **24** (eluent system: 50% EA in petroleum ether, 0.27 g, 41%). ^{1}H NMR (300 MHz, *CDCl$_3$*) *δ* ppm 1.76 - 2.00 (m, 7 H, 5-CH$_3$, piperidin-3-yl, piperidin-5-yl), 2.97 - 3.11 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 4.00 (d, *J* = 13.2 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.68 - 4.84 (m, 3 H, (Ph)CH$_2$, piperidin-4-yl), 5.53 (s, 2 H, N^{3}CH$_2$), 6.87 - 6.98 (m, 3 H, Ph, H-6), 7.22 - 7.42 (m, 5 H, Ph), 7.49 - 7.56 (m, 2 H, Ph). ^{13}C NMR (75 MHz, *CDCl$_3$*) *δ* ppm 13.25 (1 C, 5-CH$_3$), 30.04 (2 C, piperidin-3-yl, piperidin-5-yl), 47.50 (2 C, piperidin-2-yl, piperidin-6-yl), 53.13 (1 C, piperidin-4-yl), 70.87 (1 C, N^{3}CH$_2$), 72.28 (1 C, (Ph)CH$_2$), 101.04 (1 C, Ph), 110.51 (1 C, C-5), 114.88 (2 C, Ph), 119.69 (1 C, CN), 127.61 (3 C, Ph), 128.25 (2 C, Ph), 133.67 (2 C, Ph), 134.45 (1 C, C-6), 137.98 (1 C, Ph), 151.47 (1 C, C-2), 152.37 (1 C, Ph), 163.09 (1 C, C-4). HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{25}H_{26}N_4O_3$ + H]$^+$ 431.2078, found 431.2096.

*4-((4-(3-((Benzyloxy)methyl)-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)benzonitrile (**25**)* To a solution of 4-formylbenzonitrile (0.30 g , 2.3 mmol) and **5** (0.50 g, 1.5 mmol) in 1,2-dichloroethane (20 mL) was added sodium triacetoxyborohydride (0.65 g, 3.1 mmol) and the resulting solution was stirred at room temperature overnight. The reaction mixture was washed with sat. NaHCO$_3$ (50 mL), brine (50 mL), and dreid over Na$_2$SO$_4$. The concentrated residue was purified by column chromatography to yield **25** (eluent system: 50% EA in petroleum ether, 0.32 g, 46%). ^{1}H NMR (300 MHz, *CDCl$_3$*) *δ* ppm 1.79 - 1.94 (m, 7 H, 5-CH$_3$, piperidin-3-yl, piperidin-5-yl), 2.16 - 2.31 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.98 (d, *J* = 11.7 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.61 (s, 2 H, N$_{piperidyl}$CH$_2$), 4.49 - 4.63 (m, 1 H, piperidin-4-yl), 4.71 (s, 2 H, (Ph)CH$_2$), 5.51 (s, 2 H, N^{3}CH$_2$), 7.05 (d, *J* = 1.2 Hz, 1 H, H-6), 7.22 - 7.38 (m, 5 H, Ph), 7.48 (d, *J* = 8.2 Hz, 2 H, Ph), 7.58 - 7.67 (m, 2 H, Ph). ^{13}C NMR (75 MHz, *CDCl$_3$*) *δ* ppm 13.22 (1 C, 5-CH$_3$), 30.57 (2 C, piperidin-3-yl, piperidin-5-yl), 52.68 (2 C, piperidin-2-yl, piperidin-6-yl), 52.98 (1 C, piperidin-4-yl), 61.91 (1 C, N$_{piperidyl}$CH$_2$), 70.81 (1 C, N^{3}CH$_2$), 72.20 (1 C, (Ph)CH$_2$), 110.12 (1 C, C-5), 111.15 (1 C, Ph), 118.73 (1 C, CN), 127.55 (3 C, Ph), 128.19 (2 C, Ph), 129.44 (2 C, Ph), 132.16 (2 C, Ph), 134.83 (1 C, C-6), 137.98 (1 C, Ph), 143.45 (1 C, Ph), 151.47 (1 C, C-2), 163.16 (1 C, C-4). HRMS (ESI): m/z [M + H]$^+$ Calcd. for [$C_{26}H_{28}N_4O_3$ + H]$^+$ 445.2234, found 445.2187.

*6-Chloro-2-ethyl-N-(4-(4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)benzyl)imidazo[1,2-a]pyridine-3-carboxamide (**26**)* **24** (0.50 g, 1.2 mmol) was heated in TFA

(8.0 mL) at 73 ℃ for 4 h, follwed by evaporation *in vacuo* to remove the volatile. According to a literature [56], the nitrile compound (0.30 g, 0.96 mmol) and $CoCl_2$ (13 mg, 0.096 mmol) were dissolved in THF/water (4.0 mL/2.0 mL) at 0 ℃ with ice bath. $NaBH_4$ (73 mg, 1.9 mmol) was added in 8 min and the mixture kept stirring for 2 h. The reaction mixture was filtered through celite to remove the dark solid, and the filtrate was concentrated *in vacuo* to obtain oil, which was dried on oil pump overnight. The oil amine and 6-chloro-2-ethylimidazo[1,2-a]pyridine-3-carboxylic acid (0.32 g, 1.4 mmol) were dissolved in DMF (20 mL), followed by addition of EDC·HCl (0.37 g, 1.9 mmol), hydroxybenzotriazole (65 mg, 0.48 mmol) and triethylamine (0.27 mL, 1.9 mmol). After being heated at 70 ℃ for 4 h, the reaction mixture was evaporated *in vacuo* and purified to give **26** (eluent system: 5% methanol in CH_2Cl_2, 0.17 g, 28%). ^{1}H NMR (300 MHz, *DMSO-d_6*) *δ* ppm 1.24 (t, *J* = 7.5 Hz, 3 H, $\underline{CH_3}CH_2$), 1.67 - 1.84 (m, 5 H, 5-CH_3, piperidin-3a-yl, piperidin-5a-yl), 1.84 - 2.00 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.65 - 2.82 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.96 (q, *J* = 7.4 Hz, 2 H, $CH_3\underline{CH_2}$), 3.77 (d, *J* = 12.6 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.33 - 4.47 (m, 3 H, Ph$\underline{CH_2}$, piperidin-4-yl), 6.93 (d, *J* = 8.8 Hz, 2 H, Ph), 7.22 (d, *J* = 8.5 Hz, 2 H, Ph), 7.43 (dd, *J* = 9.5, 2.2 Hz, 1 H, Py), 7.60 - 7.67 (m, 2 H, H-6, Py), 8.39 (t, *J* = 5.9 Hz, 1 H, $CH_2\underline{NH}$), 9.05 (dd, *J* = 2.2, 0.7 Hz, 1 H, Py), 11.20 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d_6*) *δ* ppm 12.00 (1 C, 5-CH_3), 13.09 (1 C, $\underline{CH_3}CH_2$), 21.85 (1 C, $CH_3\underline{CH_2}$), 29.80 (2 C, piperidin-3-yl, piperidin-5-yl), 41.92 (1 C, Ph$\underline{CH_2}$), 48.61 (2 C, piperidin-2-yl, piperidin-6-yl), 52.42 (1 C, piperidin-4-yl), 108.96 (1 C, C-5), 115.85 (1 C, $\underline{C}_{imidazole}$(CO)), 116.06 (2 C, Ph), 117.18 (1 C, Py), 119.65 (1 C, (Cl)Py), 124.81 (1 C, Py), 127.02 (1 C, Py), 128.26 (2 C, Ph), 129.68 (1 C, Ph), 137.68 (1 C, C-6), 143.25 (1 C, Py), 149.81 (1 C, Ph), 150.84 (1 C, $(CH_2)\underline{C}_{imidazole}$), 150.96 (1 C, C-2), 160.44 (1 C, CONH), 163.69 (1 C, C-4). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{27}H_{29}ClN_6O_3 + H]^+$ 521.2063, found 521.2056.

*N-(4-(4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)benzyl)-3,5-dinitrobenzamide (**27**)* Following the same procedure as **26**, **24** (0.30 g, 0.70 mmol) was used as staring material. After being heated in TFA (4.0 mL) at 73 ℃ for 4 h, the deprotected intermediate and $CoCl_2$ (5.86 mg, 0.045 mmol) were dissolved in THF/water (4.0 mL/2.0 mL) with ice bath, followed by addition of $NaBH_4$ (34 mg, 0.90 mmol) and the resulting mixture kept stirring for 2 h. The reaction mixture was filtered through celite to remove the dark solid, and the filtrate was concentrated *in vacuo* to obtain oil, which was dried on oil pump overnight. To

a solution of dried amine and 3.5-dinitrobenzoic acid (0.14 g, 0.67 mmol) in DMF (10 mL) were added EDC·HCl (0.17 g, 0.89 mmol), hydroxybenzotriazole (30 mg, 0.22 mmol) and triethylamine (0.13 mL, 0.89 mmol). After being heated at 70 ℃ for 4 h, the reaction mixture was evaporated *in vacuo* and purified to give **27** (eluent system: 5% methanol in CH_2Cl_2, 58 mg, 16%). ^{1}H NMR (300 MHz, *DMSO-d*$_6$) *δ* ppm 1.72 - 1.80 (m, 5 H, 5-CH_3, piperidin-3a-yl, piperidin-5a-yl), 1.84 - 1.99 (m, 2 H, piperidin-3b-yl, piperidin-5b-yl), 2.69 - 2.79 (m, 2 H, piperidin-2a-yl, piperidin-6a-yl), 3.77 (d, *J* = 12.6 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 4.33 - 4.49 (m, 3 H, piperidin-4-yl, PhCH_2), 6.94 (d, *J* = 8.5 Hz, 2 H, Ph), 7.21 (d, *J* = 8.8 Hz, 2 H, Ph), 7.62 (d, *J* = 0.9 Hz, 1 H, H-6), 8.94 (t, *J* = 2.2 Hz, 1 H, Ph), 9.07 (d, *J* = 2.1 Hz, 2 H, Ph), 9.63 (t, *J* = 5.7 Hz, 1 H, CH_2NH), 11.20 (s, 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d*$_6$) *δ* ppm 12.00 (1 C, 5-CH_3), 29.76 (2 C, piperidin-3-yl, piperidin-5-yl), 42.73 (1 C, Ph$\underline{CH_2}$), 48.61 (2 C, piperidin-2-yl, piperidin-6-yl), 52.44 (1 C, piperidin-4-yl), 108.97 (1 C, C-5), 116.08 (2 C, Ph), 120.84 (1 C, Ph), 127.54 (2 C, Ph), 128.57 (2 C, Ph), 128.99 (1 C, Ph), 136.90 (1 C, Ph), 137.68 (1 C, C-6), 148.21 (2 C, Ph), 150.01 (1 C, Ph), 150.84 (1 C, C-2), 161.90 (1 C, CONH), 163.69 (1 C, C-4). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{24}H_{24}N_6O_7 + H]^+$ 509.1779, found 509.1778.

*N-(4-((4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)piperidin-1-yl)methyl)benzyl)-3,5-dinitrobenzamide (**28**)* Following the same procedure as **26**, **25** (0.40 g, 0.90 mmol) was used as staring material. After being heated in TFA (4.0 mL) at 73 ℃ for 4 h, the deprotected intermediate and $CoCl_2$ (10 mg, 0.077 mmol) were dissolved in THF/water (4.0 mL/2.0 mL) with ice bath, followed by addition of $NaBH_4$ (58 mg, 1.5 mmol) and the resulting solution kept stirring for 2 h. The reaction mixture was filtered through celite to remove the dark solid, and the filtrate was concentrated *in vacuo* to obtain oil, which was dried on oil pump overnight. To a solution of dried amine and 3.5-dinitrobenzoic acid (97 mg, 0.46 mmol) in DMF (10 mL) were added EDC·HCl (0.12 g, 0.60 mmol), hydroxybenzotriazole (20 mg, 0.15 mmol) and triethylamine (85 μl, 0.61 mmol). After being heated at 70 ℃ for 4 h, the reaction mixture was evaporated *in vacuo* and purified to give **28** (eluent system: 5% methanol in CH_2Cl_2, 47 mg, 10%). ^{1}H NMR (300 MHz, *DMSO-d*$_6$) *δ* ppm 1.56 - 1.68 (m, 2 H, piperidin-3a-yl, piperidin-5a-yl), 1.72 - 1.85 (m, 5 H, 5-CH_3, piperidin-3b-yl, piperidin-5b-yl), 2.01 (t, *J* = 10.8 Hz, 2 H, piperidin-2a-yl, piperidin-6a-yl), 2.87 (d, *J* = 11.1 Hz, 2 H, piperidin-2b-yl, piperidin-6b-yl), 3.44 (s, 2 H, $N_{piperidyl}CH_2$), 4.17 - 4.28 (m, 1 H, piperidin-4-yl), 4.52 (d, *J* = 5.6 Hz, 2 H, Ph$\underline{CH_2}$), 7.21 - 7.34 (m, 4 H, Ph), 7.60

(d, *J* = 1.2 Hz, 1 H, H-6), 8.94 (t, *J* = 2.1 Hz, 1 H, Ph), 9.05 - 9.10 (m, 2 H, Ph), 9.70 (t, *J* = 5.9 Hz, 1 H, CH_2NH), 11.15 (br. s., 1 H, NH). ^{13}C NMR (75 MHz, *DMSO-d*$_6$) *δ* ppm 11.99 (1 C, 5-CH_3), 29.97 (2 C, piperidin-3-yl, piperidin-5-yl), 42.98 (1 C, PhCH_2), 52.30 (2 C, piperidin-2-yl, piperidin-6-yl), 52.39 (1 C, piperidin-4-yl), 61.42 (1 C, $N_{piperidyl}CH_2$), 108.90 (1 C, C-5), 120.90 (1 C, Ph), 127.43 (2 C, Ph), 127.57 (2 C, Ph), 128.90 (2 C, Ph), 136.78 (1 C, Ph), 137.34 (2 C, Ph), 137.68 (1 C, C-6), 148.21 (2 C, Ph), 150.79 (1 C, C-2), 162.09 (1 C, CONH), 163.66 (1 C, C-4). HRMS (ESI): m/z $[M + H]^+$ Calcd. for $[C_{25}H_{26}N_6O_7 + H]^+$ 523.1936, found 523.1956.

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References

[1] WHO, Global tuberculosis report 2019, Geneva, Switzerland, (2019).

[2] K. Dheda, T. Gumbo, G. Maartens, et al., The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis, Lancet Respir. Med. 5 (2017) 291-360. https://doi.10.1016/s2213-2600(17)30079-6.

[3] H. Hoffmann, T.A. Kohl, S. Hofmann-Thiel, et al., Delamanid and bedaquiline resistance in *Mycobacterium tuberculosis* ancestral Beijing genotype causing extensively drug-resistant tuberculosis in a Tibetan refugee, Am. J. Respir. Crit. Care Med. 193 (2016) 337-340. https://doi.10.1164/rccm.201502-0372LE.

[4] Food and Drug Administration, *FDA approved drug products*. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=212862> (accessed on 14 August 2019).

[5] E.O. Johnson, E. LaVerriere, E. Office, et al., Large-scale chemical-genetics yields new

M. tuberculosis inhibitor classes, Nature. (2019). https://doi.10.1038/s41586-019-1315-z.

[6] S. Van Calenbergh, S. Pochet, H. Munier-Lehmann, Drug design and identification of potent leads against *mycobacterium tuberculosis* thymidine monophosphate kinase, Curr Top Med Chem. 12 (2012) 694-705. https://doi.10.2174/156802612799984580.

[7] I. Li de la Sierra, H. Munier-Lehmann, A.M. Gilles, et al., X-ray structure of TMP kinase from *Mycobacterium tuberculosis* complexed with TMP at 1.95 A resolution, J. Mol. Biol. 311 (2001) 87-100. https://doi.10.1006/jmbi.2001.4843.

[8] V. Vanheusden, P. Van Rompaey, H. Munier-Lehmann, et al., Thymidine and thymidine-5'-O-monophosphate analogues as inhibitors of *Mycobacterium tuberculosis* thymidylate kinase, Bioorg. Med. Chem. Lett. 13 (2003) 3045-3048. https://doi.10.1016/s0960-894x(03)00643-7.

[9] V. Vanheusden, H. Munier-Lehmann, M. Froeyen, et al., 3'-C-branched-chain-substituted nucleosides and nucleotides as potent inhibitors of *Mycobacterium tuberculosis* thymidine monophosphate kinase, J. Med. Chem. 46 (2003) 3811-3821. https://doi.10.1021/jm021108n.

[10] S. Van Poecke, H. Munier-Lehmann, O. Helynck, et al., Synthesis and inhibitory activity of thymidine analogues targeting *Mycobacterium tuberculosis* thymidine monophosphate kinase, Bioorg. Med. Chem. 19 (2011) 7603-7611. https://doi.10.1016/j.bmc.2011.10.021.

[11] L. Song, M.D.P. Risseeuw, M. Froeyen, et al., Elaboration of a proprietary thymidylate kinase inhibitor motif towards anti-tuberculosis agents, Bioorg. Med. Chem. 24 (2016) 5172-5182. https://doi.10.1016/j.bmc.2016.08.041.

[12] L. Song, R. Merceron, B. Gracia, et al., Structure guided lead generation toward nonchiral *M. tuberculosis* thymidylate kinase inhibitors, J. Med. Chem. 61 (2018) 2753-2775. https://doi.10.1021/acs.jmedchem.7b01570.

[13] M. Naik, A. Raichurkar, B.S. Bandodkar, et al., Structure guided lead generation for *M. tuberculosis* thymidylate kinase (*Mtb* TMK): discovery of 3-cyanopyridone and 1,6-naphthyridin-2-one as potent inhibitors, J. Med. Chem. 58 (2015) 753-766. https://doi.10.1021/jm5012947.

[14] V. Vanheusden, H. Munier-Lehmann, M. Froeyen, et al., Discovery of bicyclic thymidine analogues as selective and high-affinity inhibitors of *Mycobacterium tuberculosis* thymidine monophosphate kinase, J. Med. Chem. 47 (2004) 6187-6194. https://doi.10.1021/jm040847w.

[15] V. Vanheusden, H. Munier-Lehmann, S. Pochet, et al., Synthesis and evaluation of thymidine-5'-O-monophosphate analogues as inhibitors of *Mycobacterium tuberculosis* thymidylate kinase, Bioorg. Med. Chem. Lett. 12 (2002) 2695-2698. https://doi.10.1016/s0960-894x(02)00551-6.

[16] I. Van Daele, H. Munier-Lehmann, P.M. Hendrickx, et al., Synthesis and biological evaluation of bicyclic nucleosides as inhibitors of *M. tuberculosis* thymidylate kinase, Chemmedchem. 1 (2006) 1081-1090. https://doi.10.1002/cmdc.200600028.

[17] I. Van Daele, H. Munier-Lehmann, M. Froeyen, et al., Rational design of 5'-thiourea-substituted alpha-thymidine analogues as thymidine monophosphate kinase inhibitors capable of inhibiting mycobacterial growth, J. Med. Chem. 50 (2007) 5281-5292.

https://doi.10.1021/jm0706158.

[18] R. O'Shea, H.E. Moser, Physicochemical properties of antibacterial compounds: implications for drug discovery, J. Med. Chem. 51 (2008) 2871-2878. https://doi.10.1021/jm700967e.

[19] L.D. Andrews, T.R. Kane, P. Dozzo, et al., Optimization and mechanistic characterization of pyridopyrimidine inhibitors of bacterial biotin carboxylase, J. Med. Chem. 62 (2019) 7489-7505. https://doi.10.1021/acs.jmedchem.9b00625.

[20] G. Mugumbate, J.P. Overington, The relationship between target-class and the physicochemical properties of antibacterial drugs, Bioorg. Med. Chem. 23 (2015) 5218-5224. https://doi.10.1016/j.bmc.2015.04.063.

[21] M. Miethke, M.A. Marahiel, Siderophore-based iron acquisition and pathogen control, Microbiol Mol Biol Rev. 71 (2007) 413-451. https://doi.10.1128/MMBR.00012-07.

[22] T. Zheng, E.M. Nolan, Enterobactin-mediated delivery of beta-lactam antibiotics enhances antibacterial activity against pathogenic *Escherichia coli*, J. Am. Chem. Soc. 136 (2014) 9677-9691. https://doi.10.1021/ja503911p.

[23] W. Neumann, M. Sassone-Corsi, M. Raffatellu, et al., Esterase-catalyzed siderophore hydrolysis activates an enterobactin-ciprofloxacin conjugate and confers targeted antibacterial activity, J. Am. Chem. Soc. 140 (2018) 5193-5201. https://doi.10.1021/jacs.8b01042.

[24] M. Ghosh, P.A. Miller, U. Mollmann, et al., Targeted antibiotic delivery: selective siderophore conjugation with daptomycin confers potent activity against multidrug resistant *Acinetobacter baumannii* both *in vitro* and *in vivo*, J. Med. Chem. 60 (2017) 4577-4583. https://doi.10.1021/acs.jmedchem.7b00102.

[25] J.F. Fisher, S. Mobashery, M.J. Miller, Antibacterials, in: J.F. Fisher, S. Mobashery, M.J. Miller (Eds.), Topics in Medicinal Chemistry, 26 (2018). https://doi.org/10.1007/978-3-319-70839-3.

[26] D. Ferreira, A.M.L. Seca, G.A.D. C, et al., Targeting human pathogenic bacteria by siderophores: A proteomics review, J. Proteom. 145 (2016) 153-166. https://doi.10.1016/j.jprot.2016.04.006.

[27] M.J. Miller, A.J. Walz, H. Zhu, et al., Design, synthesis, and study of a mycobactin-artemisinin conjugate that has selective and potent activity against tuberculosis and malaria, J. Am. Chem. Soc. 133 (2011) 2076-2079. https://doi.10.1021/ja109665t.

[28] L. Tan, Y. Tao, T. Wang, et al., Discovery of novel pyridone-conjugated monosulfactams as potent and broad-spectrum antibiotics for multidrug-resistant Gram-negative infections, J. Med. Chem. 60 (2017) 2669-2684. https://doi.10.1021/acs.jmedchem.6b01261.

[29] M.E. Flanagan, S.J. Brickner, M. Lall, et al., Preparation, gram-negative antibacterial activity, and hydrolytic stability of novel siderophore-conjugated monocarbam diols, ACS Med. Chem. Lett. 2 (2011) 385-390. https://doi.10.1021/ml200012f.

[30] K.E. Murphy-Benenato, P.R. Bhagunde, A. Chen, et al., Discovery of efficacious *Pseudomonas aeruginosa*-targeted siderophore-conjugated monocarbams by application of a semi-mechanistic pharmacokinetic/pharmacodynamic model, J. Med. Chem. 58 (2015) 2195-2205. https://doi.10.1021/jm501506f.

[31] A. Ito, N. Kohira, S.K. Bouchillon, et al., *In vitro* antimicrobial activity of S-649266, a

catechol-substituted siderophore cephalosporin, when tested against non-fermenting Gram-negative bacteria, J. Antimicrob. Chemother. 71 (2016) 670-677. https://doi.10.1093/jac/dkv402.

[32] S. Kang, R.Y. Kim, M.J. Seo, et al., Lead optimization of a novel series of imidazo[1,2-a]pyridine amides leading to a clinical candidate (Q203) as a multi- and extensively-drug-resistant anti-tuberculosis agent, J. Med. Chem. 57 (2014) 5293-5305. https://doi.10.1021/jm5003606.

[33] L. Li, K. Lv, Y. Yang, et al., Identification of N-benzyl 3,5-dinitrobenzamides derived from PBTZ169 as antitubercular agents, ACS Med. Chem. Lett. 9 (2018) 741-745. https://doi.10.1021/acsmedchemlett.8b00177.

[34] J.A. Ferreras, A. Gupta, N.D. Amin, et al., Chemical scaffolds with structural similarities to siderophores of nonribosomal peptide-polyketide origin as novel antimicrobials against *Mycobacterium tuberculosis* and *Yersinia pestis*, Bioorg. Med. Chem. Lett. 21 (2011) 6533-6537. https://doi.10.1016/j.bmcl.2011.08.052.

[35] L. de Leseleuc, G. Harris, R. KuoLee, et al., *In vitro* and *in vivo* biological activities of iron chelators and gallium nitrate against *Acinetobacter baumannii*, Antimicrob. Agents Chemother. 56 (2012) 5397-5400. https://doi.10.1128/AAC.00778-12.

[36] V. Prachayasittikul, S. Prachayasittikul, S. Ruchirawat, et al., 8-Hydroxyquinolines: a review of their metal chelating properties and medicinal applications, Drug Des. Dev. Ther. 7 (2013) 1157-1178. https://doi.10.2147/Dddt.S49763.

[37] C.A. Lefebvre, E. Forcellini, S. Boutin, et al., Synthesis of novel substituted pyrimidine derivatives bearing a sulfamide group and their *in vitro* cancer growth inhibition activity, Bioorg. Med. Chem. Lett. 27 (2017) 299-302. https://doi.10.1016/j.bmcl.2016.11.052.

[38] S.A. Defrees, K.S. Reddy,J.M. Cassady, A selective and efficient method for the deprotection of N-benzyloxymethyl (Bom) protecting groups from pyrimidine and dihydropyrimidine ring-systems, Synth. Commun. 18 (1988) 213-220. https://doi.Doi 10.1080/00397918808077347.

[39] R.A. Cutler, A.R. Surrey, The reaction of 4,7-dichloroquinoline with acetic acid, J. Am. Chem. Soc. 72 (1950) 3394–3395. https://doi.org/10.1021/ja01164a021.

[40] K. Kubo, S. Ohyama, T. Shimizu, et al., Synthesis and structure-activity relationship for new series of 4-phenoxyquinoline derivatives as specific inhibitors of platelet-derived growth factor receptor tyrosine kinase, Bioorg. Med. Chem. 11 (2003) 5117-5133. http://doi.10.1016/j.bmc.2003.08.020.

[41] M. Heller, U.S. Schubert, Functionalized 2,2'-bipyridines and 2,2':6',2' '-terpyridines via stille-type cross-coupling procedures, J. Org. Chem. 67 (2002) 8269-8272. https://doi.10.1021/jo0260600.

[42] B. Chetan, M. Bunha, M. Jagrat, et al., Design, synthesis and anticancer activity of piperazine hydroxamates and their histone deacetylase (HDAC) inhibitory activity, Bioorg. Med. Chem. Lett. 20 (2010) 3906-3910. https://doi.10.1016/j.bmcl.2010.05.020.

[43] Y. Jian, M.D.P. Risseeuw, M. Froeyen, et al., 1-(Piperidin-3-yl)thymine amides as inhibitors of *M. tuberculosis* thymidylate kinase, J. Enzyme Inhib. Med. Chem. 34 (2019) 1730-1739. https://doi.10.1080/14756366.2019.1662790.

[44] H. Munier-Lehmann, A. Chaffotte, S. Pochet, et al., Thymidylate kinase of *Mycobacterium tuberculosis*: a chimera sharing properties common to eukaryotic and

bacterial enzymes, Protein Sci. 10 (2001) 1195-1205. https://doi.10.1110/ps.45701.

[45] W. Kabsch, XDS, Acta Crystallogr D Biol Crystallogr. 66 (2010) 125-132. https://doi.10.1107/S0907444909047337.

[46] P.V. Afonine, R.W. Grosse-Kunstleve, N. Echols, et al., Towards automated crystallographic structure refinement with phenix.refine, Acta Cryst. D. 68 (2012) 352-367. https://doi.10.1107/S0907444912001308.

[47] P. Emsley, B. Lohkamp, W.G. Scott, et al., Features and development of Coot, Acta Crystallogr D Biol Crystallogr. 66 (2010) 486-501. https://doi.10.1107/S0907444910007493.

[48] C.J. Williams, J.J. Headd, N.W. Moriarty, et al., MolProbity: More and better reference data for improved all-atom structure validation, Protein Sci. 27 (2018) 293-315. https://doi.10.1002/pro.3330.

[49] C. Blondin, L. Serina, L. Wiesmuller, et al., Improved spectrophotometric assay of nucleoside monophosphate kinase activity using the pyruvate kinase/lactate dehydrogenase coupling system, Anal. Biochem. 220 (1994) 219-221. https://doi.10.1006/abio.1994.1326.

[50] G.M. Morris, R. Huey, W. Lindstrom, et al., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785-2791. https://doi.10.1002/jcc.21256.

[51] M. Makowska-Grzyska, Y. Kim, S.K. Gorla, et al., *Mycobacterium tuberculosis* IMPDH in complexes with substrates, products and antitubercular compounds, PloS one. 10 (2015) e0138976. https://doi.10.1371/journal.pone.0138976.

[52] S. Dawadi, H.I.M. Boshoff, S.W. Park, et al., Conformationally constrained cinnolinone nucleoside analogues as siderophore biosynthesis inhibitors for tuberculosis, ACS Med. Chem. Lett. 9 (2018) 386-391. https://doi.10.1021/acsmedchemlett.8b00090.

[53] K. Hayasaka, K. Kamata, H. Nakazawa, Highly efficient olefin hydrosilylation catalyzed by iron complexes with iminobipyridine ligand, Bull. Chem. Soc. Jpn. 89 (2016) 394-404. https://doi.10.1246/bcsj.20150359.

[54] W.L. Wang, S.C. Chai, Q.Z. Ye, Synthesis and structure-function analysis of Fe(II)-form-selective antibacterial inhibitors of *Escherichia coli* methionine aminopeptidase, Bioorg. Med. Chem. Lett. 19 (2009) 1080-1083. https://doi.10.1016/j.bmcl.2009.01.011.

[55] R. Cervellati, P. Galletti, E. Greco, et al., Monocyclic beta-lactams as antibacterial agents: facing antioxidant activity of N-methylthio-azetidinones, Eur. J. Med. Chem. 60 (2013) 340-349. https://doi.10.1016/j.ejmech.2012.12.024.

[56] J.O. Osby, S.W. Heinzman, B. Ganem, Studies on the mechanism of transition-metal-assisted sodium-borohydride and lithium aluminum-hydride reductions, J. Am. Chem. Soc. 108 (1986) 67-72. https://doi.10.1021/Ja00261a011.