Structure-aided optimization of non-nucleoside M. tuberculosis thymidylate kinase inhibitors

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A B S T R A C T

Mycobacterium tuberculosis thymidylate kinase (MtTMPK) has emerged as an attractive target for rational drug desing. We recently investigated new families of non-nucleoside MtTMPK inhibitors in an effort to diversify MtTMPK inhibitor chemical space. We here report a new series of MtTMPK inhibitors by combining the Topliss scheme with rational drug design approaches, fueled by to co-crystal structures of MtTMPK in complex with developed inhibitors. These efforts furnished the most potent MtTMPK inhibitors in our assay, with two analogues displaying low micromolar MIC values against H37Rv Mtb. Prepared inhibitors address new sub- sites in the MtTMPK nucleotide binding pocket, thereby offering new insights into its druggability. We studied the role of efflux pumps as well as the impact of cell wall permeabilizers for selected compounds to potentially provide an explanation for the lack of correlation between potent enzyme inhibition and whole-cell activity.

1. Introduction

Tuberculosis (TB) in humans is caused by the bacterium Mycobacterium tuberculosis (Mtb) and has become the most lethal infectious disease ranking among the top ten causes of death worldwide [1]. In 2016, 10.4 million cases of active TB were reported while 1.3 million people were estimated to have died from Mtb infection [1]. Tuberculosis is generally manifested as a respiratory disease affecting the lungs, although other sites on the human body can also be affected. After exposure to Mtb, the host immune system may eliminate the infection; latent asymptomatic TB infection can also be established, which may develop into active TB in case of immunosuppression. Pulmonary TB is a chronic disease with typical symptoms such as fever, weight loss and cough, leading to death over the years. However, if the patient is co- infected with human immunodeficiency virus (HIV), death could occur within 4 weeks, including the diagnosis time [2]. TB contributes heavily to the mortality of HIV-infected patients. Moreover, drug-resistant TB has become a global issue with the alarming emergence of resistant strains [1]. Therefore, drugs with a novel mode of action and scaffolds are urgently needed to combat TB.

M. tuberculosis thymidine monophosphate kinase (MtTMPK) is a phosphotransferase that phosphorylates thymidine monophosphate (TMP) to thymidine diphosphate (TDP), which is then further transformed into thymidine triphosphate (TTP) [3–5]. Its essentiality for the survival of Mtb and the druggability of the TMP-binding site have been proven via high-resolution phenotypic profiling [6] and sitemap analysis [7,8], respectively. Additionally, the availability of X-ray co-crystal structures of MtTMPK [8–11] allow rational drug design.

In previous work from our group, hit compound 1 (Fig. 1) was discovered as a promising scaffold for the elaboration of MtTMPK inhibitors [11]. While retaining the thymine moiety (ring A), which mediates key interactions with the active site of the enzyme, modifications of ring B or the spacer between ring A and B were found to negatively influence the inhibitory activity. Attempts to truncate the structure by removal of ring D increased ligand

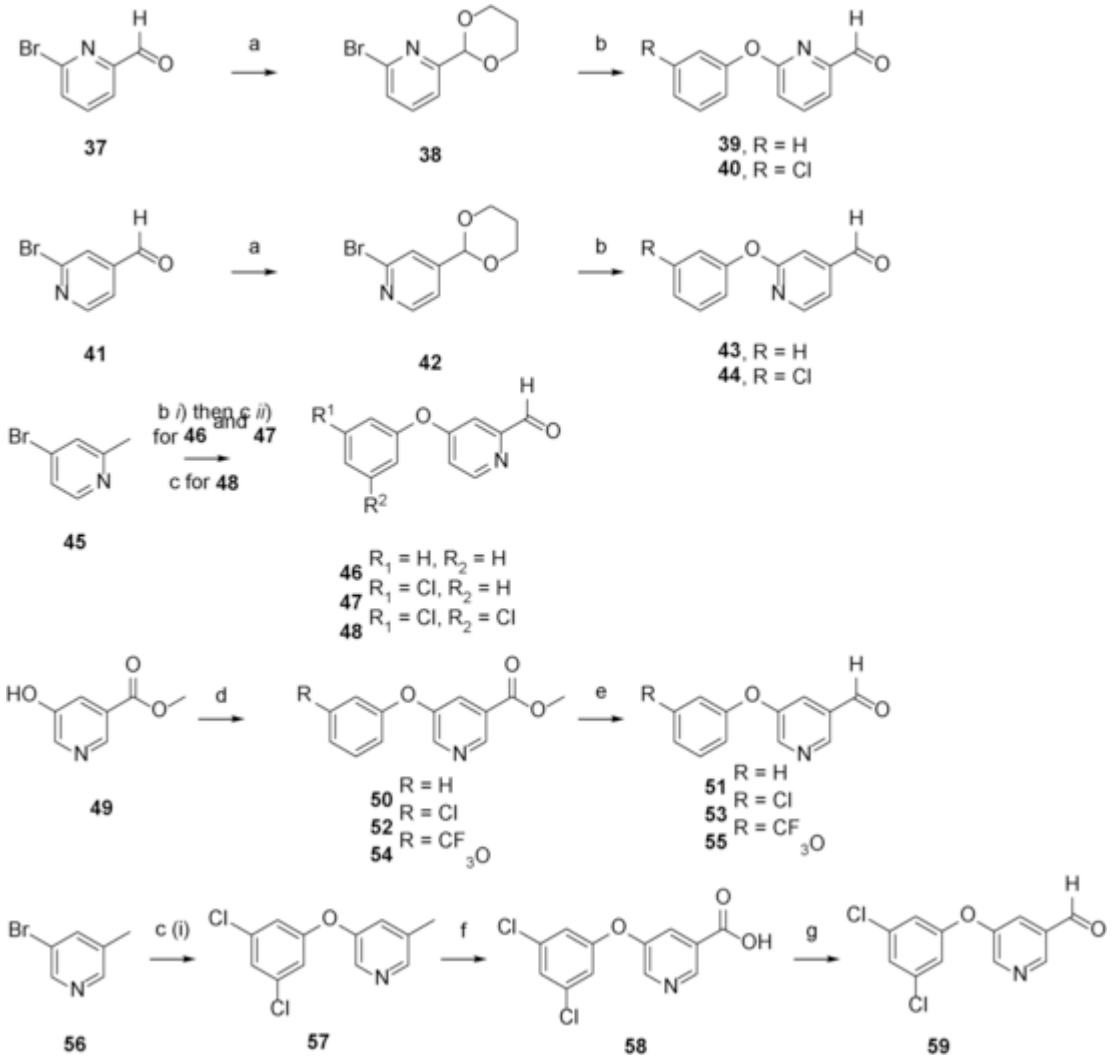
efficiency [12], but failed to afford compounds with antimycobacterial activity (Fig. 1). Further modification led to phenoxy-quinoline-type analogues (compound 2), some of which exhibited potent enzymatic activity and moderate whole-cell activities. The aim of this study was to design MtTMPK inhibitors with different physicochemical properties (i.e. increase polarity) in an effort to increase the solubility and potentially identify analogues with improved whole-cell activity [13,14].
Optimization of the tetracyclic structure (rings A-B-C-D), spanned three stages (Fig. 1). First, the phenyl ring (ring C) was replaced by a pyridyl unit, of which all possible positions of the nitrogen were investigated (series 1), while retaining the meta-relationship [11,15] of the piperidinyl and phenoxy substituent (Fig. 2, upper part). Alternatively, the terminal D-ring was replaced by a range of different rings (series 2) with diverging lipophilic and electronic properties (Fig. 2, lower part). Finally, the most promising modifications that arose from stage 1 as well as stage 2 were combined to yield a third series (Fig. 3).

2. Results and discussion

2.1. Synthesis

In general, all target analogues were synthesized by means of a sequence of reductive amination between an appropriate aromatic aldehyde and BOM-protected 4-piperidinylthymine [15], followed by acid- mediated BOM deprotection [11], to reveal the desired final compounds.

The synthetic routes to obtain the required aromatic aldehydes to prepare series 1 and 3 are depicted in Scheme 1. The successful synthetic route of the aldehyde precursor depended on the desired substitution pattern of the pyridine ring. For C-2/6 and C-2/4 substituted pyridines, the appropriate 2-bromopyridine-aldehydes (37 or 41, respectively) were first protected as cyclic acetals. Ullmann coupling with either phenol or 3-chlorophenol, followed by deprotection gave the desired aldehydes 39, 40, 43 and 44 (Scheme 1) [16]. Generally, the Ullmann coupling with 3-chlorophenol gave substantially higher yields than with phenol.



Scheme 1. Synthesis of required aldehydes for pyridine and hybrid analogues (Series 1 and 3).

This sequence (protection-Ullmann coupling-deprotection) was found unsuitable for the synthesis of the remaining pyridine isomers (46–48, 51, 53, 55 and 59), possibly due to altered electronic in the pyridine ring (data not shown). For C-4 substituted 2-pyridinecarbaldehydes, the synthesis started from 4-bromo-2- methylpyridine 45, which was reacted with the appropriate phenol under Ullmann coupling conditions [11,16]. Notably, the Ullmann reaction with 3,5-dichlorophenol required harsher reaction conditions to force coupling [17]. Next, oxidation of the methyl group with selenium dioxide yielded aldehydes 46–48.

The synthesis of 5-substituted 3-pyridinecarbaldehydes started from methyl-5-hydroxynicotinate 49, which was reacted with the appropriate phenylboronic acid under Chan-Lam coupling conditions to afford compounds 50, 52 and 54. Finally, a reduction/re-oxidation sequence, employing LiAlH4 and MnO2, furnished the desired aldehydes 51, 53

and 55. Chan-Lam coupling between 3,5-dichlorophenylboronic acid and 49, however, failed to yield the desired product, as did an Ullmann coupling between 3,5-dichloro-iodobenzene and 49 using picolinic acid as the ligand [18]. Finally, reversal of the coupling partner polarity (bromopyridine 56 and 3,5-dichlorophenol) yielded intermediate 57, under the same conditions as those used for the preparation of 48. Direct oxidation of the aromatic methyl group to the corresponding aldehyde with selenium dioxide at 120 °C failed to give the desired aldehyde [19]. However, oxidation of 57 to the corresponding carboxylic acid (58) with aq. KMnO4 was successful. Transformation of 58 to its methyl ester, followed by LiAlH4 reduction and re-oxidation with Dess- Martin periodinane furnished the desired aldehyde 59.

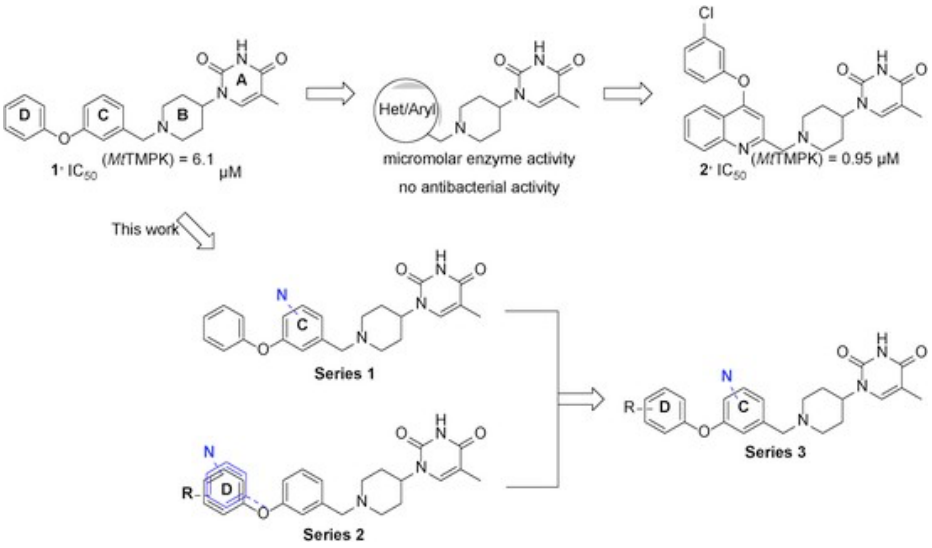


Fig. 1. Overview of previous (upper line) and current (lower line) modifications of hit compound 1 [11].

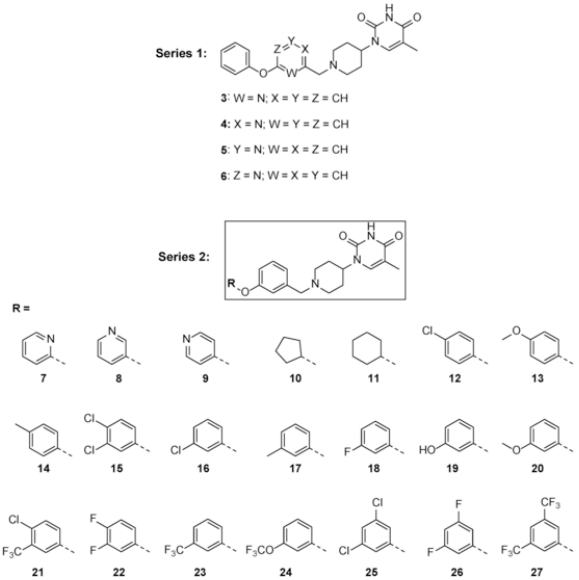
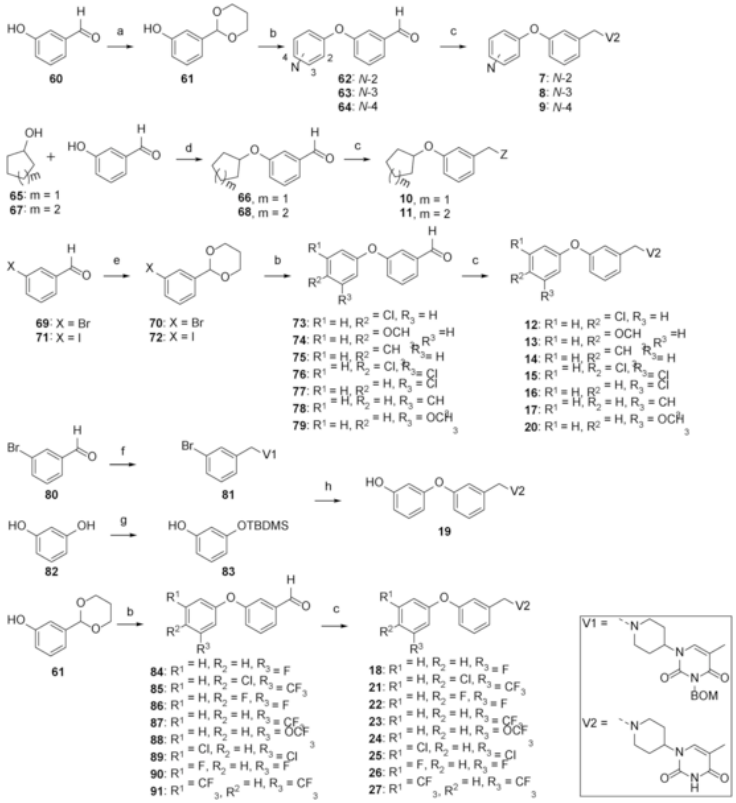


Fig. 2. Structures of prepared analogues in this study. Structures of analogues with a pyridine C-ring (upper part), and structures of analogues with a modified D-ring (lower part).

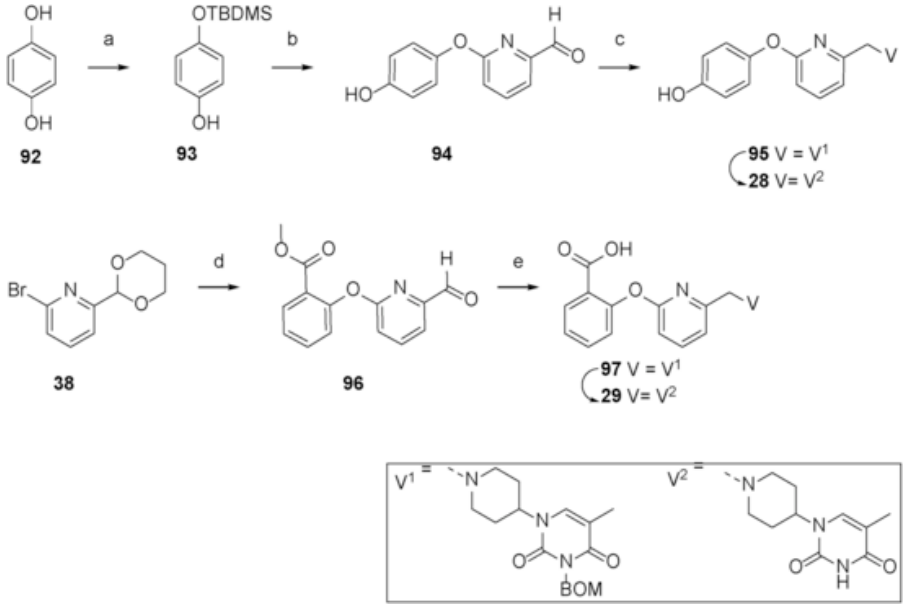
Reagents and conditions: (a) 1,3-dihydroxypropane (1.2 eq), p- toluenesulfonic acid (cat.), toluene, reflux (Dean-Stark), 3 h; (b) (i) appropriate phenol, Cs2CO3 (3 eq), N,N-dimethylglycine.HCl (0.3 eq), CuI (0.1 eq), 1,4-dioxane, dry air, 90 °C, 24 h; (ii) THF/2 M aq. HCl (V/V,1/1), 60 °C, 2–7 h; (c) (i) 3,5-dichlorophenol, K3PO4, picolinic acid, CuI, dry DMF, argon, 140 °C, 24 h; (ii) SeO2, 1,4-dioxane, 80 °C, overnight (compound 46 and 47) or 100 °C, 24 h (compound 48); (d) appropriate phenylboronic acid (3–3.5 eq), Cu(OAc)2 (2 eq), Et3N (7 eq), 4 Å molecular sieves, DCM, air, rt; (e) (i) LiAlH4, dry THF, rt; (ii) MnO2, DCM, rt; (f) KMnO4, pyridine/H2O, reflux, 7 h; (g) (i) SOCl2, MeOH, rt to reflux, overnight; (ii) LiAlH4, dry THF, rt; (iii) Dess-Martin reagent, DCM, rt.

Analogues with a modified D-ring (7–27) were synthesized as depicted in Scheme 2. For selected analogues with N-containing D-rings (7–9), 3-the appropriate pyridinyl halides and protected hydroxybenzaldehyde (61) were employed for the Ullmann coupling. The cyclo-alkyl substituted aldehydes 66 and 68 were synthesized via Mitsunobu reaction [20]. In other cases substituted phenols and protected halo aldehydes (69 and 71) were preferred. The synthesis of compound 19 involved reductive amination of 3-bromobenzaldehyde 80 with BOM- protected 1-(piperidin-4yl)thymine, followed by Ullmann coupling with mono-TBDMS-protected resorcinol 83. Conveniently, the silyl group was removed under Ullmann coupling condition, but in view of the low yield of this approach, we decided to prepare the other required aromatic aldehydes as described earlier: acetal protection, Ullmann coupling and acetal deprotection.



Scheme 2. Synthesis of D-ring modified derivatives (Series 2).

Reagents and conditions: (a) (i) Ac2O, pyridine, 0–25 °C, 1 h; (ii) 1,3-dihydroxypropane, p-toluenesulfonic acid, toluene, reflux (Dean- Stark), 3 h; (iii) 10% aq. NaOH, 2 h; (b) (i) Cs2CO3 (3 eq), N,N- dimethylglycine.HCl (0.3 eq), CuI (0.1), 1,4-dioxane, argon, 90 °C, 24 h; (ii) THF/2 M aq. HCl (V/V, 1/1), rt, 2–7 h; (c) (i) V1–H, NaBH(OAc)3, dry DCE, r.t. overnight; (ii) 80% TFA/H2O, 0.25–0.27 M l-cysteine, 72 °C, 3–12 h; (d) Ph3P, diisopropyl azodicarboxylate, dry THF, reflux, 24 h. (e) 1,3-dihydroxypropane (1.2 eq), p-toluenesulfonic acid (cat.), toluene, reflux (Dean-Stark), 3 h; (f) V1–H, NaBH(OAc)3, dry DCE, rt, overnight; (g) TBDMSCl, imidazole, DMF, rt, overnight; (h) (i) K3PO4, picolinic acid, CuI, dry DMSO, 140 °C, argon, 24 h; (ii) 80% TFA/H2O, 0.27 M l-cysteine, 72 °C, 5 h.
The methods to access derivatives of analogue 3 (28 and 29) are described in Scheme 3.



Scheme 3. Synthesis of derivatives of analogue 3 (analogue 28 and 29).

Synthesis of aldehyde 94 started from hydroquinone. First, mono-OH silyl-protection gave 93, which was used for the Ullmann coupling with 38. Sequential silyl and acetal deprotection afforded the required aldehyde 94. For the synthesis of carboxylic acid 29, we first attempted an Ullmann coupling of 38 with 2- hydroxybenzonitrile. However, final hydrolysis (after acetal deprotection) of the cyano group only gave rise to the amide intermediate, even upon heating at reflux for an extended period of time in aqueous potassium hydroxide solution (data not shown). Alternatively, Ullmann coupling with methyl 2-hydroxybenzoate, followed by ester hydrolysis furnished the desired product after reductive amination and final BOM deprotection.
Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, overnight; (b) (i) 38, Cs2CO3 (3 eq), N,N-dimethylglycine.HCl (0.3 eq), CuI (0.1 eq), 1,4-dioxane, argon, 90 °C, 24 h; (ii) 1 M TBAF in THF, THF, rt, 3 h; (iii) THF/2 M aq. HCl (V/V, 1/1), 55 °C, 3 h; (c) (i) V1–H,
NaBH(OAc)3, dry DCE, rt, overnight; (ii) 80% TFA/H2O, 0.25–0.27 M l-cysteine, 72 °C, 3–12 h; (d) (i) methyl 2-hydroxybenzoate, K3PO4, picolinic acid, CuI, dry DMSO, 90 °C, argon, 24

h; (ii) THF/2 M aq. HCl (V/V, 1/1), rt; (e) (i) V1–H, NaBH(OAc)3, dry DCE, rt, overnight; (ii) NaOH, H2O/THF, rt, 2 days; (iii) 80% TFA/H2O, 0.25 M l-cysteine, 72 °C, 3–12 h.

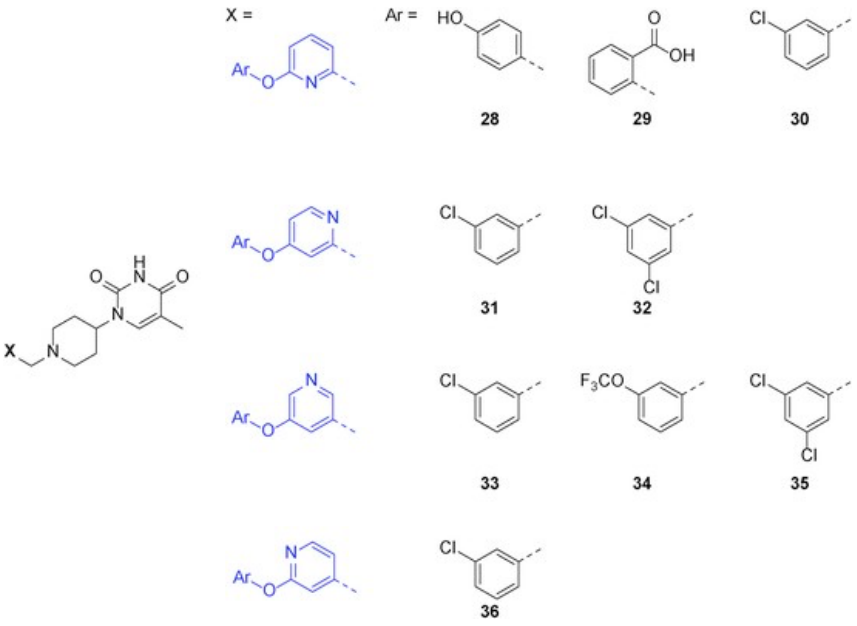
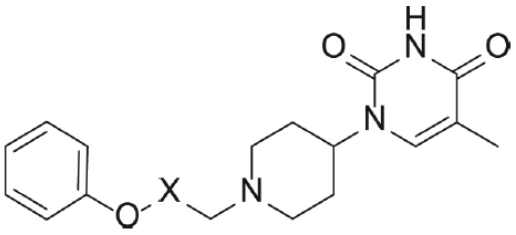


Fig. 3. Hybrid analogues (Series 3) comprised of elements investigated from Series 1 and 2.

2.2. Biological evaluation

In our previous work, structure-guided modification of compound 1 led to phenoxyquinoline analogue 2, which exhibited improved enzymatic and cellular activity [11]. This investigation also indicated that efflux pumps are, at least, partially responsible for the discrepancy between the moderate (or lack of) whole-cell activity on the one hand, and the high MtTMPK inhibitory activity of phenoxyquinoline derivatives on the other hand. Nevertheless, we also hypothesized that the main reason for this disparity could also be ascribed to low permeability of the reported MtTMPK inhibitors through the thick mycobacterial cell wall and its crystalline-like mycolate layer [21,22]. Additionally, for selected analogues in vitro assessment of the antimycobacterial activity was also hampered by poor aqueous solubility, which was particularly relevant for phenoxyquinoline analogues. To potentially address this solubility issue and to identify compounds that might accumulate better in the bacteria, we thus introduced a nitrogen atom in ring C of lead compound 1 (Fig. 2 and Table 1), to increase overall polarity. The resulting pyridine analogues 3–6 had comparable MtTMPK inhibitory activity as 1, but failed to show in vitro antitubercular activity, except for the 3,5-substituted analogue 5, which displayed weak antitubercular activity. The co-crystal structure of compound 3, the most potent enzyme inhibitor pyridine isomer of this series, with MtTMPK was successfully solved (vide infra).



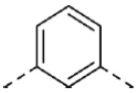
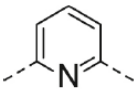
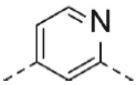
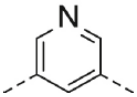
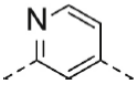
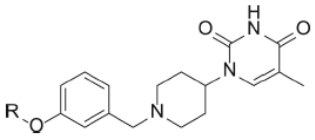
No	X	ClogP	IC_{50} (μM, *Mt*TMPK)	MIC (μM, H37Rv, Glucose)	MRC-5 EC_{50} (μM)
1		2.86	6.1 ± 0.5[15]	N.D.	N.D.
3		2.66	2.5 ± 0.3	>50	>64.0
4		1.94	8.7 ± 0.4	>50	>64.0
5		1.52	6.4 ± 0.4	37	>64.0
6		2.23	10.2 ± 1.0	>50	>64.0

Table 1. MtTMPK inhibitory activity and antimycobacterial activity (MIC) against M. tuberculosis H37Rv of analogues in Series 1. Cytotoxicity was assayed in human fibroblast cells (MRC-5). The MtTMPK inhibitory activity value for analogue 1 was taken from Ref. [15].

To further expand the SAR information, we introduced different substituents on phenyl D-ring of 1 or replaced it by pyridyl and cycloalkyl rings (Table 2). The MtTMPK inhibitory activity of the pyridyl substituted analogues (7–9) was inferior to that of 1 and the C-ring pyridine analogues of series 1. The inferior inhibitory potency of cycloalkyl substituted compounds (10 and 11) suggests that a planar D- ring is preferred. The substituents introduced on the D-ring of 1 were selected via Topliss scheme, a decision tree that relies on lipophilicity (π), electronic effect (σ) and steric effect (Es) of the aromatic substituents [23]. While the effect on the enzyme inhibitory potency of a 4- Cl-substituent (12, + π, + σ) is comparable to that of lead 1 (4-H, π = 0, σ = 0), introduction of a 4-MeO (13, - π, - σ) and 4-Me (14, + π, - σ) yielded inferior activities, indicating that substituents at the para- position are sterically not well tolerated. A 3-Cl substitution (16, + π, + σ), on the other hand, showed a significant improvement in MtTMPK inhibitory activity. Consistently, analogue pairs 15/16 (3,4-Cl2/3-Cl), 21/23 (3-CF3-4-Cl/3-CF3) and 22/18 (3,4-F2/3-F) demonstrate that MtTMPK better accommodates 3-substituted over 3,4-substituted analogues. Following application

of Topliss tree for 3-substituted analogues, analogues 23 (3-CF3) and 24 (3-CF3O) further improved the enzyme inhibitory activity, while analogues 17 (3-Me), 19 (3-OH) and 20 (3-MeO) with electron-donating groups gave weaker inhibitory activities. Most interestingly, the 3,5-disubstituted analogues 25 (3,5-Cl2) and 27 (3,5-(CF3)2) resulted in highly potent MtTMPK inhibitors. Thus we conclude that ring D prefers lipophilic, electron-withdrawing groups, preferably not in the para position. With regard to whole-cell activity against M. tuberculosis, analogues 15, 16 and 25 displayed encouraging in vitro activity. Although more potent enzyme inhibitors are clearly also more potent antitubercular compounds, the correlation is rather poor (compare analogues 25 and 27). Unfortunately, 15, 26 and 25 were poorly selective with respect to mammalian cells, limiting their development potential. Next, we verified that cytotoxicity is not due to inhibition of human TMPK (hTMPK) by assaying selected analogues (15, 21, 24 and 25), which revealed no inhibition of hTMPK up to a concentration of 40 μM.



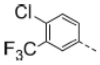
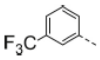
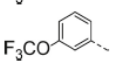
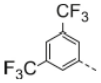
No	R	ClogP	IC_{50} (μM, *Mt*TMPK)	Mtb H37Rv MIC (μM, glucose)	MRC-5 EC_{50} (μM)
7		2.23	41 ± 7	>50	>64.0
8		1.52	27 ± 8	>50	>64.0
9		1.52	20 ± 2	N.D.	N.D.
10		2.32	32 ± 2	>50	30.3 ± 3.1
11		2.74	42 ± 3	37	23.6 ± 3.0
12		3.41	6.9 ± 0.7	12.5	25.1 ± 2.6
13		2.73	10.5 ± 0.5	*	28.0 ± 1.9
14		3.34	12 ± 1	*	[>64.0; 24.0]
15		3.97	1.1 ± 0.3	12.5	6.10 ± 0.10
16		3.41	0.89 ± 0.12	12.5	21.0 ± 3.7
17		3.34	26 ± 1	37	>64.0
18		3.01	2.9 ± 0.5	25	>64.0
19		2.47	3.3 ± 0.3	N.D.	>64.0
20		2.73	1.2 ± 0.1	25	25.3 ± 5.7
21		4.34	1.1 ± 0.1	25	5.28 ± 0.22
22		3.17	2.6 ± 0.6	19	>64.0
23		3.78	0.60 ± 0.19	19	12.4 ± 6.6
24		4.38	0.65 ± 0.06	25	5.26 ± 0.40
25		3.97	0.12 ± 0.01	12.5	6.73 ± 0.50
26		3.17	2.8 ± 0.8	>50	>64.0
27		4.7	0.14 ± 0.01	>50	N.D.

Table 2. MtTMPK inhibitory activity and antimycobacterial activity (MIC) against M. tuberculosis H37Rv of analogues in Series 2. Cytotoxicity was assayed in human fibroblast cells (MRC-5). *see Table 4 for activity of the compound against H37Rv.

To gain insights into the binding mode of inhibitor 3 we determined the crystal structure of MtTMPK in complex with 3 (PDB code 5NRN) to 2.2 Å resolution. Inhibitor 3 which behaves

as a competitive inhibitor, binds with full occupancy in the active site of the enzyme where it adopts a U-turn shaped conformation (Fig. 4).

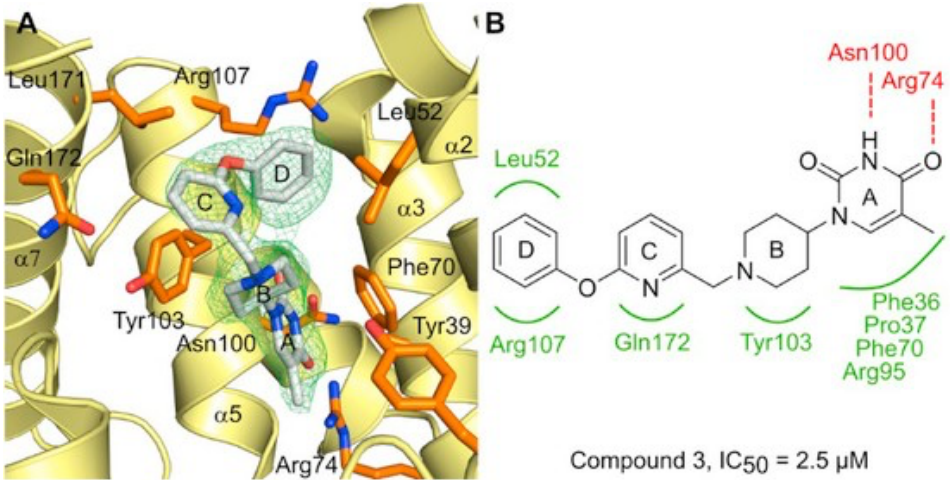


Fig. 4. Structural characterization of the binding pose of compound 3 in MtTMPK active site (deposited as PDB code 5NRN). (A) Co-crystal structure of compound 3 bound to MtTMPK at 2.2 Å resolution. The protein is depicted in pale yellow cartoon representation. Compound 3 (stick representation, carbon atoms in white) and the side chains of MtTMPK interacting residues (stick representation, carbon atoms in orange) are highlighted. The corresponding σ-weighted Fo-Fc difference electron density map (contoured to +4 σ r.m.s.d.) and calculated before adding the ligand in the refinement process is shown as a green mesh. (B) Schematic drawing of compound 3 binding site displaying key MtTMPK interacting residues (hydrogen bonds are represented with a red dashed line). Compound 3 comprises four rings named A to D.

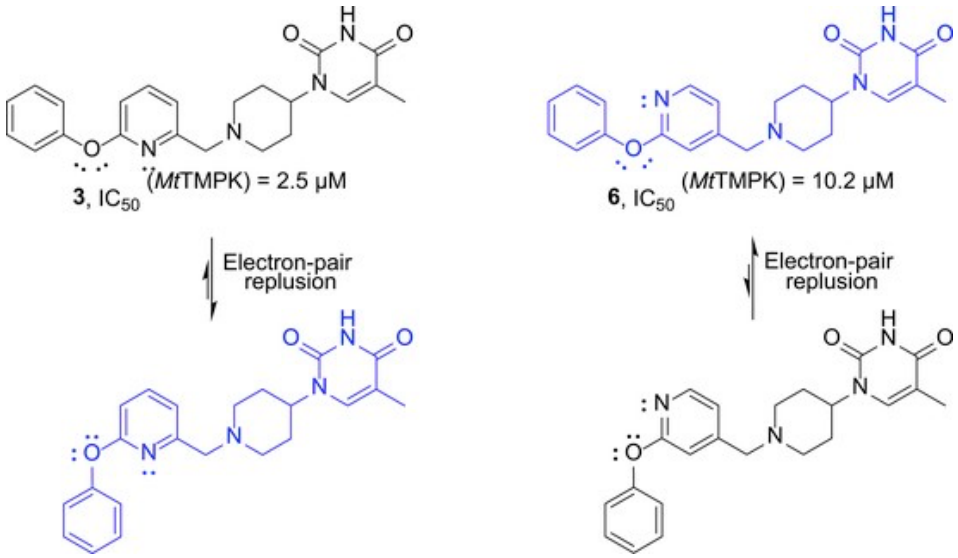


Fig. 5. Conformational preferences of analogues 3 and 6 due to electron-pair repulsion. The favoured conformation of each compound is shown in blue.

The thymine ring (ring A) interacts with residues lining the catalytic pocket in a configuration reminiscent to the one previously described for MtTMPK structures in complex with dTMP and thymine-like inhibitors. Ring A is stabilized via hydrophobic interactions between its C [5] methyl group and Arg95, Pro37 and Phe36 side chains. In addition to a π-π stacking interaction between ring A and Phe70, the inhibitor is further stabilized via hydrogen bonds linking the O [4] and N [3] groups of the thymine ring with Arg74 and Asn100 respectively. The para-piperidine ring (ring B), modeled in a chair conformation, protrudes out of the active site and is located in a similar position to the 2'-deoxyribose ring of dTMP establishing a π-alkyl interaction with the aromatic side chain of Tyr103. In chain A, the pyridyl ring (ring C) protrudes out of the active site where it does not establish any specific interaction with the enzyme. Conversely, ring C establishes a CH-alkyl interaction with residue Arg153 which belongs to the LID loop, and which is in a closed conformation in chain B (the same loop is disordered in chain A). The LID loop in chain B establishes contacts with neighboring symmetry related molecules and its conformation might be the result of crystal packing leading to some questioning about the validity of this later interaction. The benzene ring (ring D) is accommodated in a pocket formed by residues Leu52 and Arg107 and ring C provides the compound with the necessary spacer to adopt this U-turn shaped conformation.

For compound 3, the electron-pair repulsion between oxygen and nitrogen tends to stabilize a U-shaped conformer, which is preferred by the binding pocket of the enzyme (Fig. 6) [24]. For analogues 4 and 5, the influence of the electron-pair repulsion is less pronounced, while compound 6 is unlikely to adopt the U-shaped conformation. This is in agreement with the observed trend in IC50-values for these isomers.

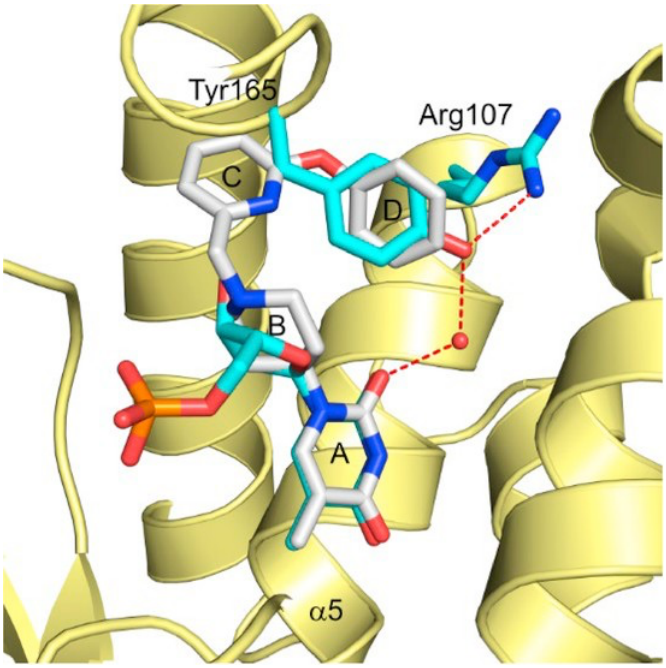
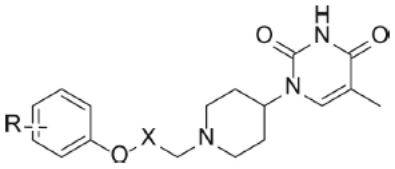


Fig. 6. Crystal structure of MtTMPK in complex with dTMP (PDB code 1G3U) showing the binding of the substrate (stick representation, carbon atoms in cyan) in the MtTMPK active site. The protein is depicted in pale-yellow as a ribbon representation. Residue Tyr165 in the LID loop and in close interaction with the substrate is depicted in stick representation with carbon atoms in cyan. The pose of 3 (stick representation, carbon atoms in white) in MtTMPK/3 co-crystal structure has been overlaid with dTMP. The superimposition shows a strict conservation of the thymine core position and a similar position for the phenol ring of Tyr165 and ring D in 3. Tyr165 establishes a hydrogen bond with Arg107 and with dTMP via a water molecule. Hydrogen bonds are represented with red dashed lines.

According to the overlay of 3 and TMP crystallographic poses in the active site of MtTMPK, the interaction of the thymine moiety of 3 with the enzyme is quasi-identical as that found in the crystal structure of the dTMP substrate with the enzyme (Fig. 6). Interestingly, the D-ring of 3 bends to occupy the position of Tyr165 in the co-crystal of dTMP. In the latter structure, the phenol ring of residue Tyr165 establishes specific interactions via its hydroxyl group with residues Arg107 and with the O [4] group of the thymine ring via a water molecule. This particular observation inspired us to synthesize analogue 28, featuring a para- hydroxyphenyl D-ring as to mimic this tyrosine. This operation resulted in a two-fold affinity gain compared to analogue 3 (Table 3).



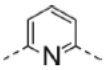
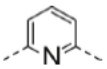
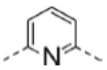
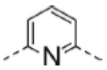
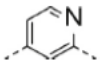
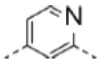
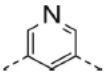
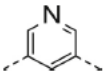
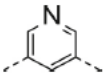
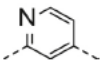
No	X	R	Clog*P*	IC_{50} (μM, *Mt*TMPK)	MIC (μM, H37Rv, glucose)	IC_{50} (μM, MRC-5)
3		–	2.66	2.5 ± 0.3	>50	>64.0
28		4-OH	2.27	1.1 ± 0.1	>50	N.D.
29		2-CO_2H	2.22	25 ± 2	>50	N.D.
30		3-Cl	3.22	0.45 ± 0.07	31	>64.0
31		3-Cl	2.50	2.8 ± 0.7	>50	>64.0
32		3,5-Cl_2	3.06	0.60 ± 0.09	34	N.D.
33		3-Cl	2.08	0.77 ± 0.09	31	[>64.0; 39.9]
34		3-CF_3O	3.05	0.81 ± 0.01	37	>64.0
35		3,5-Cl_2	2.64	0.22 ± 0.01	43	>64.0
36		3-Cl	2.79	3.1 ± 0.2	37	[>64.0; 58.9]

Table 3
MtTMPK inhibitory activity and antimycobacterial activity (MIC) against M. tuberculosis H37Rv of analogues in Series 3. Cytotoxicity was assayed in human fibroblast cells (MRC-5).

The co-crystal structure of 3 further suggested the possibility to establish an interaction with Arg153 via introducing an o-carboxylic group in its D-ring as shown in the docking model (Fig. 7). However, 29 failed to show enhanced MtTMPK inhibitory activity (Table 3).

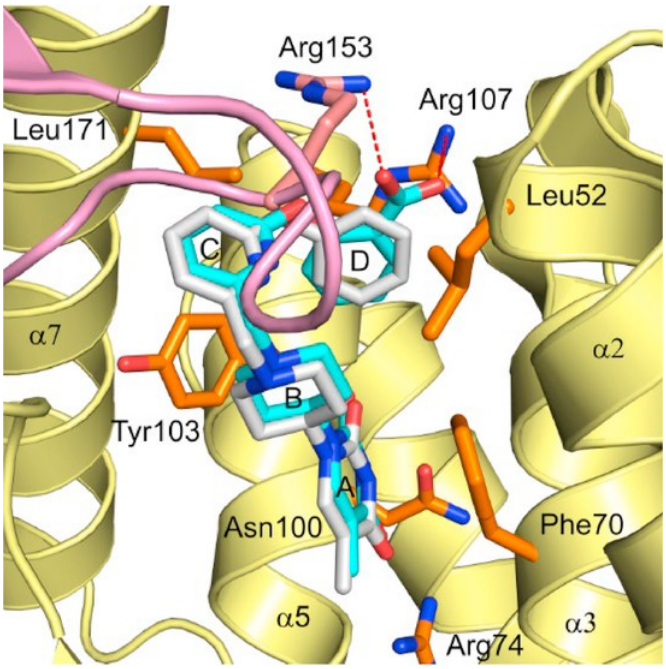


Fig. 7. Docking model of compound 29 (carbon atoms in cyan) in MtTMPK's active site, highlighting the envisaged electrostatic interaction (red dashed line) between the carboxyl moiety and Arg107 and Arg153 of the LID loop (depicted in pink) in close proximity to ring D. The co-crystal structure of MtTMPK (pale yellow ribbon representation) with compound 3 (stick representation, carbon atoms in white) was used a template. Key MtTMPK interacting residues with 3 are shown in orange for carbon atoms.

Based on the SAR within series 2, we decided to synthesize analogues 30–36, which combine a pyridine ring C with the most promising D-ring substitution pattern, e.g. 3-CF3O or 3,5-Cl2 (Table 3). Introduction of these modifications furnished the most potent enzyme inhibitors of series 3. Specifically, the 3-Cl/CF3O substituents lower the enzymatic activity by 3–8 fold compared to analogues of series 1 (3/30, 4/31, 5/33, 5/34, 6/36). Following the same SAR discovered from derivatives of series 2, 3,5-Cl2 even resulted in further improvement in enzyme inhibitory potency (31/32, 33/35), indicating that a hydrophobic and electron-poor D-ring is favorable for inhibitory activity, and thus confirming our earlier observation. A co-crystal structure of one of the potent MtTMPK inhibitors resulting from this endeavor, i.e. 33, was solved at 2.1 Å resolution (PDB code: 5NRQ) and is shown in Fig. 8.

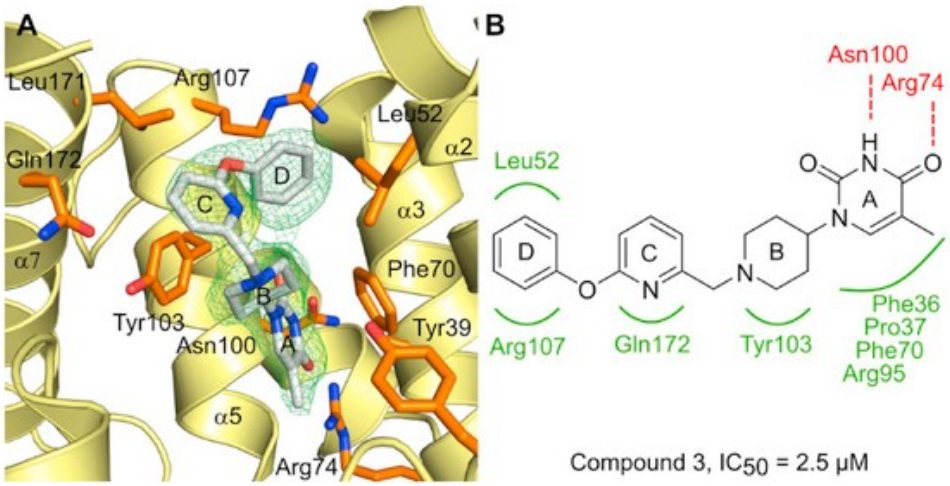


Fig. 8. Crystal structure of MtTMPK in complex with compound 33 at 2.1 Å resolution and key interactions with the enzyme (deposited as PDB code 5NRQ). (A). MtTMPK is shown in pale yellow cartoon representation. Compound 33 and the side chains of interacting residues of MtTMPK are represented as grey and orange sticks for carbon atoms, respectively. The corresponding σ-weighted Fo-Fc difference electron density map (contoured to +3 σ r.s.m.d.) and calculated before adding the ligand in the refinement process is shown as a green mesh. (B) Schematic drawing of compound 33 binding site displaying key MtTMPK residues in direct contact with 33 (hydrogen bonds are represented with a red dashed line).

Inhibitor 33, which is bound in the active site of the enzyme, was positioned in the residual Fobs-Fcalc electron density and adopts a similar U-turn shaped conformation with the one observed in 3 complexed structure (Figs. 5 and 8). The MtTMPK/33 co-crystal structure shows identical binding modes for rings A and B relative to the structure of MtTMPK in complex with 3 with all major interactions conserved. The main difference between the two bound inhibitors arises from the relative orientation of ring C and D compared to ring A and B. Indeed, there is a notable conformational difference between 3 and 33 in the corresponding co-crystal structures (Figs. 5 and 8), which is well captured by changes of 30°–40° in torsions angles around bonds between ring B and C and between ring C and D. We assume that the conformational state of 33 compared to 3 is necessary to prevent a potential intramolecular steric clash between the chloro-substituent in ring D and the O [4] group in ring A. Another difference between 3 and 33 concerns the position of the nitrogen atom in ring C, for which no specific direct interactions with the binding pocket has been detected. Of note, the conformational change observed in 33 complexed structure leads to a different positioning of the pyridyl ring (ring C) which now establishes an edge-to-face π-stacking interaction with residue Tyr103 side chain. Despite this conformational change, ring D conserved its specific CH-π stacking interaction with residue Leu52 side chain in the pocket formed by residues Leu52 and Arg107.

Although analogues in series 3 exhibit potent enzymatic activity, only moderate to weak activity against M. tuberculosis H37Rv strain was observed (Table 4). No cytotoxicity was

noted among the analogues having a pyridine ring C. Additionally, analogue 30 nor 35 inhibited hTMPK at a concentration of 40 µM and 31 µM, respectively.

Compound	MIC (µg/mL)				
	H37Rv	H37Rv + verapamil	H37Rv + PAβN	KO Tap	KO Mmr
3	>250	125–62.5	125	>250	>250
13	31.25	31.25–15.6	15.6	31.25	31.25–15.6
14	31.25–15.6	15.6	15.6	31.25–15.6	31.25–15.6
25	31.25–15.6	15.6–7.8	15.6–7.8	15.6	31.25–15.6
36	31.25–15.6	31.25–15.6	31.25	31.25	31.25

Table 4
Minimal inhibitory concentrations (MICs) of compounds against M. tuberculosis H37Rv with or without efflux pump inhibitors (verapamil, PAβN) and mutant strains (KO Tap, KO Mmr).

In an effort to explain the gap between enzymatic and cellular potency, the MIC-value of some representative compounds was determined in the presence of efflux pump inhibitors or by using mutant strains. As shown in Table 4, the antimycobacterial activities of most of the investigated compounds are not affected by efflux inhibitors verapamil or PAβN, except for compound 3, whose activity was moderately improved by the efflux inhibitor verapamil [25]. Similarly, deletion of the efflux pumps Tap (Rv1258c) [26] or Mmr (Rv3065) [27] in Mtb H37Rv strain did not significantly alter susceptibility to the assayed compounds (Table 4).
The data presented in Table 4 show that efflux is probably not able to account for the activity gap between target and Mtb bacilli of the newly prepared inhibitors in this manuscript. Additionally, we initially hypothesized that perhaps more polar compounds would be able to overcome this, we evaluated whether a correlation between calculated LogP and MIC values could be identified, as has been proposed in literature [13,28–30], however, we were unable to identity any meaningful correlation.
Interestingly, recent work from GSK [31] on structurally similar benzylpiperidines, disclosed potent mycobacterial activity, which prompted us to further investigate whether permeability is an issue of our inhibitors. The first-line antibiotic, ethambutol (EMB) [32,33] and thioridazine (TZ) [34] are known to alter membrane permeability of Mtb, and can therefore be used in co-incubation experiments to check for potential synergy, which, if identified, is indicative for issues with permeability. Thus, we co-incubated three inhibitors (3, 15 and 36) with EMB and TZ at one fourth of the MIC values (MIC EMB = 2 µg/mL and MIC TZ = 7.8 µg/mL) of the antibiotics. Unfortunately, no significant differences could be observed in the presence or absence of EMB or TZ (data not shown).

3. Conclusion

To further exploit the potential of the earlier discovered MtTMPK inhibitor 1, this study investigates the structure-activity relationship of this identified lead compound. Structure-guided and Topliss-scheme-directed modifications led to the identification of potent MtTMPK inhibitors, which are among the most potent ever identified in our assay. Two enzyme-inhibitor co-crystal structures were obtained, providing insights into a new binding mode of the biphenylether tail region in the enzyme. Unfortunately, all chemistry-related efforts to overcome the difficulties to translate active enzyme inhibitors to potent antibiotics were met with failure. Nevertheless, selected compounds such as 15 and 25 displayed interesting activity (MIC ~ 12.5 µM), they lack sufficient selectivity with respect to human fibroblasts to render them suitable candidates for further development. Additionally, we verified that the observed cytotoxicity was not due to off-target inhibition of hTMPK. Studying selected compounds in efflux pump mutant strains or by co-incubation with efflux inhibitors indicated that the tested analogues were not substrates for efflux pumps. Thus, efflux probably does not provide an explanation for the vexing problem contradicting on- target versus whole-cell activity. Further, we assayed three analogues in the presence or absence of permeability-enhancing analogues ethambutol and thioridazine, which did not result in synergism, which would be indicative for permeability issues. Future studies might entail the quantification of compound accumulation [35–37] to provide a definite answer on the conundrum of enzyme vs. whole-cell activity.

4. Experimental section

4.1. Chemistry

4.1.1. General

Solvents were purchased from standard commercial sources and of analytical grade. Building blocks and reagents were used as received without any further purification. TLC analysis was performed using precoated Alugram Silica Gel F254 plates from Machery-Nagel. Spots were examined under ultraviolet light at 254 nm. Column chromatography was carried out on a Reveleris X2 (Grace-Büchi) automated flash unit using the disposable silica gel cartridges. 1H and 13C NMR spectra were recorded in CDCl3 or DMSO-d6 on a Varian Mercury 300/75 MHz spec trometer. Chemical shifts are given in parts per million (ppm δ), δ rela tive to residual solvent peak or TMS for 1H and 13C. Structural assign ment was confirmed with 1H–1H gCOSY, 1H–13C gHSQC and 1H–13C gHMBC. Exact mass measurements (HRMS) were performed on a Waters LCT Premier XE™ Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray™ interface. Samples were infused in a CH3CN/H2O (1:1) mixture at 100 µL/min. Preparative reversed phase HPLC chromatography was carried out using a Phenomenex Luna C-18 (21.2 × 250 mm) column using a liner gradient from 10% MeCN to 100% MeCN in

10 mM ammonium bicarbonate solution over 20 min and at a flow rate of 17.5 mL/min. The purity of all final compounds was determined by LC-MS analyses on a Waters Alliance 2695 XE separation module using a Phenomenex Kinetex EVO C18, 5 μm 100 × 2.1 mm column and a gradient system of HCOOH in H2O (0.1%, v/v)/HCOOH in CH3CN (0.1%, v/v) at a flow rate of 0.6 mL/min, 05:95 to 0:100 (5–100% CH3CN) in 8 min. All final compounds were shown to have a measured purity higher than 95%.
cLogP values were calculated using ChemDraw Professional, version 15.0.0.106.

4.1.2. General procedure for the synthesis of final compounds

According a procedure in literature [11], a suspension of compound 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione [15] (1 eq), substituted aromatic aldehyde (1–2 eq) and sodium triacetoxyborohydride (1.5–4 eq) in dry 1,2-dichloroethane (~0.03 M) was stirred at room temperature under argon overnight or for 48 h. The reaction mixture was evaporated and dried with oil pump for 0.5 h. The residue was purified by column chromatography (10% ethyl acetate/hexane + 0.8% Et3N – 100% ethyl acetate + 0.8% Et3N or 100% CH2Cl2 - 10% MeOH/CH2Cl2 or 100% ethyl acetate – 5% MeOH/ethyl acetate in linear gradient elution) to afford pure intermediate. The intermediate was dissolved with 80% TFA/H2O (~0.01 M), and l-cysteine hydrochloride (~0.27 M) was added to the reaction mix- ture. The reaction mixture was stirred at 72 °C for 5 h–16 h. HRMS was used to monitor the reaction progress. Once the starting material was consumed completely, the reaction mixture was cooled to room temper- ature and evaporated. The residue was dissolved with the solvent mixture (1–2 mL, MeCN/t-BuOH/H2O, v/v/v = 1/1/1 or acetic acid or H2O) and purified with RP high-performance liquid chromatography (HPLC, Phenomenex Luna C-18, a liner gradient from 10% MeCN to 100% MeCN in H2O over 20 min (10 mM ammonium bicarbonate) and a flow rate of 17.5 mL/min. After lyophilization, all final products were obtained as white powder.

5-Methyl-1-(1-((6-phenoxypyridin-2-yl)methyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (3): Following the general procedure for the synthesis of final compounds: 3-((benzyloxy)methyl)-5-methyl- 1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (92 mg, 0.28 mmol),
6-phenoxypicolinaldehyde 39 (110 mg, 0.56 mmol), sodium triacetoxyborohydride (120 mg, 0.56 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l- cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 3 (45 mg, 41%). 1H NMR (300 MHz,
DMSO-d6) δ: 1.63 (d, J = 9.67 Hz, 2H), 1.74–1.95 (m, 5H), 2.06–2.20
(m, 2H), 2.91 (d, J = 11.72 Hz, 2H), 3.48 (s, 2H), 4.17–4.33 (m, 1H),
6.84 (d, J = 8.20 Hz, 1H), 7.07–7.15 (m, 2H), 7.16–7.24 (m, 2H),
7.36–7.47 (m, 2H), 7.64 (d, J = 1.17 Hz, 1H), 7.83 (dd, J = 8.05,
7.47 Hz, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.01,
30.01 (2C), 52.24, 52.39 (2C), 62.57, 108.90, 109.40, 117.58, 120.65
(2C), 124.27, 129.67 (2C), 137.69, 140.39, 150.80, 154.14, 157.52,

mixture to yield compound 4 (62 mg, 44%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.37 Hz, 2H), 1.74–1.93 (m, 5H), 2.15 (t, J = 11.42 Hz, 2H), 2.93 (d, J = 11.42 Hz, 2H), 3.60 (s, 2H), 4.19–4.34 (m, 1H), 6.77 (dd, J = 5.56, 2.64 Hz, 1H), 7.03 (d, J = 2.64 Hz, 1H), 7.15–7.22 (m, 2H), 7.26–7.34 (m, 1H), 7.46–7.54 (m, 2H), 7.61 (d, J = 0.88 Hz, 1H), 8.37 (d, J = 5.56 Hz, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.01, 29.96 (2C), 52.22, 52.48 (2C), 62.98, 108.91, 110.43, 110.58, 120.50 (2C), 125.37, 130.44 (2C), 137.64, 150.72, 150.79, 153.79, 161.17, 163.65, 164.54.

162.26, 163.65. HRMS (ESI): calculated for [C22H24N4O3 + H]+, 393.1921; found 393.1924.

5-Methyl-1-(1-((4-phenoxypyridin-2-yl)methyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (4): Following the general procedure for the synthesis of final compounds: 3-((benzyloxy)methyl)-5-methyl- 1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (120 mg, 0.36 mmol), 4-phenoxypicolinaldehyde 46 (70.8 mg, 0.360 mmol), sodium triacetoxyborohydride (113 mg, 0.530 mmol) in dry 1,2-dichloroethane (5 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (32 mL) and l-cysteine hydrochloride (1.52 g, 8.64 mmol) was added to the reaction

5-Methyl-1-(1-((5-phenoxypyridin-3-yl)methyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (5): Following the general procedure for the synthesis of final compounds: 3-((benzyloxy)methyl)-5-methyl- 1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (98.0 mg, 0.298 mmol), 5-phenoxynicotinaldehyde 51 (80.0 mg, 0.402 mmol), sodium triacetoxyborohydride (126 mg, 0.595 mmol) in dry 1,2- dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 5 (52 mg, 44%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.67 Hz, 2H), 1.74–1.92 (m, 5H), 2.09 (t, J = 10.84 Hz, 2H), 2.90 (d, J = 10.84 Hz, 2H), 3.56 (s, 2H), 4.18–4.34 (m, 1H), 7.03–7.12 (m, 2H), 7.20 (tt, J = 7.43, 1.06 Hz, 1H), 7.34–7.39 (m, 1H), 7.40–7.48 (m, 2H), 7.63 (s, 1H), 8.27 (d, J = 1.76 Hz, 1H), 8.33 (s, 1H), 11.20 (s, 1 H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.89 (2C), 52.21 (3C), 58.13, 108.91, 118.58 (2C), 124.04, 125.69, 130.27 (2C), 135.47, 137.66, 139.55, 144.91, 150.79, 153.06, 156.11, 163.65. HRMS (ESI): calculated for [C22H24N4O3 + H]+, 393.1921; found 393.1928.

5-Methyl-1-(1-((2-phenoxypyridin-4-yl)methyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (6): Following the general procedure for the synthesis of final compounds: 3-((benzyloxy)methyl)-5-methyl- 1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (110 mg, 0.33 mmol),
2-phenoxyisonicotinaldehyde 43 (80 mg, 0.40 mmol), sodium triacetoxyborohydride (106 mg, 0.50 mmol) in dry 1,2-dichloroethane (5 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-

cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 6 (56 mg, 43%). 1H NMR (300 MHz,
DMSO-d6) δ: 1.67 (d, J = 9.37 Hz, 2H), 1.76–1.99 (m, 5H), 2.07–2.20
(m, 2H), 2.92 (d, J = 11.42 Hz, 2H), 3.56 (s, 2H), 4.20–4.37 (m, 1H),
6.99 (s, 1H), 7.05–7.15 (m, 3H), 7.17–7.25 (m, 1H), 7.36–7.46 (m,
2H), 7.66 (d, J = 1.17 Hz, 1H), 8.08 (d, J = 5.27 Hz, 1H), 11.10 (br.
s., 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.00, 29.99 (2C), 52.19,
52.43 (2C), 60.02, 108.94, 110.72, 119.07, 121.11 (2C), 124.36,
129.61 (2C), 137.67, 147.15, 150.82, 152.39, 153.99, 163.36, 163.67.
HRMS (ESI): calculated for [C22H24N4O3 + H]+, 393.1921; found 393.1927.
5-Methyl-1-(1-(3-(pyridin-2-yloxy)benzyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (7): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy) methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (148.2 mg, 0.45 mmol), 3-(pyridin-2-yloxy)benzaldehyde 62
(134 mg, 0.675 mmol), sodium triacetoxyborohydride (190 mg,
0.90 mmol) in dry 1,2-dichloroethane (15 mL) using methanol - CH2Cl2 eluent system to obtain the intermediate, which was dissolved with TFA (15 mL) and yielded compound 7 (130 mg, 74%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.96 Hz, 2H), 1.73–1.94 (m, 5H), 1.98–2.16 (m, 2H), 2.93 (d, J = 11.13 Hz, 2H), 3.52 (br. s., 2H), 4.27 (ddd, J = 11.94, 8.13, 3.95 Hz, 1H), 6.93–7.22 (m, 5H), 7.32–7.41 (m, 1H), 7.65 (s, 1H), 7.85 (ddd, J = 8.35, 7.18, 2.05 Hz, 1H), 8.16 (ddd, J = 4.98, 2.05, 0.88 Hz, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.95, 29.96 (2C), 52.30 (3C), 61.15, 108.90, 111.48, 119.00, 119.63, 121.05, 124.73, 129.34, 137.67, 140.15, 140.57, 147.46, 150.78, 153.90, 163.01, 163.64. HRMS (ESI): calculated for [C22H24N4O3 + H]+, 393.1921; found, 393.1922.
5-Methyl-1-(1-(3-(pyridin-3-yloxy)benzyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (8): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy) methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione
(126 mg, 0.384 mmol), 3-(pyridin-3-yloxy)benzaldehyde 63 (153 mg,
0.768 mmol), sodium triacetoxyborohydride (163 mg, 0.768 mmol) in dry 1,2-dichloroethane (10 mL) using methanol CH2Cl2 eluent system to obtain the intermediate, which was dissolved with TFA (15 mL) and yielded compound 8 (69 mg, 46%). 1H NMR (300 MHz,
DMSO-d6) δ: 1.64 (d, J = 9.67 Hz, 2H), 1.73–1.93 (m, 5H), 1.97–2.14
(m, 2H), 2.91 (d, J = 11.72 Hz, 2H), 3.51 (s, 2H), 4.26 (tt,
J = 12.05, 3.92 Hz, 1H), 6.94 (dt, J = 8.13, 1.21 Hz, 1H), 7.01–7.07

(m, 1H), 7.14 (d, J = 7.91 Hz, 1H), 7.32–7.47 (m, 3H), 7.64 (d, J = 1.17 Hz, 1H), 8.38 (br. s., 2H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.97, 29.97 (2C), 52.30 (3C), 61.11, 108.90, 117.15, 118.72, 124.41, 124.72 (br. s., 1C) 125.50, 129.95, 137.66, 140.79, 141.25, 144.46, 150.79, 155.98 (2C), 163.64. HRMS (ESI): calculated for [C22H24N4O3 + H]+, 398.1921; found, 393.1939.

5-Methyl-1-(1-(3-(pyridin-4-yloxy)benzyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (9): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy) methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (148 mg, 0.45 mmol), 3-(pyridin-4-yloxy)benzaldehyde 64 (135 mg, 0.675 mmol), sodium triacetoxyborohydride (191 mg, 0.90 mmol) in dry 1,2-dichloroethane (15 mL) using methanol - CH2Cl2 eluent system to obtain the intermediate, which was dissolved with TFA (10 mL) and yielded compound 9 (45 mg, 26%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.37 Hz, 2H), 1.73–1.95 (m, 5H), 2.00–2.15 (m, 2H), 2.92 (d, J = 11.13 Hz, 2H), 3.55 (s, 2H), 4.27 (ddd, J = 11.94, 7.98, 4.10 Hz, 1H), 6.93 (d, J = 3.22 Hz, 2H), 7.04–7.10 (m, 1H), 7.10–7.15 (m, 1H), 7.25 (d, J = 7.62 Hz, 1H), 7.40–7.49 (m, 1H), 7.65 (d, J = 1.17 Hz, 1H), 8.49 (br. s., 2H), 11.19 (br. s., 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.96, 29.97 (2C), 52.29 (3C), 60.96, 108.89, 112.06 (br. s. 2C), 119.12, 120.47, 125.73, 130.17, 137.68, 141.51, 150.79 (2C), 151.54, 153.55, 163.64, 163.98. HRMS (ESI): calculated for [C22H24N4O3 + H]+, 393.1921; found, 393.1910.

1-(1-(3-(Cyclopentyloxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (10): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (131 mg, 0.399 mmol),3-(cyclopentyloxy) benzaldehyde 66 (114 mg, 0.598 mmol), sodium triacetoxyborohydride (127 mg, 0.598 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - hexane eluent system to obtain the intermediate, which was dissolved with TFA (10 mL) and yielded compound 10 (86 mg, 56%). 1H NMR (300 MHz, DMSO-d6) δ: 1.54–2.11 (m, 17H), 2.92 (d, J = 10.84 Hz, 2H), 3.46 (s., 2H), 4.16–4.34 (m, 1H), 4.73–4.84 (m, 1H), 6.74–6.89 (m, 3H), 7.16–7.26 (m, 1H), 7.65 (s, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.00, 23.60 (2C), 29.98 (2C), 32.27 (2C), 52.33 (3C), 61.58 (1C), 78.38, 108.90, 113.51, 115.89, 120.59, 129.12, 137.69, 140.01 (br. s., 1C), 150.80, 157.58, 163.67. HRMS (ESI): calculated for [C22H29N3O3 + H]+, 384.2282; found, 384.2280.

1-(1-(3-(Cyclohexyloxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (11): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (132 mg, 0.400 mmol),3-(cyclohexyloxy) benzaldehyde 68 (123 mg, 0.600 mmol), sodium triacetoxyborohydride (127 mg, 0.600 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - hexane eluent system to

obtain the intermediate, which was dissolved with TFA (10 mL) and yielded compound 11 (50.4 mg, 32%). 1H NMR (300 MHz, DMSO-d6) δ: 1.19–1.79 (m, 14H), 1.79–1.97 (m, 4H), 1.97–2.15 (m, 2H), 2.91 (d, J = 11.13 Hz, 2H), 3.46 (s, 2H), 4.17–4.40 (m, 2H), 6.76–6.90 (m, 3H), 7.16–7.25 (m, 1H), 7.65 (d, J = 0.88 Hz, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.74, 22.88 (2C), 24.90, 29.75 (2C), 31.08 (2C), 52.08 (2C), 52.14, 61.32, 73.81, 108.67, 113.57, 116.07, 120.50, 128.91, 137.44, 139.84, 150.56, 156.98, 163.41. HRMS (ESI): calculated for [C23H31N3O3 + H]+, 398.2438; found, 398.2435.

1-(1-(3-(4-Chlorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (12): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (120.0 mg, 0.364 mmol), 3-(4-chlorophenoxy) benzaldehyde 73 (169 mg, 0.728 mmol), sodium triacetoxyborohydride (154 mg, 0.728 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate hexane eluent system to ob tain the intermediate, which was dissolved with TFA (15 mL) and yielded compound 12 (80.0 mg, 52%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.37 Hz, 2H), 1.72–1.95 (m, 5H), 1.98–2.16 (m, 2H), 2.91 (d, J = 11.13 Hz, 2H), 3.51 (s, 2H), 4.26 (ddd, J = 11.86, 8.05, 4.10 Hz, 1H), 6.92 (dd, J = 8.20, 1.76 Hz, 1H), 6.98–7.07 (m, 3H), 7.12 (d, J = 7.62 Hz, 1H), 7.31–7.40 (m, 1H), 7.40–7.48 (m, 2H), 7.64 (s, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.96 (2C), 52.30 (3C), 61.14, 108.91, 117.37, 118.93, 120.04 (2C), 124.26, 127.05, 129.87 (3C), 137.66, 141.14, 150.80, 155.78, 156.16, 163.65. HRMS (ESI): calculated for [C23H24ClN3O3 + H]+, 426.1579; found, 426.1597.

1-(1-(3-(4-Methoxyphenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (13): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (153 mg, 0.465 mmol), 3-(4-methoxyphenoxy) benzaldehyde 74 (159 mg, 0.700 mmol), sodium triacetoxyborohydride (197 mg, 0.93 mmol) in dry 1,2-dichloroethane (15 mL) using ethyl acetate - hexane eluent system to obtain the intermediate, which was dissolved with TFA (10 mL) and yielded compound 13 (90.3 mg, 46%).1H NMR (300 MHz, DMSO-d6) δ: 1.58–1.70 (m, 2H), 1.72–1.91 (m, 5H), 1.98–2.10 (m, 2H), 2.89 (d, J = 11.42 Hz, 2H), 3.46 (s, 2H), 3.75 (s, 3H), 4.17–4.34 (m, 1H), 6.78 (ddd, J = 8.05, 2.49, 0.88 Hz, 1H), 6.89–6.92 (m, 1H), 6.93–7.05 (m, 5H), 7.28 (t, J = 7.91 Hz, 1H), 7.63 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.97, 29.97 (2C), 52.32 (3C), 55.39, 61.31, 108.90, 115.06 (2C), 115.76, 117.45, 120.58 (2C), 122.95, 129.54, 137.65, 140.72, 149.42, 150.79, 155.55, 157.97, 163.64. HRMS (ESI): calculated for

[C24H27N3O4 + H]+, 422.2074; found, 422.2055.

5-Methyl-1-(1-(3-(p-tolyloxy)benzyl)piperidin-4-yl)pyrimidine- 2,4(1H,3H)-dione (14): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5- methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (114 mg, 0.34 mmol), 3-(p-tolyloxy)benzaldehyde 75 (146 mg, 0.69 mmol), sodium triacetoxyborohydride (146 mg, 0.69 mmol) in dry 1,2- dichloroethane (10 mL) using ethyl acetate - hexane eluent system

to obtain the intermediate, which was dissolved with TFA (15 mL) and yielded compound 14 (45.4 mg, 79%). 1H NMR (300 MHz,
DMSO-d6) δ: 1.64 (d, J = 10.25 Hz, 2H), 1.72–1.92 (m, 5H), 2.04
(t, J = 10.98 Hz, 2H), 2.29 (s, 3H), 2.90 (d, J = 10.84 Hz, 2H),
3.48 (s, 2H), 4.17–4.33 (m, 1H), 6.84 (d, J = 7.91 Hz, 1H),
6.87–6.97 (m, 3H), 7.05 (d, J = 7.62 Hz, 1H), 7.19 (d,
J = 8.20 Hz, 2H), 7.26–7.36 (m, 1H), 7.64 (s, 1H), 11.19 (s, 1H).
13C NMR (75 MHz, DMSO-d6) δ: 11.97, 20.23, 29.97 (2C), 52.30
(3C), 61.26, 108.90, 116.58, 118.22, 118.73, 123.41, 129.62,
130.38 (2C), 132.53, 137.66, 140.82, 150.79, 154.21, 157.14,
163.64. HRMS (ESI): calculated for [C24H27N3O3 + H]+, 406.2125; found, 406.2116.

1-(1-(3-(3,4-Dichlorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (15): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (152 mg, 0.460 mmol), 3-(3,4-dichlorophenoxy)
benzaldehyde 76 (184 mg, 0.690 mmol), sodium triacetoxyborohydride (195 mg, 0.920 mmol) in dry 1,2-dichloroethane (15 mL) using ethyl acetate - hexane eluent system to obtain the intermediate, which was dissolved with TFA (15 mL) and yielded compound 15 (64.5 mg, 31%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.37 Hz, 2H), 1.72–1.94 (m, 5H),
1.99–2.15 (m, 2H), 2.91 (d, J = 11.42 Hz, 2H), 3.52 (s,
2H), 4.26 (ddd, J = 11.94, 7.98, 4.10 Hz, 1H), 6.95–7.03
(m, 2H), 7.04–7.08 (m, 1H), 7.17 (d, J = 7.62 Hz, 1H),
7.29 (d, J = 2.64 Hz, 1H), 7.35–7.43 (m, 1H), 7.59–7.67
(m, 2H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ:
11.98, 29.99 (2C), 52.28 (3C), 61.03, 108.91, 117.77, 118.45,
119.26, 120.04, 124.82, 125.16, 130.03, 131.60,
131.98,
137.66, 141.32, 150.80, 155.50, 156.56, 163.65. HRMS (ESI):
calculated for [C23H23Cl2N3O3 + H]+, 460.1189; found, 460.1178.

1-(1-(3-(3-Chlorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (16): Following the general procedure for the synthesis of final compounds (method 1): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4
(1H,3H)-dione (100.0 mg, 0.300 mmol), 3-(3-chlorophenoxy)
benzaldehyde 77 (141 mg, 0.600 mmol), sodium triacetoxyborohydride (127 mg, 0.600 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - hexane eluent system

to ob tain the intermediate, which was dissolved with TFA (10 mL) and yielded compound 16 (63 mg, 50%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.37 Hz, 2H), 1.73–1.94 (m, 5H), 1.99–2.14 (m, 2H), 2.91 (d, J = 10.54 Hz, 2H), 3.52 (s, 2H), 4.17–4.35 (m, 1H), 6.96 (dd, J = 8.20, 2.05 Hz, 2H), 7.05 (d, J = 1.76 Hz, 2H), 7.12–7.23 (m, 2H), 7.34–7.45 (m, 2H), 7.64 (s, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.01, 29.98 (2C), 52.30 (3C), 61.10, 108.96, 116.82, 117.86, 118.07, 119.37, 123.19, 124.71, 130.03, 131.52, 133.98, 137.69, 141.21, 150.83, 155.69, 158.04, 163.70. HRMS (ESI): calculated for [C23H24Cl N3O3 + H]+, 426.1579; found, 426.1578.

5-Methyl-1-(1-(3-(m-tolyloxy)benzyl)piperidin-4-yl) pyrimidine-2,4(1H,3H)-dione (17): Following the general proce dure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (165 mg, 0.500 mmol), 3-(m-tolyloxy)benzaldehyde 78 (212 mg, 1.00 mmol), sodium triacetoxyborohydride (212 mg, 1.00 mmol) in dry 1,2-dichloroethane (15 mL) using ethyl acetate - hexane eluent system to obtain the intermediate as colorless gel (220.0 mg, 83.7%). The obtained intermediate (120 mg, 0.228 mmol) was dissolved with 80% TFA/H2O (24 mL) and l- cysteine hydrochloride (1.02 g, 6.48 mmol) was added to the reac tion mixture to yield compound 17 (72.0 mg, 78%). 1H NMR (300 MHz, DMSO-d6) δ: 1.64 (d, J = 9.37 Hz, 2H), 1.73–1.92 (m, 5H), 1.99–2.12 (m, 2H), 2.29 (s, 3H), 2.91 (d, J = 11.42 Hz, 2H), 3.49 (s, 2H), 4.18–4.34 (m, 1H), 6.75–6.90 (m, 3H), 6.92–7.01 (m, 2H), 7.08 (d, J = 7.91 Hz, 1H), 7.26 (t, J = 7.91 Hz, 1H), 7.30–7.37 (m, 1H), 7.64 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 20.93, 29.98 (2C), 52.31 (3C), 61.23, 108.90, 115.51, 117.10, 118.71, 118.99, 123.72, 124.06, 129.69, 129.74, 137.67, 139.70, 140.88, 150.80, 156.63, 156.69, 163.65. HRMS (ESI): calculated for [C24H27N3O3 + H]+, 406.2125; found, 406.2127.

1-(1-(3-(3-Fluorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (18): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (92.2 mg, 0.280 mmol), 3-(3-fluorophenoxy) benzaldehyde 84 (121 mg, 0.560 mmol), sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 18 (58.7 mg, 51%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.37 Hz, 2H), 1.73–1.93 (m, 5H), 1.99–2.13 (m, 2H), 2.91 (d, J = 11.42 Hz, 2H), 3.51 (s, 2H), 4.17–4.34 (m, 1H), 6.78–6.89 (m, 2H), 6.92–7.01 (m, 2H), 7.02–7.07 (m,

1H), 7.15 (d, J = 7.62 Hz, 1H), 7.34–7.46 (m, 2H), 7.64 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.99 (2C), 52.31 (3C), 61.10, 105.65 (d, JCF = 25.50 Hz), 108.90, 109.91 (d, JCF = 21.80 Hz), 113.97 (d, JCF = 2.30 Hz), 117.74, 119.26, 124.55, 129.92, 131.32 (d, JCF = 9.00 Hz), 137.67, 141.21, 150.80, 155.69, 158.40 (d, JCF = 11.52 Hz), 162.87 (d, JCF = 243.80 Hz), 163.65, several signals overlapped. 19F NMR (282 MHz, DMSO-d6) δ: 111.02 to −110.90 (m). HRMS (ESI): calculated for [C23H24FN3O3 + H]+, 410.1874; found 410.1881.

1-(1-(3-(3-Hydroxyphenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (19): Following the general procedure for the synthesis of final compounds: 3-((benzyloxy) methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (132 mg, 0.400 mmol), 3-bromobenzaldehyde 80 (148 mg, 0.800 mmol), sodium triacetoxyborohydride (169 mg, 0.800 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate 81 (106 mg, 53%). According to a modified literature procedure [18], the suspension of the intermediate 81 (106 mg, 0.213 mmol), 83 (240 mg, 1.07 mmol), K3PO4 (158 mg, 0.746 mmol), picolinic acid (9.2 mg, 0.075 mmol) and CuI (8.1 mg, 0.043 mmol) in dry DMSO (3 mL) yielded the intermediate, which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 19 (21 mg, 24%). 1H NMR (300 MHz, CDCl3) δ: 1.65 (d, J = 9.96 Hz, 2H), 1.72–1.92 (m, 5H), 2.07 (s, 2H), 2.92 (d, J = 10.54 Hz, 2H), 3.51 (s, 2H), 4.19–4.34 (m, 1H), 6.34–6.38 (m, 1H), 6.41 (m, 1H), 6.52 (m, 1H), 6.90 (dd, J = 8.05, 1.90 Hz, 1H), 7.00 (s, 1H), 7.09 (d, J = 7.62 Hz, 1H), 7.15 (t, J = 8.20 Hz, 1H), 7.34 (t, J = 9.00 Hz, 1H), 7.63 (s, 1H), 9.58 (s, 1H), 11.19 (s, 1H). 13CNMR (75 MHz, DMSO-d6) δ: 12.00, 29.93 (2C), 52.28 (3C), 61.17, 105.33, 108.82, 108.93, 110.43, 117.55, 119.07, 123.93, 129.70, 130.41, 137.67, 141.30 (1C, it cannot be found from CNMR, but can be observed in the 1H–13C gHMBC spectrum), 150.80, 156.40, 157.98, 158.81, 163.67.

1-(1-(3-(3-Methoxyphenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (20): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (148 mg, 0.450 mmol), 3-(3-methoxyphenoxy) benzaldehyde 79 (205 mg, 0.900 mmol), sodium triacetoxyborohydride (191 mg, 0.900 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H2O (40 mL) and l-cysteine hydrochloride (1.70 g, 10.8 mmol) was added to the reaction mixture to yield compound 20 (70.0 mg, 37%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 10.25 Hz, 2H), 1.73–1.93 (m, 5H), 1.96–2.17 (m, 2H), 2.92 (d, J = 8.79 Hz, 2H), 3.40–3.60 (m, 2H), 3.73 (s, 3 H), 4.19–4.35 (m, 1H), 6.49–6.60 (m, 2H), 6.72 (ddd,

J = 8.35, 2.34, 0.73 Hz, 1H), 6.91 (d, J = 8.20 Hz, 1H), 7.01 (br. s., 1H), 7.10 (d, J = 7.62 Hz, 1H), 7.28 (t,
J = 8.20 Hz, 1H), 7.31–7.40 (m, 1H), 7.63 (br. s., 1H),
11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.89, 29.86
(2C), 52.13–52.13 (3C), 55.15, 61.07, 104.37, 108.82, 108.90,
110.24, 117.28, 118.82, 123.90, 129.66, 130.41,
137.57,
140.80, 150.69, 156.25, 157.84, 160.59, 163.56. HRMS (ESI):
calculated for [C24H27N3O4+ H]+, 422.2074; found, 422.2092.

1-(1-(3-(4-Chloro-3-(trifluoromethyl)phenoxy)benzyl) piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (21): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)
pyrimidine-2,4(1H,3H)-dione (92 mg, 0.28 mmol), 3-(4-chloro-3-
(trifluoromethyl)phenoxy)benzaldehyde 85 (168 mg, 0.560 mmol),
sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2- dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 21 (73.5 mg, 53%). 1H NMR (300 MHz, DMSO-d6) δ:
1.64 (d, J = 9.37 Hz, 2H), 1.73–1.93 (m, 5H),
2.00–2.14 (m, 2H), 2.91 (d, J = 11.42 Hz, 2H), 3.53 (s, 2H), 4.26 (m,
1H), 7.02 (ddd, J = 8.05, 2.49, 0.88 Hz, 1H), 7.07–7.11 (m, 1H), 7.19
(d, J = 7.62 Hz, 1H), 7.29 (dd, J = 8.79, 3.22 Hz, 1H), 7.38–7.46 (m,
2H), 7.62 (d, J = 1.17 Hz, 1H), 7.72 (d, J = 8.79 Hz, 1H). 13C NMR
(75 MHz, DMSO-d6) δ: 12.00, 29.99 (2C), 52.27 (3C), 61.01, 108.93,
117.21 (q, JCF = 5.30 Hz), 117.97, 119.45, 120.56, 123.19, 124.19,
125.11, 127.90 (q, JCF = 31.30 Hz), 130.16, 133.41, 137.63, 141.43,
150.83, 155.24, 156.17, 163.70, several signals overlapped or could not be found. 19F NMR (282 MHz, DMSO-d6) δ: 61.68. HRMS (ESI): calculated for [C24H23ClF3N3O3 + H]+, 494.1453; found 494.1460.

1-(1-(3-(3,4-Difluorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (22): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (92.2 mg, 0.280 mmol), 3-(3,4-difluorophenoxy)
benzaldehyde 86 (131 mg, 0.560 mmol), sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 22 (75.6 mg, 63%). 1H NMR (300 MHz, DMSO-d6) δ: 1.63 (d,
J = 9.67 Hz, 2H), 1.72–1.90 (m, 5H), 1.98–2.12 (m, 2H),
2.90 (d, J = 11.72 Hz, 2H), 3.49 (s, 2H), 4.17–4.32 (m,
1H), 6.79–6.87 (m, 1H), 6.87–6.93 (m, 1H), 6.99–7.03 (m,
1H), 7.12 (d, J = 7.62 Hz, 1H), 7.18 (ddd, J = 11.72,
6.74, 2.93 Hz, 1H), 7.32–7.39 (m, 1H), 7.45 (dt, J = 10.54,
9.23 Hz, 1H), 7.63 (d, J = 1.17 Hz, 1H), 11.18 (s, 1H).

13C NMR (75 MHz, DMSO-d6) δ: 11.97, 29.99 (2C), 52.31 (3C), 61.10, 108.30–109.57 (m), 108.91, 114.84 (dd, JCF = 6.91, 3.45 Hz), 117.07, 118.16 (d, JCF = 18.43 Hz), 118.70, 124.30, 129.89, 137.67, 141.20, 145.75 (dd, JCF = 239.30, 12.80 Hz), 149.72 (dd, JCF = 246.00, 15.00 Hz), 150.80, 153.07 (dd, JCF = 8.06, 2.30 Hz), 156.25, 163.67, several signals overlapped or could not be found. 19F NMR (282 MHz, DMSO-d6) δ: 145.33–145.07 (m), −135.46–135.20 (m). HRMS (ESI): calculated for [C23H23F2N3O3 + H]+, 428.1780; found, 428.1773.

5-Methyl-1-(1-(3-(3-(trifluoromethyl)phenoxy)benzyl) piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (23): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (92.2 mg, 0.280 mmol), 3-(3-(trifluoromethyl) phenoxy)benzaldehyde 87 (149 mg, 0.560 mmol), sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 23 (84.1 mg, 65%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.67 Hz, 2H), 1.73–1.91 (m, 5H), 1.99–2.15 (m, 2H), 2.92 (d, J = 11.13 Hz, 2H), 3.53 (s, 2H), 4.26 (t, J = 12.01 Hz, 1H), 6.99 (dd, J = 8.05, 1.90 Hz, 1H), 7.07 (s, 1H), 7.18 (d, J = 7.32 Hz, 1H), 7.25–7.34 (m, 2H), 7.36–7.44 (m, 1H), 7.49 (d, J = 7.91 Hz, 1H), 7.58–7.68 (m, 2H) 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.95 (2C), 52.27 (3C), 61.04, 108.93, 114.33 (q, JCF = 3.40 Hz), 117.95, 119.48, 119.68 (q, JCF = 3.40 Hz), 121.97, 124.88, 125.54, 130.10, 130.69 (d, JCF = 31.5 Hz) 131.45, 137.64, 141.26, 150.80, 155.46, 157.59, 163.67, several signals overlapped or could not be found. 19F NMR (282 MHz, DMSO-d6) δ: 61.27. HRMS (ESI): calculated for [C24H24F3N3O3 + H]+, 460.1843; found, 460.1837.

5-Methyl-1-(1-(3-(3-(trifluoromethoxy)phenoxy)benzyl) piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (24): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (49.4 mg, 0.150 mmol), 3-(3-(trifluoromethoxy) phenoxy)benzaldehyde 88 (84.7 mg, 0.300 mmol), sodium triacetoxyborohydride (63.6 mg, 0.300 mmol) in dry 1,2-dichloroethane (6 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reac tion mixture to yield compound 24 (30.3 mg, 42%). 1H NMR (300 MHz, DMSO-d6) δ: 1.64 (d, J = 9.37 Hz, 2H), 1.74–1.92 (m, 5H), 2.00–2.12 (m, 2H), 2.91 (d, J = 12.01 Hz, 2H), 3.52 (s, 2H), 4.18–4.34 (m, 1H), 6.95–7.03 (m, 3H), 7.05–7.08 (m, 1H),

7.09–7.15 (m, 1H), 7.17 (d, J = 7.62 Hz, 1H), 7.36–7.43 (m, 1H), 7.47–7.55 (m, 1H), 7.63 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). 13CNMR (75 MHz, DMSO-d6) δ: 11.97, 29.98 (2C), 52.28 (3C), 61.07, 108.91, 110.79, 115.26, 116.74, 117.92, 119.45, 119.96 (q, JCF = 255.00 Hz) 124.82, 130.03, 131.51, 137.63, 141.29, 149.23, 150.80, 155.40, 158.33, 163.65.19F NMR (282 MHz, DMSO-d6) δ: 56.86. HRMS (ESI): calculated for [C24H24F3N3O4 + H]+, 476.1792; found 476.1810.

1-(1-(3-(3,5-Dichlorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (25): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (92.2 mg, 0.280 mmol), 3-(3,5-dichlorophenoxy) benzaldehyde 89 (150 mg, 0.560 mmol), sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 25 (85.4 mg, 66%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.08 Hz, 2H), 1.73–1.94 (m, 5H), 2.00–2.14 (m, 2H), 2.91 (d, J = 11.42 Hz, 2H), 3.53 (s, 2H), 4.20–4.33 (m, 1H), 6.99–7.05 (m, 3H), 7.07–7.11 (m, 1H), 7.19 (d, J = 7.91 Hz, 1H), 7.36 (t, J = 1.90 Hz, 1H), 7.41 (t, J = 7.50 Hz, 1H), 7.64 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.99 (2C), 52.28 (3C), 60.98, 108.91, 116.81 (2C), 118.16, 119.63, 122.79, 125.23, 130.15, 134.94 (2C), 137.66, 141.43, 150.80, 154.92, 158.75, 163.65. HRMS (ESI): calculated for [C23H23Cl2N3O3 + H]+, 460.1189; found 460.1191.

1-(1-(3-(3,5-Difluorophenoxy)benzyl)piperidin-4-yl)-5- methylpyrimidine-2,4(1H,3H)-dione (26): Following the general procedure for the synthesis of final compounds (method 2): 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (92.2 mg, 0.280 mmol), 3-(3,5-difluorophenoxy) benzaldehyde 90 (131 mg, 0.560 mmol), sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 26 (65.8 mg, 57%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.96 Hz, 2H), 1.73–1.94 (m, 5H), 2.00–2.13 (m, 2H), 2.92 (d, J = 11.42 Hz, 2H), 3.53 (s, 2H), 4.27 (m, 1H), 6.65–6.77 (m, 2H), 6.94–7.04 (m, 2H), 7.07–7.12 (m, 1H), 7.20 (d, J = 7.62 Hz, 1H), 7.37–7.45 (m, 1H), 7.64 (d, J = 1.17 Hz, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.99 (2C), 52.30 (3C), 61.03, 98.63 (t, JCF = 26.30 Hz, 2C), 101.67 (dd, JCF = 19.50, 9.00 Hz),

108.91, 118.16, 119.66, 125.17, 130.07, 137.66, 141.43,
150.80, 154.88, 159.36 (t, JCF = 14.30 Hz), 163.07 (dd,
JCF = 243.80, 15.30 Hz, 2C), 163.65, several signals overlapped. 19F NMR (282 MHz, DMSO-d6) δ: 108.41–108.14 (m). HRMS (ESI): calculated for [C23H23F2N3O3 + H]+, 428.1780;
found 428.1786.

1-(1-(3-(3,5-Bis(trifluoromethyl)phenoxy)benzyl)piperidin-4- yl)-5-methylpyrimidine-2,4(1H,3H)-dione (27): Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (86.2 mg, 0.260 mmol),3-(3,5-bis
(trifluoromethyl)phenoxy)benzaldehyde 91 (167 mg,
0.500 mmol), sodium triacetoxyborohydride (110 mg,
0.520 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (32 mL) and l- cysteine hydrochloride (1.4 g, 8.9 mmol) was added to the reaction mixture to yield compound 27 (43.0 mg, 31%). 1H NMR (300 MHz, CD3OD) δ: 1.76–1.97 (m, 7H), 2.19 (td, J = 11.86, 2.34 Hz, 2H), 3.03 (d, J = 11.72 Hz, 2H), 3.60 (s, 2H), 4.33–4.45 (m, 1H), 7.06 (ddd, J = 8.05, 2.49, 0.88 Hz, 2H), 7.15–7.18 (m, 1H), 7.27 (d, J = 7.91 Hz, 1H), 7.42–7.49 (m, 4H), 7.67 (s, 1H). 13C NMR (75 MHz, CD3OD) δ: 10.87, 29.84 (2C), 52.36 (2C), 53.10, 61.47, 110.06, 115.54–115.69 (m), 117.57 (d, JCF = 3.46 Hz, 2C), 124.82 (q, JCF = 270.00 Hz, 2C), 118.64, 120.25, 125.99, 130.08, 132.96 (q, JCF = 33.80 Hz, 2C), 137.62, 140.92, 151.41, 155.16, 159.24, 164.84, several signals overlapped or cannot be found. 19F NMR (282 MHz, CD3OD) δ: 64.58. HRMS (ESI): calculated for [C25H23F6N3O3 + H]+, 528.1716; found 528.1718.

1-(1-((6-(4-Hydroxyphenoxy)pyridin-2-yl)methyl)piperidin-4- yl)-5-methylpyrimidine-2,4(1H,3H)-dione (28): Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (86.6 mg, 0.263 mmol), 6-(4-hydroxyphenoxy)
picolinaldehyde 94 (85.0 mg, 0.395 mmol), sodium triacetoxyborohydride (111 mg, 0.526 mmol) in dry 1,2-dichloroethane

(10 mL) using ethyl acetate - methanol eluent system to ob tain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 28 (54.8 mg, 51%). 1H NMR (300 MHz, DMSO-d6) δ: 1.64 (d, J = 9.37 Hz, 2H), 1.74–1.94 (m, 5H), 2.13 (t,
J = 11.13 Hz, 2H), 2.92 (d, J = 11.13 Hz, 2H), 3.47 (s,
2H), 4.17–4.33 (m, 1H), 6.70 (d, J = 9.00 Hz, 1H),
6.74–6.81 (m, 2H), 6.89–6.97 (m, 2H), 7.13 (d,
J = 9.00 Hz, 1H), 7.65 (s, 1H), 7.76 (t, J = 9.00 Hz,
1H), 9.34 (s, 1H), 11.20 (s, 1H). 13C NMR (75 MHz,
DMSO-d6) δ: 12.01, 29.99 (2C), 52.25, 52.46 (2C), 62.68,
108.36, 108.91, 115.87 (2C), 116.85, 122.03 (2C), 137.69,

140.10, 145.89, 150.80, 154.16, 157.35, 163.18, 163.67. HRMS (ESI): calculated for [C22H24N4O4 + H]+, 409.1870; found 409.1874.

2-((6-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) piperidin-1-yl)methyl)pyridin-2-yl)oxy)benzoic acid (29) Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (116 mg, 0.352 mmol), methyl 2-((6-formylpyridin- 2-yl)oxy)benzoate 96 (125 mg, 0.488 mmol), sodium triacetoxyborohydride (207 mg, 0.975 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - methanol eluent system to obtain the picolinate intermediate, which was dissolved in THF (8 mL). NaOH (141 mg, 3.52 mmol) in H2O (4 mL) was added to the reaction mixture at room temperature. The solution was stirred at room tem- perature for 2 days and 2 M aq. HCl was used to adjust the pH to 3. The suspension was concentrated to offer the picolinic acid intermediate, which was dissolved with 80% TFA/H2O (32 mL) and l- cysteine hydrochloride (1.36 g, 8.64 mmol) was added to the reac tion mixture to yield compound 29 (82.4 mg, 54%). 1H NMR (300 MHz, D2O) δ: 1.81–2.03 (m, 7H), 3.00–3.17 (m, 2H), 3.48 (d, J = 12.59 Hz, 2H), 4.21 (s, 2H), 4.47–4.60 (m, 1H), 6.98 (d, J = 8.20 Hz, 1H), 7.05 (d, J = 7.32 Hz, 1H), 7.12 (dd, J = 8.05, 1.03 Hz, 1H), 7.26 (td, J = 7.62, 1.17 Hz, 1H), 7.34 (d, J = 0.88 Hz, 1H), 7.43 (td, J = 7.76, 1.76 Hz, 1H), 7.56 (dd, J = 7.62, 1.76 Hz, 1H), 7.79 (dd, J = 8.35, 7.47 Hz, 1H). 13C NMR (75 MHz, D2O) δ: 11.35, 27.02 (2C), 50.65 (2C), 52.07, 59.09, 111.29, 111.87, 118.71, 122.60, 125.52, 128.79, 130.86, 132.91, 138.42, 141.52, 146.60, 149.59, 151.91, 163.25, 166.29, 174.75. HRMS (ESI): calculated for [C22H24N4O5 + H]+, 437.1819; found 437.1821.

1-(1-((6-(3-Chlorophenoxy)pyridin-2-yl)methyl)piperidin-4- yl)-5-methylpyrimidine-2,4(1H,3H)-dione (30): Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (92.2 mg, 0.280 mmol), 6-(3-chlorophenoxy) picolinaldehyde 40 (130 mg, 0.560 mmol), sodium triacetoxyborohydride (119 mg, 0.560 mmol) in dry 1,2-dichloroethane (10 mL) using ethyl acetate - hexane eluent system to ob tain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 30 (50.2 mg, 42%). 1H NMR (300 MHz, DMSO-d6) δ: 1.63 (d, J = 9.08 Hz, 2H), 1.74–1.95 (m, 5H), 2.08–2.23 (m, 2H), 2.92 (d, J = 11.13 Hz, 2H), 3.50 (s, 2H), 4.25 (m, 1H), 6.93 (d, J = 7.62 Hz, 1H), 7.10 (m, 1H), 7.19–7.30 (m, 3H), 7.39–7.48 (m, 1H), 7.63 (d, J = 0.88 Hz, 1H), 7.82–7.91 (m, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.01, 30.01 (2C), 52.21, 52.33 (2C), 62.43, 108.91, 109.86, 118.24, 119.48, 120.84, 124.26, 131.02, 133.46, 137.64, 140.67, 150.79, 154.97, 157.41, 161.65, 163.65. HRMS (ESI): calculated for [C22H23ClN4O3 + H]+, 427.1531; found 427.1552.

1-(1-((4-(3-Chlorophenoxy)pyridin-2-yl)methyl)piperidin-4- yl)-5-methylpyrimidine-2,4(1H,3H)-dione (31): Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (96.5 mg, 0.290 mmol), 4-(3-chlorophenoxy) picolinaldehyde 47 (90.0 mg, 0.390 mmol), sodium triace- toxyborohydride (93.1 mg, 0.440 mmol) in dry 1,2- dichloroethane (10 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (32 mL) and l-cysteine hydrochloride (1.52 g, 8.64 mmol) was added to the reaction mixture to yield compound 31 (57.2 mg, 46%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.67 Hz, 2H), 1.75–1.93 (m, 5H), 2.17 (t, J = 11.28 Hz, 2H), 2.94 (d, J = 11.42 Hz, 2H), 3.62 (s, 2H), 4.19–4.35 (m, 1H), 6.84 (dd, J = 5.71, 2.49 Hz, 1H), 7.07 (d, J = 2.64 Hz, 1H), 7.17 (m, 1H), 7.31–7.41 (m, 2H), 7.48–7.55 (m, 1H), 7.61 (d, J = 1.17 Hz, 1H), 8.41 (d, J = 5.56 Hz, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.01, 29.98 (2C), 52.17, 52.45 (2C), 62.88, 108.91, 110.84 (2C), 119.17, 120.62, 125.37, 131.83, 134.22, 137.61, 150.79, 150.91, 154.83, 161.39, 163.65, 163.96. HRMS (ESI): calculated for [C22H23ClN4O3 + H]+, 427.1531; found 427.1547.

1-(1-((4-(3,5-Dichlorophenoxy)pyridin-2-yl)methyl)piperidin- 4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (32): Following the general procedure for the synthesis of final compounds: 3((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (85.6 mg, 0.260 mmol), 4-(3,5-dichlorophenoxy) picolinaldehyde 48 (69.7 mg, 0.260 mmol), sodium triacetoxyborohydride (110 mg, 0.520 mmol) in dry 1,2-dichloroethane (5 mL) using ethyl acetate - methanol eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 32 (34.2 mg, 29%). 1H NMR (300 MHz, DMSO-d6) δ: 1.60–1.72 (m, 2H), 1.78 (d, J = 0.59 Hz, 3H), 1.81–1.93 (m, 2H), 2.19 (t, J = 10.84 Hz, 2H), 2.96 (d, J = 11.72 Hz, 2H), 3.65 (s, 2H), 4.21–4.37 (m, 1H), 6.92 (dd, J = 5.71, 2.49 Hz, 1H), 7.12 (d, J = 2.64 Hz, 1H), 7.37 (d, J = 2.05 Hz, 2H), 7.55 (t, J = 1.90 Hz, 1H), 7.61 (d, J = 0.88 Hz, 4H), 8.44 (d, J = 5.56 Hz, 1H), 11.21 (s, 4H). 13C NMR (75 MHz, DMSO-d6) δ: 12.03, 29.93 (2C), 52.08, 52.40 (2C), 62.71, 108.93, 111.13, 111.16, 119.60 (2C), 125.14, 135.17 (2C), 137.60, 150.80, 151.06, 155.47, 161.44, 163.47, 163.65. HRMS (ESI): calculated for [C22H22Cl2N4O3 + H]+, 461.1142; found 461.1141.

1-(1-((5-(3-Chlorophenoxy)pyridin-3-yl)methyl)piperidin-4- yl)-5-methylpyrimidine-2,4(1H,3H)-dione (33): Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (82.3 mg, 0.25 mmol),5-(3-chlorophenoxy) nicotinaldehyde 53 (116 mg, 0.500 mmol), sodium triacetoxy- borohydride (106 mg, 0.500 mmol) in dry 1,2-dichloroethane (8 mL) using ethyl acetate - methanol eluent

system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 33 (52.0 mg, 49%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.67 Hz, 2H), 1.74–1.93 (m, 5H), 2.10 (t, J = 10.98 Hz, 2H), 2.91 (d, J = 11.13 Hz, 2H), 3.58 (s, 2H), 4.19–4.35 (m, 1H), 7.04 (ddd, J = 8.27, 2.42, 1.03 Hz, 1H), 7.18 (t, J = 2.20 Hz, 1H), 7.22–7.29 (m, 1H), 7.41–7.49 (m, 2H), 7.63 (d, J = 0.88 Hz, 1H), 8.32 (d, J = 2.34 Hz, 1H), 8.37 (s, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.00, 29.92 (2C), 52.17 (3C), 58.04, 108.91, 116.99, 118.45, 123.90, 126.36, 131.66, 134.13, 135.69, 137.64, 139.95, 145.58, 150.79, 152.33, 157.33, 163.65. HRMS (ESI): calculated for [C22H23ClN4O3 + H]+, 427.1531; found 427.1545.

5-Methyl-1-(1-((5-(3-(trifluoromethoxy)phenoxy)pyridin-3-yl) methyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (34): According to a literature procedure [38], methyl 5-(3-(trifluoromethoxy)phenoxy)nicotinate 54 (165 mg, 0.528 mmol) was reduced by LiAlH4 and oxidized by MnO2 sequentially to offer the crude intermediate 5-(3- (trifluoromethoxy)phenoxy)nicotinaldehyde 55, which was used for next step directly. Following the general procedure for the synthesis of final compounds: 3-((benzyloxy) methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (85.6 mg, 0.260 mmol),the obtained crude intermediate, sodium triacetoxyborohydride (110 mg, 0.520 mmol) in dry 1,2-dichloroethane (5 mL) using ethyl acetate - methanol eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l- cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 34 (34.3 mg, 28%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d, J = 9.67 Hz, 2H), 1.75–1.91 (m, 5H), 2.10 (t, J = 10.98 Hz, 2H), 2.91 (d, J = 11.42 Hz, 2H), 3.58 (s, 2H), 4.18–4.34 (m, 1H), 7.06–7.12 (m, 2H), 7.19 (dt, J = 8.20, 1.03 Hz, 1H), 7.44–7.48 (m, 1H), 7.51–7.58 (m, 1H), 7.62 (d, J = 1.17 Hz, 1H), 8.33 (d, J = 2.64 Hz, 1H), 8.39 (d, J = 1.17 Hz, 1H) 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 11.98, 29.92 (2C), 52.17 (3C), 58.04, 108.91, 111.31, 116.04, 116.99, 121.66 (t, JCF = 225.00 Hz), 126.58, 131.74, 135.73, 137.60, 140.07, 145.79, 149.26, 149.29, 150.79, 152.08, 157.61,

163.64, several signals cannot be found. 19F NMR (282 MHz, CD3OD) δ: 56.87. HRMS (ESI): calculated for [C23H23F3N4O4 + H]+, 477.1744; found 477.1745.

1-(1-((5-(3,5-Dichlorophenoxy)pyridin-3-yl)methyl)piperidin- 4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (35): According to a literature procedure [39], thionyl chloride (0.99 mL, 1.4 mmol) was added dropwise to a solution of 5-(3,5-dichlorophenoxy)nicotinic acid 58 (194 mg, 0.684 mmol) in methanol (10 mL) to offer the crude nicotinate intermediate. which was first reduced by LiAlH4, followed by oxidation with Dess-Martin reagent according to a literature procedure [38] to give nicotinaldehyde intermediate 59. Following the general procedure for the synthesis of final compounds: 3- ((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (71.1 mg, 0.216 mmol), 5-(3,5-dichlorophenoxy)
nicotinaldehyde 59, sodium triacetoxyborohydride (183 mg, 0.862 mmol) in dry 1,2-dichloroethane (5 mL) using ethyl acetate - methanol eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g, 8.1 mmol) was added to the reaction mixture to yield compound 35 (34.5 mg, 35%). 1H NMR (300 MHz, DMSO-d6) δ: 1.65 (d,
J = 9.37 Hz, 2H), 1.76–1.92 (m, 5H), 2.10 (t, J = 10.69 Hz, 2H),
2.91 (d, J = 11.42 Hz, 2H), 3.59 (s, 2H), 4.21–4.33 (m, 1H), 7.17
(d, J = 1.76 Hz, 2H), 7.43 (t, J = 1.90 Hz, 1H), 7.48–7.52 (m, 1H),
7.63 (d, J = 0.88 Hz, 1H), 8.36 (d, J = 2.64 Hz, 1H), 8.41 (d, J = 1.46 Hz, 1H), 11.19 (br. s., 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.00, 29.95 (2C), 52.16 (3C), 57.98, 108.91, 117.29 (2C), 123.61,
126.80, 135.08, 135.84, 137.63, 140.16, 146.11, 150.79, 151.78,
158.01, 163.65. HRMS (ESI): calculated for [C22H22Cl2N4O3 + H]+,
461.1142; found 461.1143.

1-(1-((2-(3-Chlorophenoxy)pyridin-4-yl)methyl)piperidin-4- yl)-5-methylpyrimidine-2,4(1H,3H)-dione (36): Following the general procedure for the synthesis of final compounds: 3-

((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4 (1H,3H)-dione (96.5 mg, 0.290 mmol), 2-(3-chlorophenoxy)
isonicotinaldehyde 44 (83.6 mg, 0.350 mmol), sodium triace-
toxyborohydride (93.2 mg, 0.440 mmol) in dry 1,2- dichloroethane (5 mL) using ethyl acetate - hexane eluent system to obtain the intermediate which was dissolved with 80% TFA/H2O (30 mL) and l-cysteine hydrochloride (1.3 g,
8.1 mmol) was added to the reaction mixture to yield compound 36 (59.0 mg, 48%). 1H NMR (300 MHz, DMSO-d6) δ: 1.67 (d, J = 9.37 Hz, 2H), 1.75–1.98 (m, 5H), 2.14 (t, J = 10.98 Hz, 2H), 2.93 (d, J = 11.72 Hz, 2H), 3.58 (s, 2H), 4.21–4.36 (m, 1H), 7.05 (s, 1H), 7.09–7.16 (m, 2H), 7.24–7.32 (m, 2H), 7.39–7.48 (m, 1H), 7.67 (d, J = 1.17 Hz, 1H), 8.10 (d, J = 4.69 Hz, 1H), 11.20 (s, 1H). 13C NMR (75 MHz, DMSO-d6) δ: 12.00, 29.99 (2C), 52.17, 52.42 (2C), 59.96, 108.94, 110.91, 119.51, 120.01, 121.37, 124.45, 131.00, 133.44, 137.66, 147.14, 150.82, 152.69, 154.79, 162.86, 163.67. HRMS (ESI): calculated for [C22H23ClN4O3 + H]+, 427.1531; found 427.1539.

2-Bromo-6-(1,3-dioxan-2-yl)pyridine (38): According to a literature procedure [40], 38 was obtained as white solid. 1H NMR (300 MHz, CDCl3) δ: 1.46 (dtt, J = 13.55, 2.53, 2.53, 1.39, 1.39 Hz,
1H), 2.15–2.32 (m, 1H), 3.96–4.06 (m, 2H), 4.24–4.32 (m, 2H), 5.53
(s, 1H), 7.43–7.50 (m, 1H), 7.56–7.63 (m, 2H). HRMS (ESI): calculated for [C9H10BrNO2 + H]+, 243.9968; found, 243.9960.

4.1.3. General procedure for Ullmann coupling

According to a modified literature procedure [16], a suspension of the protected aromatic aldehyde (1 eq), substituted aromatic halide or substituted phenol (3 eq), Cs2CO3 (3 eq), and N,N- dimethylglycine.hydrochloride (0.3 eq) and CuI (0.1 eq) in dry 1,4- dioxane (~0.3 M) was heated at 90 °C under argon or under air (with a drying tube) for 24 h. After cooling to room temperature, CH2Cl2 (3 times the suspension volume) was added to the reaction mixture, followed by filtration through Celite®. Next, work-up was effected by employing either one of the following methods: Method 1: the filtrate was evaporated and the obtained residue was dissolved with 5 M aq. NaOH (the suspension volume) and extracted with CH2Cl2 (two times the suspension volume) for three times. The combined organic layers were washed with 5 N aq. NaOH (two times the suspension volume) for two times. TLC was used to check the presence of product in the water layer. If the combined water layers contain product, they need to be re- extracted with CH2Cl2 (two times the suspension volume) for three times. Next, all the organic layers are combined, washed with water (3 times the suspension volume) and brine (3 times the suspension volume) sequentially. After drying over Na2SO4 and filtration, the concentrated residue was purified with column chromatography (100% hexane – 10% ethyl acetate/hexane in linear gradient elution or 100% toluene – 10% ethyl acetate/toluene in linear gradient elution). Method 2: the filtrate was evaporated and the residue was dried with oil pump high vacuum for 0.5 h, the residue was purified with column chromatography (100% hexane/ethyl acetate – 90% hexane/ethyl acetate in linear gradient elution or 100% toluene/ethyl acetate – 90% toluene/ethyl acetate in linear gradient elution) to offer the desired intermediate, which was used directly for the acetal hydrolysis step.

4.1.4. General procedure for acetal hydrolysis

The obtained Ullmann coupling intermediate was dissolved with 2 M aq. HCl/THF (v/v = 1/1, 3/4 or 4/3, ~0.15 M). The reaction mixture was stirred at room temperature or at 50–60 °C for 2–7 h. TLC was used to monitor the reaction progress. There are two methods for the work-up procedure. Method 1: the reaction mixture was diluted with ethyl acetate (1.5 times the solvent volume) and brine (0.5 times the solvent volume). The organic layer was separated and washed with sat. aq. NaHCO3 (0.5 times the solvent volume) and brine (0.5 times the sol-

vent volume) sequentially. After drying over Na2SO4 and filtration, the concentrated residue was purified with column chromatography (100% hexane – 10% ethyl acetate/hexane in linear gradient elution or 100% toluene – 10% ethyl acetate/toluene in linear gradient elution). Method 2: the reaction mixture was neutralized with sat. aq.

NaHCO3 solution (0.5 times the solvent volume), followed by extraction with ethyl acetate (the solvent volume) for three times. The combined organic layers were washed with water (the solvent volume) and brine (the solvent volume) sequentially. After drying over Na2SO4 and filtration, the concentrated residue was purified with column chromatography (100% hexane – 10% ethyl acetate/hexane in linear gradient elution or 100% toluene – 10% ethyl acetate/toluene in linear gradient elution).

6-Phenoxypicolinaldehyde (39): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 2), the suspension of 2-bromo-4-(1,3-dioxan-2-yl)pyridine 38 (0.49 g, 2.0 mmol), phenol (0.56 g, 6.0 mmol), Cs2CO3 (2.0 g,
6.0 mmol), N,N-dimethylglycine hydrochloride (84 mg, 0.60 mmol) and CuI (38.1 mg, 0.2 mmol) in dry 1,4-dioxane (8 mL) under air with a drying tube yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (20 mL/15 mL) at room temperature. The reaction mixture was heated at 50 °C to offer compound 39 as light yellow solid (129 mg, 32%). 1H NMR (300 MHz, CDCl3) δ: 7.12 (dd, J = 8.20, 0.88 Hz, 1H), 7.17–7.23 (m, 2H), 7.24–7.29 (m, 1H), 7.40–7.47 (m, 2H), 7.69 (dd, J = 7.32, 0.88 Hz, 1H), 7.85 (ddd, J = 8.20, 7.32, 0.88 Hz, 1H), 9.85 (d, J = 0.59 Hz, 1H). HRMS (ESI): calculated for [C12H9NO2 + H]+, 200.0706; found, 200.0714.

6-(3-Chlorophenoxy)picolinaldehyde (40): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 2), the suspension of 2-bromo-4-(1,3-dioxan- 2-yl)pyridine 38 (0.49 g, 2.0 mmol), 3-chlorophenol (0.77 g, 6.0 mmol), Cs2CO3 (3.0 g, 6.0 mmol), N,N-dimethylglycine hydrochloride (84 mg, 0.60 mmol) and CuI (38 mg, 0.20 mmol) in dry 1,4-dioxane (8 mL) under air with a drying tube yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (20 mL/15 mL) at room temperature. The reaction mixture was heated at 50 °C to offer compound 40 as light yellow gel (280 mg, 54%). 1H NMR (300 MHz, CDCl3) δ: 7.08–7.12 (m, 1H), 7.16 (dd, J = 7.91, 0.88 Hz, 1H),
7.21–7.25 (m, 2H), 7.32–7.39 (m, 1H), 7.72 (dd, J = 7.32, 0.88 Hz,
1H), 7.86–7.92 (m, 1H), 9.83 (d, J = 0.59 Hz, 1H). HRMS (ESI): calculated for [C12H9ClNO2 + H]+, 234.0316; found, 234.0325.

2-Bromo-4-(1,3-dioxan-2-yl)pyridine (42): According to a literature procedure [40], compound 42 was obtained as colorless oil. 1H NMR (300 MHz, CDCl3) δ: 1.43–1.53 (m, 1H), 2.11–2.29 (m, 1H), 3.92–4.03 (m, 2H), 4.23–4.32 (m, 2H), 5.45 (s, 1H), 7.32–7.37 (m, 1H),
7.61 (d, J = 0.59 Hz, 1H), 8.36 (dd, J = 4.98, 0.59 Hz, 1H). HRMS
(ESI): calculated for [C9H10BrNO2 + H]+, 243.9968; found, 243.9973. 2-Phenoxyisonicotinaldehyde (43): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 2), the suspension of 2-bromo-4-(1,3-dioxan-2-yl)pyridine 42 (0.38 g, 1.6 mmol), phenol (0.44 g, 4.7 mmol), Cs2CO3 (1.5 g,
4.7 mmol), N,N-dimethylglycine hydrochloride (65 mg, 0.47 mmol) and CuI (30 mg, 0.16 mmol) in dry 1,4-dioxane (4 mL) under air with a drying tube yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) at room temperature. The reaction mixture was heated at 50 °C to offer compound 43 as colorless oil (80 mg, 26%). 1H NMR (300 MHz, CDCl3) δ: 7.13–7.19 (m, 2H), 7.22–7.29 (m,
1H), 7.30 (dd, J = 1.32, 0.73 Hz, 1H), 7.40–7.47 (m, 3H), 8.41 (d,

J = 5.27 Hz, 1H), 10.05 (s, 1H). HRMS (ESI): calculated for [C12H9NO2 + H]+, 200.0706; found, 200.0712.

4- Phenoxypicolinaldehyde (46): Following the general procedure of Ullmann coupling (work-up method 2), 4-bromo-2- methylpyridine 45 (0.86 g, 5.0 mmol), phenol (1.4 g, 15 mmol), Cs2CO3 (4.9 g, 15 mmol), N,N-dimethylglycine hydrochloride (210 mg, 1.5 mmol) and CuI (95 mg, 0.50 mmol) in dry 1,4-dioxane (10 mL) under air with a drying tube yielded the intermediate as colorless oil (380 mg, 41%), the obtained intermediate (148 mg, 0.800 mmol) and SeO2 (178 mg, 1.60 mmol) was dissolved in 1,4- dioxane (6 mL) and the suspension was heated at 80 °C for overnight. After cooling to room temperature, the mixture was filtered through Celite® and the filtrate was evaporated and purified with column chromatography (100% hexane - 20% ethyl acetate/hexane in linear gradient elution) to offer compound 46 as yellow gel (20 mg, 17%). 1H NMR (300 MHz, CDCl3) δ: 7.06–7.13 (m, 3H), 7.27–7.33 (m, 1H),
7.42–7.50 (m, 3H), 8.63 (d, J = 5.56 Hz, 1H), 10.03 (s, 1H). 13C NMR (75 MHz, CDCl3) δ: 109.42, 115.97, 120.84 (2C), 126.03,
130.47 (2C), 151.70, 153.50, 154.86, 165.95, 192.93. HRMS (ESI):
calculated for [C12H9NO2 + H]+, 200.0706; found, 200.0703.

4-(3-Chlorophenoxy)picolinaldehyde (47): Following the general procedure of Ullmann coupling (work-up method 2), 4-bromo-2- methylpyridine 45 (0.86 g, 5.0 mmol), 3-chlorophenol (1.9 g,
15 mmol), Cs2CO3 (4.9 g, 15 mmol), N,N-dimethylglycine hydrochloride (209 mg, 1.50 mmol) and CuI (95 mg, 0.50 mmol) in dry 1,4- dioxane (10 mL) under air with a drying tube yielded the intermediate as colorless oil (641 mg, 58%), the obtained intermediate (176 mg, 0.800 mmol) and SeO2 (177 mg, 1.60 mmol) were dissolved in 1,4- dioxane (6 mL) and the suspension was heated at 80 °C for overnight. After cooling to room temperature, the mixture was filtered through Celite® and the filtrate was evaporated and purified with column chromatography (100% hexane - 20% ethyl acetate/hexane in linear gradient elution) to offer compound 47 as colorless gel (30.0 mg, 16%). 1H NMR (300 MHz, CDCl3) δ: 6.98–7.04 (m, 1H), 7.09 (dd, J = 5.56,
2.64 Hz, 1H), 7.13 (t, J = 2.05 Hz, 1H), 7.27–7.31 (m, 1H), 7.36–7.43
(m, 1H), 7.43–7.47 (m, 1H), 8.66 (dd, J = 5.56, 0.59 Hz, 1H), 10.04
(s, 1H). 13C NMR (75 MHz, CDCl3) δ: 109.54, 116.11, 119.01, 121.39,
126.30, 131.23, 135.78, 151.87, 154.21, 155.00, 165.35, 192.74.
HRMS (ESI): calculated for [C12H8ClNO2 + H]+, 234.0316; found, 234.0312.

4-(3,5-Dichlorophenoxy)picolinaldehyde (48): According to a literature procedure [17], a suspension of 4-bromo-2-methylpyridine 45 (0.52 g, 3.0 mmol), 3,5-dichlorophenol (0.98 g, 6.0 mmol), K3PO4
(1.3 g, 6.0 mmol), picolinic acid (74 mg, 0.60 mmol) and CuI (57 mg,
0.30 mmol) in dry DMF (5 mL) yielded the intermediate 4-(3,5- dichlorophenoxy)-2-methylpyridine as white solid (0.31 g, 41%). 1H NMR (300 MHz, CDCl3) δ: 2.54 (s, 3H), 6.68–6.74 (m, 2H), 6.99 (d, J = 1.76 Hz, 2H), 7.23 (t, J = 1.76 Hz, 1H), 8.41 (d, J = 5.57 Hz, 1H). 13C NMR (75 MHz, CDCl3) δ: 24.49, 110.08, 111.99, 119.15 (2C), 125.28, 135.92, 150.90, 155.55, 160.97, 163.66. HRMS (ESI): calculated for [C12H9Cl2NO + H]+, 254.0134; found, 254.0125.

According to a modified literature procedure [41], the suspension of 4-

(3,5dichlorophenoxy)-2-methylpyridine (160 mg, 0.63 mmol) and SeO_2 (210 mg, 1.9 mmol) in 1,4-dioxane (5 mL) was heated at 100 °C for 24 h. After cooling to room temperature, the reaction mixture was filtered through Celite®. The filtrate was evaporated and purified with column chromatography (100% hexane – 10% ethyl acetate/hexane) to offer compound 48 as white solid (34.7 mg, 21%). 1H NMR (300 MHz, $CDCl_3$) δ: 7.03 (d, J = 1.76 Hz, 2H), 7.12 (dd, J = 5.56, 2.64 Hz, 1H), 7.30 (t, J = 1.90 Hz, 1H), 7.47 (d, J = 2.64 Hz, 1H), 8.70 (d, J = 5.56 Hz, 15H), 10.05 (s, 1H). 13C NMR (75 MHz, $CDCl_3$) δ: 109.79, 116.30, 119.63 (2C), 126.36, 136.39, 152.05, 154.66, 155.14, 164.72, 192.55. HRMS (ESI): calculated for $[C_{12}H_7Cl_2NO_2 + H]^+$, 267.9927; found, 267.9925.

Methyl 5-phenoxynicotinate (50): According to a literature procedure [42], to the reaction mixture of methyl 5-hydroxynicotinate 49
(0.77 g, 5.0 mmol), phenylboronic acid (2.1 g, 18 mmol), $Cu(OAc)_2$
(1.8 g, 10 mol) and 4 Å molecular sieves (2.5 g) in CH_2Cl_2 (40 mL) was added Et_3N (5 mL). The reaction mixture was stirred at room temperature under air with a drying tube for 24 h. The crude product was purified by silica chromatography (10% ethyl acetate/hexane – 50% ethyl acetate/hexane in linear gradient) to offer compound 50 as colorless gel (163 mg, 20%). 1H NMR (300 MHz, $CDCl_3$) δ: 3.93 (s, 3H), 7.02–7.07 (m, 2H), 7.21 (tt, J = 7.36, 1.14 Hz, 1H), 7.37–7.44 (m,
2H), 7.84 (dd, J = 2.93, 1.76Hz, 1H), 8.57 (d, J = 2.93 Hz, 1H), 8.95 (d, J = 1.76 Hz, 1H).

5- Phenoxynicotinaldehyde (51): According to a literature procedure [38], methyl 5-phenoxynicotinate 50 (183 g, 0.800 mmol) was used for reduction by $LiAlH_4$ and oxidation by MnO_2 sequentially to offer compound 51 as light yellow gel (64 mg, 40%). 1H NMR (300 MHz, $CDCl_3$) δ: 7.04–7.10 (m, 2H), 7.21–7.28 (m, 1H), 7.39–7.46
(m, 2H), 7.65 (dd, J = 2.93, 1.76 Hz, 1H), 8.66 (d, J = 2.64 Hz, 1H),
8.79 (d, J = 1.76 Hz, 1H), 10.08 (s, 1H). HRMS (ESI): calculated for $[C_{12}H_9NO_2 + H]^+$, 200.0706; found, 200.0709.

Methyl 5-(3-chlorophenoxy)nicotinate (52): According to a literature procedure [42], to the suspension of methyl 5- hydroxynicotinate 49 (0.38 g, 2.5 mmol), 3-chlorophenylboronic acid (1.17 g, 7.50 mmol), $Cu(OAc)_2$ (0.91 g, 5.0 mol) and 4 Å molecular sieves (1.3 g) in CH_2Cl_2 (20 mL) was added Et_3N (2.5 mL). The reaction mixture was stirred at room temperature under air with a drying tube for 24 h to offer compound 52 as light yellow gel (112 mg, 17%). 1H NMR (300 MHz, $CDCl_3$) δ: 3.95 (s, 3H), 6.93
(ddd, J = 8.20, 2.34, 0.88 Hz, 1H), 7.04 (t, J = 2.20 Hz, 1H),
7.16–7.20 (m, 1H), 7.27–7.35 (m, 1H), 7.86 (dd, J = 2.64, 1.76 Hz,
1H), 8.58 (d, J = 2.64 Hz, 1H), 9.00 (d, J = 1.46 Hz, 1H). 13C NMR (75 MHz, $CDCl_3$) δ: 52.63, 117.16, 119.52, 124.81, 126.07,
127.00, 130.97, 135.57, 145.29, 145.73, 153.17, 156.63, 165.12.
HRMS (ESI): calculated for $[C_{13}H_{10}ClNO_3 + H]^+$, 264.0422; found, 264.0431.

5-(3-Chlorophenoxy)nicotinaldehyde (53): According to a literature procedure [38], methyl 5-(3-chlorophenoxy)nicotinate 52 (171 mg, 0.650 mmol) was reduced by $LiAlH_4$ and oxidized by MnO_2 sequentially to offer compound 53 as colorless gel (20.0 mg, 13%). 1H NMR (300 MHz, $CDCl_3$) δ: 6.96 (ddd, J = 8.13, 2.42,
0.88 Hz, 1H), 7.07 (t, J = 2.20 Hz, 1H), 7.18–7.25 (m, 1H), 7.35 (t,
J = 8.20 Hz, 1H), 7.69 (dd, J = 2.78, 1.61 Hz, 1H), 8.66 (d,

J = 2.64 Hz, 1H), 8.81–8.87 (m, 1H), 10.10 (s, 1H). HRMS (ESI):
calculated for [C12H8ClNO2 + H]+, 234.0316; found, 234.0327.
Methyl 5-(3-(trifluoromethoxy)phenoxy)nicotinate (54): According to a literature procedure [42], to a reaction mixture of methyl 5-hydroxynicotinate 49 (0.36 g, 2.4 mmol), (3-(trifluoromethoxy)phenyl)boronic acid (1.45 g, 7.04 mmol), Cu(OAc)2 (0.85 g, 4.7 mol) and 4 Å molecular sieves (1.18 g) in CH2Cl2 (10 mL) was added Et3N (2.35 mL). The reaction mixture was stirred at room temperature under air with a drying tube for 24 h to offer the compound 54 as light yellow gel (37.0 mg, 5%).1H NMR (300 MHz, CDCl3) δ: 3.95 (s, 3H), 6.91–6.98 (m, 2H), 7.07 (dt, J = 8.20,
1.03 Hz, 1H), 7.37–7.45 (m, 1H), 7.91 (dd, J = 2.78,
1.61 Hz, 1H), 8.59 (d, J = 2.93 Hz, 1H), 9.02 (d,
J = 1.46 Hz, 1H). HRMS (ESI): calculated for [C15H12F3NO3 + H]+, 312.0842; found, 312.0855.
3-(3,5-Dichlorophenoxy)-5-methylpyridine (57): According to a literature procedure [17], a suspension of 3-bromo-5-methylpyridine 56 (0.52 g, 3.0 mmol), 3,5-dichlorophenol (0.73 g, 4.5 mmol), K3PO4
(1.3 g, 6.0 mmol), picolinic acid (74 mg, 0.60 mmol) and CuI (57 mg,
0.30 mmol) in dry DMF (6 mL) yielded the compound 57 as white solid (0.35 g, 46%). 1H NMR (300 MHz, CDCl3) δ: 2.37 (s, 3H), 6.88 (d, J = 2.05 Hz, 2H), 7.12 (t, J = 1.76 Hz, 1H), 7.15–7.18 (m, 1H), 8.23 (br. s., 1H), 8.30 (s, 1H). 13C NMR (75 MHz, CDCl3) δ: 18.75, 117.46 (2C), 124.36, 127.83, 135.32, 136.33 (2C), 139.60, 146.86, 152.48,
158.63. HRMS (ESI): calculated for [C12H9Cl2NO + H]+, 254.0134; found, 254.0129.
5-(3,5-Dichlorophenoxy)nicotinic acid (58): According to a literature procedure [43], to a gently refluxing solution of 57 (0.30 g,
1.2 mmol) in pyridine (8.4 mL) and H2O (6 mL) was added KMnO4 (2.2 g, 14 mmol) in several portions to offer the product 58 (0.19 g, 56%) as white solid. 1H NMR (300 MHz, DMSO-d6) δ: 7.26–7.30 (m, 2H), 7.47 (s, 1H), 7.85 (dd, J = 2.93, 1.76 Hz, 1H), 8.70 (d,
J = 2.64 Hz, 1H), 8.92 (d, J = 1.46 Hz, 1H), 13.65 (br. s., 1H).).
13C NMR (75 MHz, DMSO-d6) δ: 118.01 (2C), 124.24, 126.39, 127.95,
135.14 (2C), 145.27, 145.93, 152.16, 157.38, 165.56.
3-(1,3-Dioxan-2-yl)phenol (61): To the solution of 3- hydroxybenzaldehyde 60 (25 g, 0.20 mol) in pyridine (100 mL) was added acetic anhydride (25 mL, 0.27 mmol) under argon in an ice bath. The reaction mixture was stirred at room temperature for 1 h, followed by dilution with cold brine (200 mL) in an ice bath. The solution was extracted with ethyl acetate (2 × 100 mL). The combined organic layer was washed with 1.2 M aq. HCl solution (2 × 100 mL), sat. NaHCO3 solution (2 × 100 mL) and brine (100 mL) sequentially. After drying over Na2SO4, evaporation in vacuum gave the crude intermediate, which was refluxing with 1,3-propanediol (19 g,
0.24 mol) in toluene (150 mL) containing p-toluenesulfonic acid (0.86 g, 4.5 mmol). Water was removed with a Dean-Stark trap. After 2 h, the production of water ceased and the mixture was allowed to cool to ambient temperature. A solution of 10% NaOH in water was added and the mixture was stirred vigorously for 2 h the pH was adjusted to 8 with concentrated HCl solution. The mixture was extracted with ethyl acetate (3 × 100 mL). The

combined organic layers were dried over Na2SO4 and evaporated. The residue was purified with column chromatography (3% ethyl acetate/toluene – 8% ethyl acetate/ toluene in linear gradient elution) to offer compound 61 as white solid (15.8 g, 43%). 1H NMR (300 MHz, CDCl3) δ: 1.45 (m, 1H), 2.23 (m, 1H), 3.93–4.04 (m, 2H), 4.23–4.31 (m, 2H), 4.86 (s, 1H), 5.46 (s, 1H), 6.77–6.81 (m, 1H), 6.97 (dd, J = 2.64, 1.46 Hz, 1H), 7.01–7.06 (m, 1H), 7.19–7.25 (m, 1H). HRMS (ESI): calculated for [C10H12O3 + H]+, 181.0859; found 181.0852.

3-(Pyridin-2-yloxy)benzaldehyde (62): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 2), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (0.72 g, 4.0 mmol), 2-bromopyridine (1.9 g, 12 mmol), Cs2CO3 (3.9 g, 12 mmol), N,N-dimethylglycine hydrochloride (170 mg, 1.2 mmol) and CuI (76.2 mg, 0.400 mmol) in dry 1,4-dioxane (15 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (15 mL/15 mL) to offer compound 62 as a colorless oil (0.67 g, 90%). 1H NMR (300 MHz, CDCl3) δ: 6.99 (dt, J = 8.20, 0.88 Hz, 1H), 7.05 (ddd, J = 7.32, 4.98, 0.88 Hz, 1H), 7.40–7.46 (m, 1H), 7.57 (t, J = 7.76 Hz, 1H), 7.66 (dd, J = 2.05, 1.46 Hz, 1H), 7.70–7.77 (m, 2H), 8.19 (ddd, J = 4.98, 2.05, 0.88 Hz, 1H), 10.01 (s, 1H). HRMS (ESI): calculated for [C12H19NO2 + H]+, 200.0706; found 200.0699.

3-(Pyridin-3-yloxy)benzaldehyde (63): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 2), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (1.08 g, 6 mmol), 3-bromopyridine (2.8 g, 18 mmol), Cs2CO3 (5.9 g, 18 mmol), N,N-dimethylglycine hydrochloride (0.25 g, 1.8 mmol) and CuI (114 mg, 0.600 mmol) in dry 1,4-dioxane (15 mL) under argon yielded the intermediate, which was dissolved with 2 N HCl/THF (5 mL/5 mL) to offer compound 63 as colorless oil (139 mg, 12%). 1H NMR (300 MHz, CDCl3) δ: 7.29–7.36 (m, 3H), 7.48–7.50 (m, 1H), 7.55 (t, J = 7.76 Hz, 1H), 7.67 (dt, J = 7.40, 1.28 Hz, 1H), 8.43–8.46 (m, 2H), 9.98 (s, 1H). HRMS (ESI): calculated for [C12H19NO2 + H]+, 200.0706; found 200.0711.

3-(Pyridin-4-yloxy)benzaldehyde (64): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (1.1 g, 6 mmol), 4-bromopyridine hydrochloride (3.5 g, 18 mmol), Cs2CO3 (8.8 g, 27 mmol), N,N-dimethylglycine hydrochloride (0.25 g, 1.8 mmol) and CuI (114 mg, 0.600 mmol) in dry 1,4-dioxane (15 mL) under argon yielded the intermediate, which was dissolved with 2 N HCl/THF (12 mL/12 mL) to offer compound 64 as a colorless oil (0.50 g, 42%). 1H NMR (300 MHz, CDCl3) δ: 6.85–6.89 (m, 2H), 7.38 (ddd, J = 7.98, 2.56, 1.17 Hz, 1H), 7.58–7.65 (m, 2H), 7.75–7.79 (m, 1H), 8.50–8.54 (m, 2H), 10.02 (s, 1H). HRMS (ESI): calculated for [C12H19NO2 + H]+, 200.0706; found 200.0701.

3-(Cyclopentyloxy)benzaldehyde (66): According to a literature procedure [44], the compound 66 was obtained as colorless oil. 1H NMR (300 MHz, CDCl3) δ: 1.62–2.02 (m, 8H), 4.83 (tt, J = 5.57, 2.78 Hz, 1H), 7.10–7.17 (m, 1H), 7.36 (dt, J = 2.64, 0.88 Hz, 1H), 7.38–7.46 (m, 2H), 9.96 (s, 1H). HRMS (ESI): calculated for [C12H14O2 + H]+, 191.1067; found 191.1062.

3-(Cyclohexyloxy)benzaldehyde (68): According to a literature procedure [44], the compound 68 was obtained as colorless oil. 1H NMR (300 MHz, CDCl3) δ: 1.31–1.64 (m, 6H), 1.75–1.88 (m, 2H),
1.92–2.06 (m, 2H), 4.28–4.39 (m, 1H), 7.13–7.20 (m, 1H), 7.36–7.46
(m, 3H), 9.96 (s, 1H). HRMS (ESI): calculated for [C13H16O2 + H]+, 205.1223; found 205.1215.
2-(3-Bromophenyl)-1,3-dioxane (70): According to a literature procedure [40], 70 was obtained as colorless gel. 1H NMR (300 MHz, CDCl3) δ: 1.41 (m, 1H), 2.08–2.27 (m, 1H), 3.88–4.00 (m, 2H),
4.17–4.30 (m, 2H), 5.44 (s, 1H), 7.17–7.24 (m, 1H), 7.36–7.47 (m,
2H), 7.65 (t, J = 1.76 Hz, 1H). HRMS (ESI): calculated for [C10H11BrO2 + H]+, 243.0015; found 243.0012.
2-(3-Iodophenyl)-1,3-dioxane (72): According to a literature procedure [40], 72 was obtained as colorless oil. 1H NMR (300 MHz, CDCl3) δ: 1.43 (m, 1H), 2.20 (m, 1H), 3.90–4.02 (m, 2H), 4.20–4.30
(m, 2H), 5.43 (s, 1H), 7.05–7.12 (m, 1H), 7.40–7.46 (m, 1H), 7.66 (dq,
J = 7.91, 0.98 Hz, 1H), 7.85 (t, J = 1.76 Hz, 1H). HRMS (ESI): calculated for [C10H11IO2 + H]+, 290.9876; found 290.9867.
3-(4-Chlorophenoxy)benzaldehyde (73): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-bromophenyl)-1,3-dioxane 70 (1.5 g, 6.0 mmol), 4-chlorophenol (2.3 g, 18 mmol), Cs2CO3 (5.9 g,
18 mmol), N,N-dimethylglycine hydrochloride (0.25 g, 1.8 mmol) and CuI (114 mg, 0.600 mmol) in dry 1,4-dioxane (10 mL) under argon yielded the intermediate, which was dissolved with 2 M HCl/THF (20 mL/20 mL) to offer compound 73 as a colorless oil (0.47 g, 33%). 1H NMR (300 MHz, CDCl3) δ: 6.94–7.01 (m, 2H), 7.25–7.30 (m, 1H),
7.31–7.37 (m, 2H), 7.45 (dd, J = 2.64, 1.46 Hz, 1H), 7.52 (t,
J = 7.91 Hz, 1H), 7.60–7.65 (m, 1H), 9.97 (s, 1H). HRMS (ESI): calculated for [C13H9ClO2 + MeCN + H]+, 274.0629; found 247.0611.
3-(4-Methoxyphenoxy)benzaldehyde (74): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-bromophenyl)-1,3dioxane 70 (0.24 g, 1.0 mmol), 4-methoxyphenol (0.37 g,
3.0 mmol), Cs2CO3 (0.98 g, 3.0 mmol), N,N-dimethylglycine hydrochloride (42 mg, 0.30 mmol) and CuI (20 mg, 0.10 mmol) in dry 1,4-dioxane (4 mL) under argon yielded the intermediate, which was dissolved with 2 M aq HCl/THF (5 mL/5 mL) to offer compound 74 as a colorless oil (88 mg, 39%). 1H NMR (300 MHz, CDCl3) δ: 3.84 (s, 3H), 6.90–6.96 (m, 2H), 6.99–7.05 (m, 2H), 7.23–7.28 (m, 1H),
7.39–7.41 (m, 1H), 7.45–7.51 (m, 1H), 7.54–7.58 (m, 1H), 9.95 (s,
1H). HRMS (ESI): calculated for [C14H12O3 + H]+, 229.0859; found 229.0849.
3-(p-Tolyloxy)benzaldehyde (75): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-bromophenyl)-1,3-dioxane 70 (1.6 g, 6.5 mmol), p-cresol (2.1 g, 20 mmol), Cs2CO3 (6.4 g, 20 mmol), N,N-dimethylglycine hydrochloride (0.27 g, 2.0 mmol) and CuI (124 mg, 0.650 mmol) in dry 1,4-dioxane (15 mL) under argon yielded the intermediate, which was

dissolved with 2 M aq. HCl/ THF (12 mL/12 mL) to offer compound 75 as yellow oil (0.72 g, 52%). 1H NMR (300 MHz, CDCl3) δ: 2.36 (s, 3H), 6.91–6.97 (m, 2H),
7.15–7.21 (m, 2H), 7.24–7.29 (m, 1H), 7.41–7.43 (m, 1H), 7.48 (t,
J = 7.76Hz, 1H), 7.55–7.59 (m, 1H), 9.95 (s, 1H). HRMS (ESI): calculated for [C14H12O2 + H]+, 213.0910; found 213.0903.

3-(3,4-Dichlorophenoxy)benzaldehyde (76): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-bromophenyl)-1,3- dioxane 70 (1.46 g, 6 mmol), 3,4-dichlorophenol (2.93 g, 18 mmol), Cs2CO3 (5.86 g, 18 mmol), N,N-dimethylglycine hydrochloride (0.25 g, 1.8 mmol) and CuI (114.3 mg, 0.6 mmol) in dry 1,4-dioxane (15 mL) under argon yielded the intermediate, which was dissolved with 2 N HCl/THF (8 mL/8 mL) to offer compound 76 as a white solid (220.8 mg, 14%). 1H NMR (300 MHz, CDCl3) δ: 6.89 (dd,
J = 8.79, 2.93 Hz, 1H), 7.13 (d, J = 2.64 Hz, 1H), 7.27–7.32 (m,
1H), 7.43 (d, J = 8.79 Hz, 1H), 7.47–7.50 (m, 1H), 7.55 (t,
J = 7.76 Hz, 1H), 7.67 (dt, J = 7.62, 1.32 Hz, 1H), 9.99 (s, 1H).
HRMS (ESI): calculated for [C13H8Cl2O2 + H]+, 266.9974; found 266.9968.

3-(3-Chlorophenoxy)benzaldehyde (77): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-bromophenyl)-1,3-dioxane
70 (0.73 g, 3.0 mmol), 3-chlorophenol (1.2 g, 9.0 mmol), Cs2CO3 (2.9 g, 9.0 mmol), N,N-dimethylglycine hydrochloride (0.13 g,
0.90 mmol) and CuI (57 mg, 0.30 mmol) in dry 1,4-dioxane (10 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (5 mL/5 mL) to offer compound 77 as a colorless oil (147 mg, 21%). 1H NMR (300 MHz, CDCl3) δ: 6.92 (ddd, J = 8.20, 2.49, 1.03 Hz, 1H), 7.02 (t, J = 2.20 Hz, 1H), 7.12–7.16 (m, 1H),
7.26–7.32 (m, 2H), 7.47–7.50 (m, 1H), 7.54 (t, J = 7.76 Hz, 1H), 7.65
(dt, J = 7.62, 1.32 Hz, 1H), 9.98 (s, 1H). HRMS (ESI): calculated for [C13H9ClO2 + H]+, 233.0364; found 233.0369.

3-(m-Tolyloxy)benzaldehyde (78): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-iodophenyl)-1,3-dioxane 72 (1.5 g, 5.0 mmol), m-cresol (1.6 g, 15 mmol), Cs2CO3 (4.9 g, 15 mmol), N,N-dimethylglycine hydrochloride (209 mg, 1.50 mmol) and CuI (95 mg, 0.50 mmol) in dry 1,4-dioxane (20 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (20 mL/20 mL) to offer compound 78 as a colorless oil (534 mg, 50%). 1H NMR (300 MHz, CDCl3) δ: 2.34 (s, 3H), 6.80–6.87 (m, 2H),
6.97 (dd, J = 7.62, 0.59 Hz, 1H), 7.21–7.29 (m, 2H), 7.43–7.51 (m,
2H), 7.56–7.60 (m, 1H), 9.94 (s, 1H). HRMS (ESI): calculated for [C14H12O2 + H]+, 213.0910; found 213.0902.

3-(3-Methoxyphenoxy)benzaldehyde (79): Following the general procedure of Ullmann coupling (work-up method 1) and the acetal hydrolysis (method 1), the suspension of 2-(3-bromophenyl)-1,3- dioxane 70 (0.73 g, 3.0 mmol), 3-methoxyphenol (1.1 g, 9.0 mmol), Cs2CO3 (2.9 g, 9.0 mmol), N,N-dimethylglycine hydrochloride (0.13 g, 0.90 mmol) and CuI (57 mg, 0.30 mmol) in dry 1,4-dioxane (10 mL) under argon yielded the intermediate,

which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer compound 79 as a colorless oil (255 mg, 37%). 1H NMR (300 MHz, CDCl3) δ: 3.79 (s,
3H), 6.58–6.64 (m, 2H), 6.69–6.74 (m, 1H), 7.23–7.32 (m, 2H),
7.47–7.53 (m, 2H), 7.59–7.63 (m, 1H), 9.96 (s, 1H). HRMS (ESI): calculated for [C14H12O3 + H]+, 229.0859; found 229.0862.

3-((tert-Butyldimethylsilyl)oxy)phenol (83): According to a literature procedure [45], to a solution of resorcinol 82 (2.75 g,
25.0 mmol) in dry DMF (20 mL) was added the mixture of imidazole (2.55 g, 37.5 mmol), t-butyldimethylsilyl chloride (3.77 g,
25.0 mmol). The desired product 83 was obtained as a colorless gel (2.80 g, 50%). 1H NMR (300 MHz, CDCl3) δ: 0.19 (s, 6H), 0.97 (s, 9H), 6.33–6.37 (m, 1H), 6.43 (dd, J = 8.20, 2.34 Hz, 2H), 7.06 (t, J = 8.05 Hz, 1H). HRMS (ESI): calculated for [C12H20O2Si + H]+, 225.1305; found, 225.1308.

3-(3-Fluorophenoxy)benzaldehyde (84): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (0.32 g, 1.8 mmol), 1-fluoro-3-iodobenzene (1.2 g, 5.4 mmol), Cs2CO3
(1.8 g, 5.4 mmol), N,N-dimethylglycine hydrochloride (75 mg,
0.54 mmol) and CuI (34 mg, 0.10 mmol) in dry 1,4-dioxane (4 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer compound 84 as a yellow oil (215 mg, 55%). 1H NMR (300 MHz, CDCl3) δ: 6.72–6.74 (m, 1H),
6.85–6.90 (m, 2H), 7.32–7.36 (m, 2H), 7.50–7.57 (m, 2H), 7.66 (d,
J = 7.62 Hz, 1 H), 9.98 (s, 1H). HRMS (ESI): calculated for [C13H9FO2 + H]+, 217.0659; found 217.0667.

3-(4-Chloro-3-(trifluoromethyl)phenoxy)benzaldehyde (85): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3- (1,3-dioxan-2-yl)phenol 61 (0.50 g, 2.8 mmol), 1-chloro-4-iodo-2-(trifluoromethyl)benzene (2.8 g, 8.4 mmol), Cs2CO3 (2.7 g,
8.4 mmol), N,N-dimethylglycine hydrochloride (0.12 g, 0.84 mmol) and CuI (53 mg, 0.28 mmol) in dry 1,4-dioxane (10 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer compound 85 as a light yellow solid (0.45 g, 53%). 1H NMR (300 MHz, CDCl3) δ: 7.09–7.15 (m, 1H), 7.28–7.33 (m, 1H), 7.36 (d, J = 2.93 Hz, 1H), 7.45–7.52 (m, 2H), 7.57 (t, J = 7.76 Hz, 1H), 7.69 (dt, J = 7.47, 1.39 Hz, 1H), 9.99 (s, 1H). HRMS (ESI): calculated for [C14H8ClF3O2 + H]+, 301.0238; found, 301.0229.

3-(3,4-Difluorophenoxy)benzaldehyde (86): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (0.32 g, 1.8 mmol), 1,2-difluoro-4-iodobenzene (1.3 g, 5.4 mmol),
Cs2CO3 (1.7 g, 5.4 mmol), N,N-dimethylglycine hydrochloride (75 mg,
0.54 mmol) and CuI (34 mg, 0.10 mmol) in dry 1,4-dioxane (4 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer compound 86 as a colorless oil (132 mg, 31%). 1H NMR (300 MHz, CDCl3) δ: 6.73–6.81 (m, 1 H) 6.88 (ddd, J = 10.98, 6.59, 2.93 Hz, 1H), 7.11–7.21 (m, 1H), 7.26–7.31 (m, 1H), 7.45 (dd, J = 2.64,

1.46 Hz, 1H), 7.53 (t, J = 7.91 Hz, 1H), 7.62–7.67 (m, 1H), 9.98 (s, 1H). HRMS (ESI): calculated for [C13H8F2O2 + H]+, 235.0565; found 235.0559.

3-(3-(Trifluoromethyl)phenoxy)benzaldehyde (87): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl) phenol 61 (0.50 g, 2.8 mmol), 1-iodo-3-(trifluoromethyl)benzene (2.3 g, 8.4 mmol), Cs2CO3 (2.7 g, 8.4 mmol), N,N-dimethylglycine hydrochloride (0.12 g, 0.84 mmol) and CuI (53 mg, 0.28 mmol) in dry 1,4-dioxane (4 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (15 mL/15 mL) to offer compound 87 as a colorless oil (0.52 g, 67%). 1H NMR (300 MHz, CDCl3) δ: 7.17–7.22 (m, 1H), 7.26–7.34 (m, 2H), 7.39–7.52 (m, 3H), 7.55 (t, J = 7.76 Hz, 1H), 7.65–7.70 (m, 1H), 9.99 (s, 1H). HRMS (ESI): calculated for [C14H9F3O2 + H]+, 267.0627; found 267.0632.

3-(3-(Trifluoromethoxy)phenoxy)benzaldehyde (88): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hyrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl) phenol 61 (0.27 g, 1.5 mmol), 1-iodo-3-(trifluoromethoxy)benzene (1.3 g, 4.5 mmol), Cs2CO3 (1.5 g, 4.5 mmol), N,N-dimethylglycine hydrochloride (63 mg, 0.45 mmol) and CuI (79 mg, 0.15 mmol) in dry 1,4-dioxane (8 mL) under air with a drying tube or under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer 88 as a colorless gel (25 mg, 6%). 1H NMR (300 MHz, CDCl3) δ: 6.90 (dt, J = 2.20, 0.95 Hz, 1H), 6.92–6.97 (m, 1H), 6.99–7.04 (m, 1H), 7.31 (ddd, J = 8.13, 2.56, 1.03 Hz, 1H), 7.37 (t, J = 8.20 Hz, 1H), 7.50–7.58 (m, 2H), 7.64–7.69 (m, 1H), 9.98 (s, 1H). HRMS (ESI): calculated for [C14H9F3O3 + H]+, 283.0577; found, 283.0585.

3-(3,5-Dichlorophenoxy)benzaldehyde (89): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (0.27 g, 1.5 mmol), 1,3-dichloro-5-iodobenzene (1.2 g, 4.5 mmol), Cs2CO3 (1.5 g, 4.5 mmol), N,N-dimethylglycine hydrochloride (63 mg, 0.45 mmol) and CuI (29 mg, 0.15 mmol) in dry 1,4-dioxane (4 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer compound 89 as a white solid (160 mg, 67%). 1H NMR (300 MHz, CDCl3) δ: 6.90 (d, J = 1.76 Hz, 2H), 7.15 (t, J = 1.76 Hz, 1H), 7.31 (ddd, J = 7.98, 2.56, 1.17 Hz, 1H), 7.51 (dd, J = 2.05, 1.46 Hz, 1H), 7.57 (t, J = 7.76 Hz, 1H), 7.68–7.73 (m, 1H), 10.00 (s, 1H). HRMS (ESI): calculated for [C13H8Cl2O2 + H]+, 266.9974; found, 266.9982.

3-(3,5-Difluorophenoxy)benzaldehyde (90): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan-2-yl)phenol 61 (0.27 g, 1.5 mmol), 1,3-difluoro-5-iodobenzene (1.1 g, 4.5 mmol), Cs2CO3 (1.5 g, 4.5 mmol), N,N-dimethylglycine hydrochloride (63 mg, 0.45 mmol) and CuI (29 mg, 0.15 mmol) in dry 1,4-dioxane (4 mL) under argon yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (10 mL/10 mL) to offer compound 90 as a light yellow oil (133 mg, 38%). 1H NMR (300 MHz, CDCl3) δ: 6.47–6.63 (m, 3H), 7.31–

7.36 (m, 1H), 7.53–7.61 (m, 2H), 7.71 (dt, J = 7.54, 1.21 Hz, 1H), 10.00 (s, 1H). HRMS (ESI): calculated for [C13H8F2O2 + H]+, 235.0565; found 235.0573.

3-(3,5-Bis(trifluoromethyl)phenoxy)benzaldehyde (91): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hydrolysis (method 1), the suspension of 3-(1,3-dioxan- 2-yl)phenol 61 (0.27 g, 1.5 mmol), 1-iodo-3,5-bis(trifluoromethyl) benzene (1.5 g, 4.5 mmol), Cs2CO3 (1.5 g, 4.5 mmol), N,N- dimethylglycine hydrochloride (63 mg, 0.45 mmol) and CuI (29 mg,
0.15 mmol) in dry 1,4-dioxane (8 mL) under air with a drying tube yielded the intermediate, which was dissolved with 2 M aq. HCl/THF (15 mL/15 mL) to offer 91 as a colorless oil (167 mg, 33%). 1H NMR (300 MHz, CDCl3) δ: 7.32–7.37 (m, 1H), 7.42 (d, J = 0.59 Hz, 2H), 7.55 (dd, J = 2.64, 1.46 Hz, 1H), 7.59–7.66 (m, 2H), 7.74–7.78 (m, 1H), 10.02 (s, 1H). HRMS (ESI): calculated for [C15H8F6O2 + H]+, 335.0501; found, 335.0511.

4-((tert-Butyldimethylsilyl)oxy)phenol (93): According to a literature procedure [45], to the solution of hydroquinone 92 (2.2 g, 20 mmol) in dry DMF (15 mL) was added the mixture of imidazole (2.0 g, 30 mmol), t-butyldimethylsilyl chloride (3.0 g, 20 mmol). The desired product was offered as a colorless gel (2.0 g, 45%). 1H NMR (300 MHz, CDCl3) δ: 0.16 (s, 6H), 0.97 (s, 9H), 4.50 (br. s, 1H), 6.70 (d, J = 1.17 Hz, 4H). HRMS (ESI): calculated for [C12H20O2Si + H]+, 225.1305; found, 225.1316.

6-(4-Hydroxyphenoxy)picolinaldehyde (94): Following the general procedure of Ullmann coupling (work-up method 2) and the acetal hyrolysis (method 1), the suspension of 2-bromo-6-(1,3-dioxan-2- yl)pyridine 38 (0.49 g, 2.0 mmol), 4-((tert-butyldimethylsilyl)oxy) phenol 93 (1.4 g, 6.0 mmol), Cs2CO3 (2.0 g, 6.0 mmol), N,N- dimethylglycine hydrochloride (84 mg, 0.60 mmol) and CuI (38 mg,
0.20 mmol) in dry 1,4-dioxane (10 mL) under air with a drying tube was heated at 90 °C for 6 h to yield the intermediate (370 mg, 16%). following a modified literature procedure [46], 1.0 M TBAF-THF solution (0.52 mL, 0.52 mmol) was added slowly dropwise to a stirred solution of the obtained intermediate (182 mg, 0.469 mmol) in dry THF (10 mL). The solution was stirred for 3 h at room temperature followed by dilution with ethyl acetate (50 mL) and washed with sat. aq. NH4Cl solution (50 mL) and brine (50 mL) sequentially. The organic layer was dried over Na2SO4 and evaporated to obtain the crude intermediate. Following the general procedure of acetal hydrolysis (method 2). The crude intermediate was dissolved with THF (6 mL) and 2 M aq. HCl (8 mL) at room temperature. The reaction mixture was heated at 65 °C for 2.5 h to offer compound 94 as a yellow gel (85 mg, 85%).
1H NMR (300 MHz, CDCl3) δ: 6.85–6.91 (m, 2H), 7.03–7.11 (m, 3H),
7.67 (dd, J = 7.32, 0.88 Hz, 1H), 7.80–7.87 (m, 1H), 9.85 (d, J = 0.88 Hz, 1H). 13C NMR (75 MHz, CDCl3) δ: 115.94, 116.35 (3C), 122.49 (2C), 140.27, 146.89, 150.83, 152.97, 164.37, 192.95. HRMS
(ESI): calculated for [C12H9NO3 + H]+, 216.0655; found, 216.0658.

Methyl 2-((6-formylpyridin-2-yl)oxy)benzoate (96): According to a literature procedure [18], the suspension of 2-bromo-4-(1,3-

dioxan-2-yl)pyridine 38 (0.49 g, 2.0 mmol), methyl 2-
hydroxybenzoate (0.46 g, 3.0 mmol), K3PO4 (0.85 g, 4.0 mmol), picolinic acid (49 mg, 0.40 mmol), and CuI (38 mg, 0.20 mmol) in dry DMSO (4 mL) under argon yielded the

intermediate, which was dissolved with 2 M aq. HCl/THF (8 mL/6 mL) at room temperature. The reaction mixture was heated at 50 °C to offer compound 96 as a light yellow gel (125 mg, 24%). 1H NMR (300 MHz, CDCl3) δ: 3.69 (s, 3H), 7.19 (dd, J = 8.20, 0.88 Hz, 1H), 7.23–7.27 (m, 1H), 7.35 (td,
J = 7.62, 1.17 Hz, 1H), 7.58–7.67, (m, 2H), 7.87 (ddd, J = 8.20,
7.32, 0.88 Hz, 1H), 8.03 (dd, J = 7.62, 1.76 Hz, 1H), 9.71 (d,
J = 0.59 Hz, 1H). 13C NMR (75 MHz, CDCl3) δ: 52.02, 116.04,
124.09, 124.23, 125.46, 131.78, 133.81, 140.16, 150.48, 152.57,
164.02, 165.43, 192.86. HRMS (ESI): calculated for [C14H11NO4 + H]+, 258.0761; found, 258.0765.

4.2. Biology

4.2.1. Expression and purification of M. tuberculosis thymidylate kinase (MtTMPK)

Recombinant MtTMPK was produced in E. coli and purified to homogeneity as previously described including slight modifications [47]. E.coli BLi5 competent cells were transformed with the bacterial expression vector pHL50 containing the cDNA sequence encoding full length wild-type MtTMPK (uniprot: P9WKE1). Transformed cells were selected on LB agar plates containing 100 μg/ml carbenicillin and 34 μg/ml chloramphenicol and directly used to inoculate 1 L of 2xYT medium supplemented with the same antibiotics. The cells were grown at 37 °C and allowed to reach the late exponential phase of growth (absorbance of 1.5 at 600 nm) at which point 1 mM isopropyl-1-thio-β-d- thiogalactoside (IPTG) was added to the culture medium. After an additional 3-h incubation period at 37 °C, cells were harvested by centrifugation at 8000×g at 4 °C and stored at −80 °C until purification. For purification, cells from 1 L culture were resuspended in 50 ml cold lysis buffer containing 50 mM Tris HCl pH 8.0 supplemented with antiprotease cocktail (Roche) and lysed by sonication. After centrifugation at 20000×g for 30 min at 4 °C, the filtered bacterial lysate was injected on a 5 ml pre-equilibrated Blue-Sepharose column. The column was washed with lysis buffer and the protein eluted with a 0–2 M NaCl gradient (50 mM Tris HCl pH 8.0, 0–2 M NaCl). Fractions containing MtTMPK 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 polishing and desalting steps. MtTMPK fractions were combined and Tris(2-carboxyethyl)-phosphine (TCEP) was added to 1 mM final concentration before flash-freezing of the protein samples in liquid nitrogen.

4.2.2. MtTMPK enzyme inhibitory assay

The compounds were dissolved in DMSO. The assays were performed at fixed concentrations of ATP (0.5 mM) and dTMP (0.05 mM), and at varying concentrations of tested compound (between 0.0008 and 0.6 mM) using the spectrophotometric assay described by Blondin et al.
[48] The reaction medium contains 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 2 mM MgCl2, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each of coupling enzymes (lactate dehydrogenase, pyruvate kinase and nucleoside diphosphate kinase). IC50 values were calculated using KaleidaGraph.

4.2.3. Protein crystallization

Cocrystallization conditions of MtTMPK-inhibitor complexes were screened at 20 °C by the sitting-drop vapor-diffusion method using a 1:1 protein: solution volume ratio and available sparse-matrix crystallization screens. First, thawed MtTMPK samples were concentrated to 6–8 mg/ml and the compound of interest added to the concentrated protein stock. The protein/inhibitor sample was incubated on ice for 1 h before setting up crystallization trials. Specific crystallization conditions for each cocrystal are described below.

For the MtTMPK/3 complex, the ligand stock solution at 50 mM in 50/50 (v/v) pure DMSO/pure isopropanol solution was added to the concentrated protein solution stock to reach a final inhibitor concentration of 1 mM. To further increase the solubility of analogue 3, a 50/50 (v/v) MtTMPK buffer/pure isopropanol solution was added to the protein/inhibitor sample to increase final isopropanol concentration from 1% (v/v) to 1.5% (v/v). An initial hit was obtained in condition 36 from crystal screen II (0.1 M HEPES pH 7.5, 4.3 M NaCl). The crystallization condition was further optimized and the best diffracting crystal obtained after a few weeks at room temperature using the crystallization condition 0.1 M HEPES pH 6.8, 4.45 M NaCl. The crystal was exposed to a cryoprotection buffer obtained by the combination of mother liquor supplemented with 1 mM compound 3 and 10% (v/v) ethylene glycol before being mounted and flash frozen in liquid nitrogen.
Initial efforts to obtain MtTMPK/33 cocrystals led to the obtention of crystals with very limited diffraction power. Better crystals were obtained using the cross-seeding approach starting from in-house grown MtTMPK crystals obtained with compound 33 related inhibitors. In short, MtTMPK cocrystals diffracting to limited resolution (3–4 angström) and initially grown in 0.1 M HEPES pH 6.8, 4.40 M NaCl were crushed in 50 µl of stabilizing solution (0.1 M HEPES pH 6.8,
4.50 M NaCl, 1% (v/v) DMSO, 1% (v/v) isopropanol, 0.5 mM compound 33) using the seed bead kit (Hampton Research) and serial dilution of seeds with stabilizing solution were performed. The MtTMPK/33 sample was prepared as previously described for compound 3 complex but using a lower protein concentration (6.0 mg/ml). Crystallization trials were set-up at room temperature using the contemporary seeding approach by mixing 0.3 µl of MtTMPK/compound 33 sample with 0.2 µl of crystallization solution and 0.1 µl of undiluted or diluted seed
stock. The best diffracting crystal was obtained after a few weeks at room temperature using the undiluted seed stock and crystallization solution consisting of 0.1 M HEPES pH 6.8, 4.45 M NaCl. This crystal was exposed to a cryoprotection buffer (mother liquor supplemented with 1 mM compound 33 and 25% (w/v) d-glucose) before being mounted and flash frozen in liquid nitrogen.
4.2.3.1. Data collection, structure determination and refinement. X-ray diffraction data for MtTMPK/3 cocrystal were collected on Proxima1 beamline at Soleil synchrotron (Paris, France) at a wavelength of 0.9184 Å and a temperature of 100 K on a Dectris Pilatus 6 M pixel detector. X-ray measurement for MtTMPK/33 cocrystal were recorded on ID23-2 beamline at ESRF (Grenoble, France) at a wavelength of 0.87290 Å and a temperature of 100 K on a Dectris Pilatus 6 M pixel detector.
All datasets were indexed, processed and scaled using XDS/XSCALE and mtz conversion was performed with XDSCONV keeping five percent of randomly selected reflections for the

Rfree set [49]. All structures were solved by molecular replacement with PDB input file 4UNR using MOLREP program from the CCP4 suite (Supporting Information, Table S1) [50]. Structures were further refined by one cycle of rigid- body refinement in BUSTER [51] followed by positional and individual isotropic B-factor refinement in BUSTER and PHENIX [52]. Models were manually improved during the course of the crystallographic refinement using the graphic program COOT [53]. For both structures, torsion NCS restraints where used throughout the refinement. Ligand coordinates were generated with the Grade Web Server (http://grade. globalphasing.org), which was also used for restraint generation. Ligand molecules were modeled in sigma-weighted Fo-Fc difference electron density maps in the course of the refinement. TLS refinement with one TLS group definition per chain was applied in the latest stage of the refinement procedure after having all atoms B-factor reset to the Wilson B-factor value. The real-space correlation coefficient (RSCC) is reported as an objective measure of the fit of inhibitor coordinates to electron density and was calculated using Twilight program [54]. Discovery studio visualizer version 16.1.0 (Dassault Systèmes BIOVIA, San Diego:

Dassault Systèmes) was used to analyze and describe the binding mode of inhibitors within the protein. The quality of the final crystal structures was assessed with MOLPROBITY [55] prior to deposition at the PDB database under the codes 5NRN (MtTMPK/3), and 5NRQ (MtTMPK/33). Molecular images were generated with PyMOL (The Py- MOL Molecular Graphics System, Schrödinger, LLC). Data collection and refinement statistics are presented in Table S1.

4.2.4. Molecular docking

The molecular modeling calculation was performed using the software packages AutoDock 4.2 on Windows Cygwin and AutodockTools 1.5.6 [56]. The solved X-ray structure of ligand 3 in complex with MtTMPK (PDB entry 5NRN) was used in all docking experiments. The 2D chemical structures and PDB files of compound 3 were drawn and created using ChemDraw 15 and ChemBioDraw 15 separately. The PDBQT file of ligand 3 and MtTMPK were prepared by AutodockTools- 1.5.6, which includes atomic partial charges, atom types and the information of the ligand torsional degrees. For the docking, a default grid spacing of 0.375 Å and 60 × 60 × 60 number of grid points were used, which centered the box on the active site of MtTMPK (e.g. the typical π-π stacking between Phe-70 and thymine ring of the ligand) [57].

The Genetic Algorithm-Local Search (GA-LS) method was adopted using default settings. 50 possible conformations were generated by Autodock 4.2 for each docking. A manual selection procedure combining visual inspection in Chimera guided by the Ligplot analysis together with the predicted free energy found for each conformation was used to validate the docked conformations.

4.2.5. In vitro cytotoxicity assay

In vitro cytotoxicity on the MRC-5 Homo sapiens long fibroblast cell line (ATCC® CCL-171™) was performed as previously described [58]. Briefly, the MRC-5 cells were cultured in 75 cm2 sterile Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum in a 5% CO2 atmosphere at 37 °C. When a semi-confluent layer of cells was formed, the cells were trypsinized, washed with sterile PBS, seeded into a

transparent, flat-bottomed 96- well plate at a density of 4 × 104 cells per well and left for recovery at 37 °C, 5% CO2 for at least 24 h. For each compound, a two-fold serial dilution was made in complete DMEM with final concentrations ranging from 128 µM to 0.5 µM. Subsequently, the MRC-5 cells were exposed to the compounds (in triplicate) by adding 100 µL of the serial dilutions to the wells. Test plates were incubated for 3 days in an atmosphere of 5% CO2 at 37 °C. For the resazurin assay, the cells were washed 2 times with 200 µL PBS and 100 µL resazurin working solution was added per well. Subsequently, the plates were left for incubation at 37 °C, 5% CO2 for 3 h. To monitor the viable cell number after compound exposure, each well was analyzed using a microplate fluorometer equipped with a 560 nm excitation/590 nm emission filter set. Tamoxifen was included as positive control (IC50 = 11.1 µM).

4.2.6. Whole-cell activity against M. tuberculosis (H37Rv)

MIC determination was performed as previously described [59]. In brief, test compounds were dissolved in DMSO at a 10 mM stock concentration. M. tuberculosis H37Rv cells (ATCC 27294) were cultured until they reached OD650nm 0.2–0.3, subsequently diluted 1000-fold in medium for the MIC determination. Compounds were 2-fold serially diluted in medium into clear, sterile round-bottom 96-well plates (Nunclon) at 50 µL per well in duplicate with a concentration range spanning 100–0.049 µM. An equal volume of the diluted cells equaling to approximately 1 × 104 bacteria per well was then added to each well. Plates were incubated for 1 week at 37 °C after which they were read with an enlarging inverted mirror plate reader. The MIC was taken as the lowest concentration that completely inhibited growth. The growth medium was 7H9/glucose/casitone/Tyloxapol, which consisted of (per liter) 4 g glucose, 4.7 g Middlebrook 7H9 broth base, 0.8 g NaCl, 0.3 g Bacto™ casitone and 0.5 mL Tyloxapol. Isoniazid was included as a positive control, and DMSO as negative control.

4.2.7. Efflux-pump studies with M. tuberculosis (H37Rv)

Experiments to study efflux were conducted as previously described [11]. Briefly, they employed M. tuberculosis strains H37Rv (reference), and KO-mutants KO-Tap [26], KO-Mmr [27], which lack the genes encoding for efflux pumps Tap and Mmr, respectively. Mycobacteria were cultured in Middlebrook 7H9 medium, supplemented with 10% ADC (albumin, dextrose, catalase supplement, BD) and 0.05% Tween80, at 37 °C. KO-strains were cultured in the same medium as mentioned, with additional supplementation of hygromycin (50 µg/mL). MIC values ware determined using resazurin [60]. Assays were performed in 96-well plates, employing a doubling dilution series starting at 500 µg/ mL. Compounds were added in 100 µL at the desired concentration (medium: Middlebrook 7H9 with 10% ADC and 0.5% glycerol supplementation). Then, bacteria were added (100 µL of a suspension containing 105 CFU/mL (determined by optical density, culture in exponential growth phase). After incubation for 6 days at 37 °C, 30 µL resazurin solution (0.01% W/V) was added to each well, and plates incubated for another 48 h at 37 °C. Assessment of colour change (blue to pink) is indicative for bacterial growth. MIC is defined as the lowest drug concentration that prevents colour change.

For assays with efflux pump inhibitors, verapamil was added at a final concentration of 40 µg/mL and PAβN at 15 µg/mL.
Changes in MIC between strains/experimental conditions were considered significant when the change was at least a 4-fold with respect to the reference strains or reference condition.

Supporting Information
Details on the data collection and refinement statistics for the cocrystal structures can be found in the Supporting Information.
NMR spectra of prepared final compounds can be found in the Supporting Information.
PDB codes

Authors will release the atomic coordinates and experimental data upon publication of the manuscript for publication: MtTMPK-3 (PDB code: 5NR5); MtTMPK-33 (PDB code: 5NRQ).

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations
ADC albumin dextrose and catalase supplement ATCC American type culture collection
BOM benzyloxymethyl
cfu/mL colony forming units per milliliters DMEM Dulbecco's modified Eagle's medium
dTMP thymidine monophosphate
EMB ethambutol
HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid IPTG isopropyl-1-thio-β-D-thiogalactoside
LB lysogeny broth
KO-Mmr H37Rv knock out for Mmr efflux pump KO-Tap H37Rv knock out for Tap efflux pump Mtb Mycobacterium tuberculosis
MtTMPK Mycobacterium tuberculosis thymidylate kinase NCS noncrystallographic symmetry
NRU neutral red uptake
PAβN phenylalanine-arginine β-naphthylamide RSCC real-space correlation coefficient

RLU relative light units
TBDMSCl tert-butyldimethylsilyl chloride TCEP tris(2-carboxyethyl)-phosphine TLS translation-libration-screw
TMP deoxythymidine monophosphate TDP deoxythymidine diphosphate dTTP deoxythymidine triphosphate
TZ thioridazine.

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