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Elaboration of a Proprietary Thymidylate Kinase Inhibitor Motif Towards Anti-Tuberculosis Agents

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Elaboration of a Proprietary Thymidylate Kinase Inhibitor Motif Towards Anti-Tuberculosis Agents

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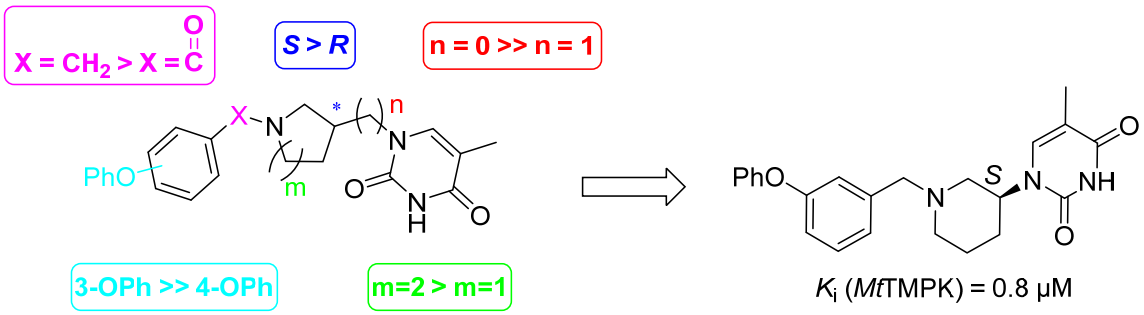
Keywords: thymine derivatives; *Mycobacterium tuberculosis*; thymidine monophosphate kinase; inhibitors

Abbreviations: HIV, human immunodeficiency virus; LTBI, latent tuberculosis infection; MDR-TB, multidrug-drug resistant tuberculosis; Mtb, Mycobacterium tuberculosis; TMPK, thymidine monophosphate kinase; dTMP, thymidine 5'-monophosphate; dTDP, thymidine 5'-diphosphate; dTTP, thymidine 5'-triphosphate; *XDR-TB, extensively drug-resistant tuberculosis.*

Graphical Abstract



Abstract

We report the design and synthesis of a series of non-nucleoside *Mtb*TMPK inhibitors (**1** - **14**) based on the gram-positive bacterial TMPK inhibitor hit compound **1**. A practical synthesis was developed to access these analogues. Several compounds show promising *Mtb*TMPK inhibitory potency and allow the establishment of a structure–activity relationship, which is helpful for further optimization.

1. Introduction

According to the global tuberculosis (TB) report in 2015, worldwide TB causes more deaths than any other disease caused by a single infectious agent. As a result, TB is ranked as the most lethal infectious disease on par with the human immunodeficiency virus HIV.[1] It is estimated that one-third of the world population is infected with asymptomatic latent TB (LTBI), with around 10% lifetime risk of developing active TB. This is especially relevant for persons with a compromised immune system[2] (one-third HIV deaths were due to TB in 2015[1]). For drug-susceptible active TB, treatment requires a standard 6 month course of 4 frontline drugs, which frequently results in poor treatment compliance. Moreover, due to the inappropriate treatment, multidrug-drug resistant (MDR) TB is now widespread (MDR-TB is defined as a rifampicin-resistant form of TB that has additional resistance to isoniazid[3]). *Mycobacterium tuberculosis* strains of the Beijing lineage are responsible for the massive spread of MDR-TB in Eurasia.[4] Furthermore extensively drug-resistant (XDR) TB has been reported by 105 countries by the end of 2014 (XDR-TB is defined as a form of MDR-TB that does not respond to any fluoroquinolone

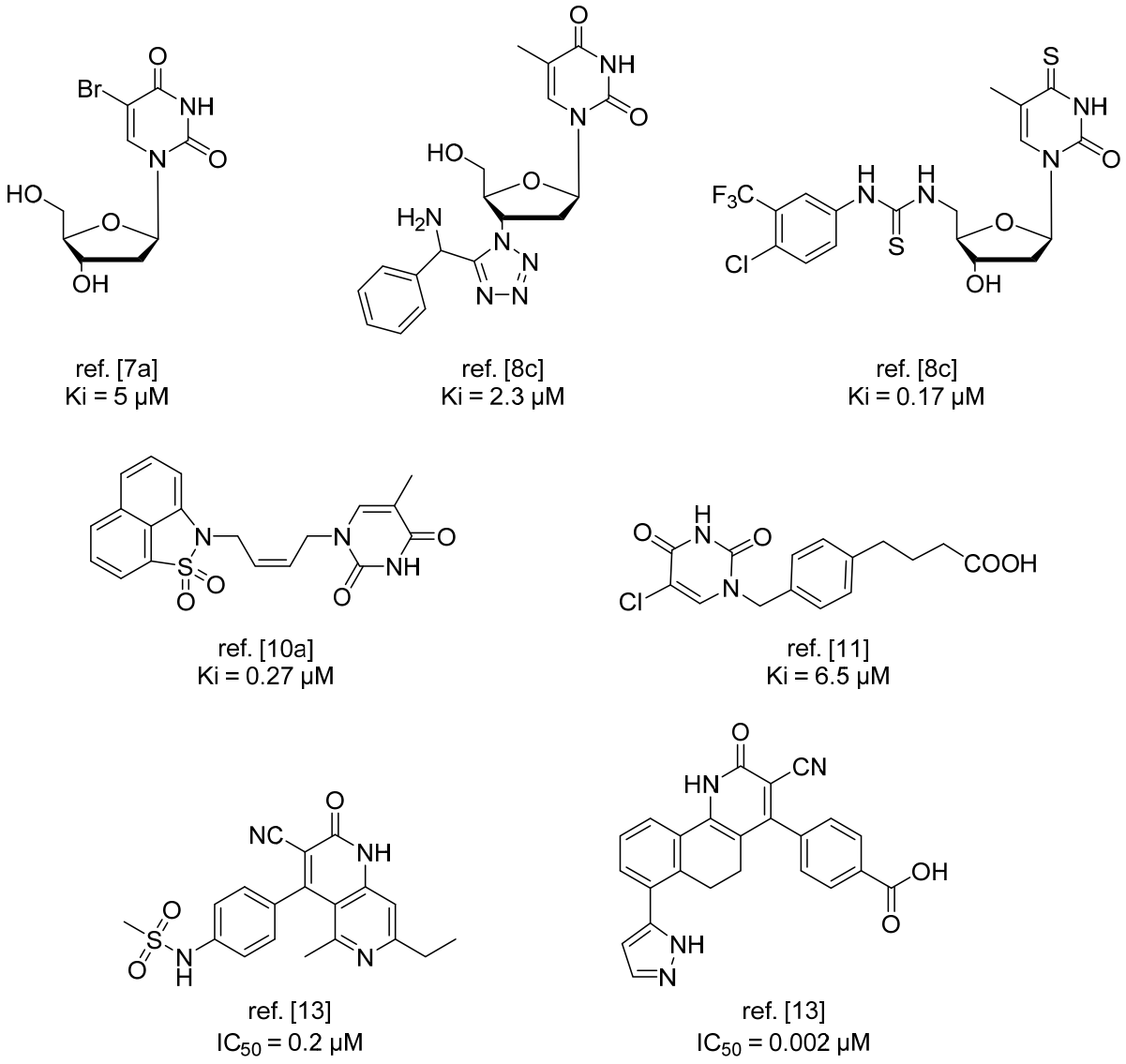
and at least one second-line injectable drug[3]). Therefore, there is an urgent need for effective and new TB drugs to shorten the treatment regime and cure MDR-TB and XDR-TB.

Thymidylate kinase (TMPK, also called thymidine 5'-monophosphate kinase) phosphorylates thymidine 5'-monophosphate (dTMP) to thymidine 5'-diphosphate (dTDP) utilizing ATP as its preferred phosphoryl donor. The synthesis of dTDP is unique in that it requires TMPK, while other deoxynucleoside diphosphates are directly produced by ribonucleotide reductase. Further phosphorylation of dTDP gives thymidine triphosphate (dTTP), which is one of the building blocks of DNA. TMPKs are subdivided into two types based on the position of basic residues within the active site.[5] Notably, *M. tuberculosis* TMPK (*Mtb*TMPK) does not belong to type I nor to the type II enzyme, revealing its unique catalytic mechanism.[6] Additionally, it also has a mere 22% sequence identity to human TMPK.[5] Moreover, TMPK is the last specific enzyme for the synthesis of dTTP. Therefore, *Mtb*TMPK inhibitors have the potential for being effective and selective anti-TB and anti-MDRTB drugs with low toxicity.

The current *Mtb*TMPK inhibitors can be categorized into two types: thymine-like inhibitors and non-thymine-like inhibitors. The thymine-like inhibitors can be further subdivided into nucleoside and non-nucleoside inhibitors. The nucleoside family is mainly composed of thymidine analogues with modifications at position 5 of the thymine base[7] and at the 3'- and/or 5'-positions of the sugar ring,[8] which give K_i values in the low micromolar range and poor anti-bacterial activity.[9] In the non-nucleoside family, most of the analogues still bear the thymine heterocyclic head with either an acyclic tail[10] or a substituted benzyl at the *N*-1 position of the thymine ring.[11] Some inhibitors of this family have submicromolar K_i values (Figure 1).[12] Two recently discovered 3-cyanopyridone and 1,6-naphthyridin-2-one *Mtb*TMPK inhibitors that show nanomolar inhibitory activity have a non-thymine-like structure.[13]

Compared to the well-explored nucleoside family, non-nucleoside *Mtb*TMPK inhibitors warrant further investigation particularly since some of them offer promising antimycobacterial activity, e.g. the sulfoxide- and sulfone-containing cyanopyridone derivatives.[13]

Figure 1. Representative *Mtb*TMPK inhibitors

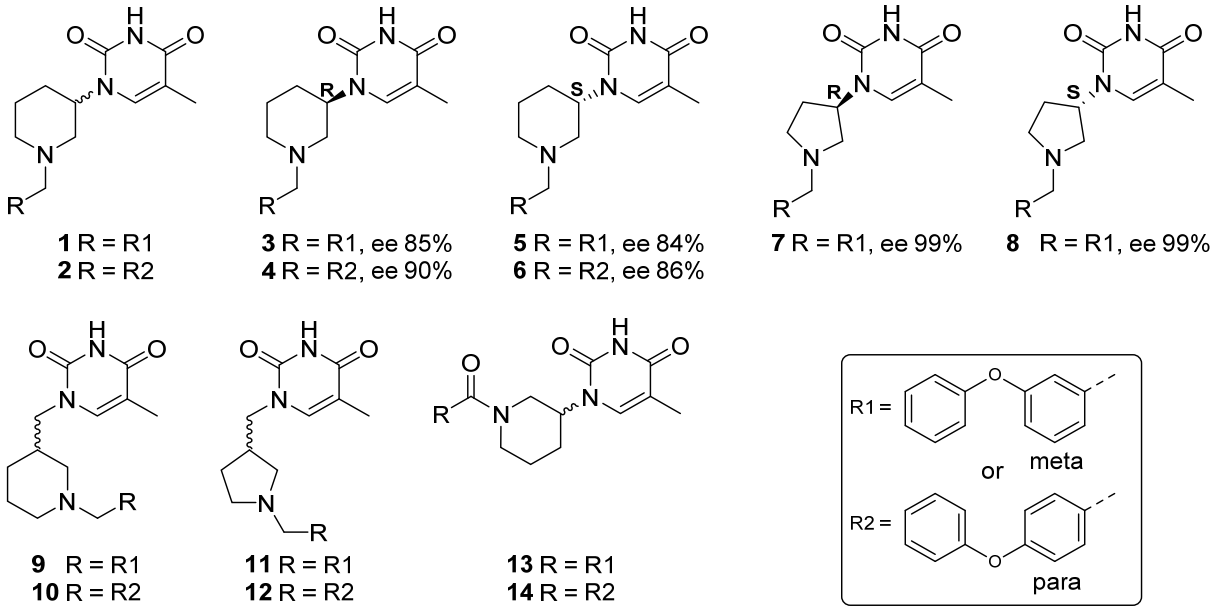


In 2012, gram-positive bacterial TMPK inhibitors with picomolar activity were reported and further optimization of their physico-chemical properties and pharmacokinetics afforded a class

of promising antibacterial agents.[14] Since no evaluation of these analogues on *Mtb*TMPK was reported, we set out to resynthesize compound **1**, which featured as the parent inhibitor for lead generation, and found it to be a fairly potent *Mtb*TMPK inhibitor (Ki = 1.5 μM), comparable with the most potent nucleoside *Mtb*TMPK inhibitors.[9]

Encouraged by this result, we decided to further investigate its SAR (Figure 2). We first changed the configuration of the lipophilic biaryl ether from the meta- to para-position of the internal phenyl group (compound **2**). In order to explore the chiral preference of the enzyme, the pure enantiomers of **1** and compound **2** (compounds **3**-**6**) were prepared. In two more analogues (**7** and **8**) the piperidine ring was substituted for a pyrrolidine ring. To explore the effect of additional distance and flexibility between the thymine and piperidinyl or pyrrolidinyl rings, analogues having a methylene linkage inserted between these heterocycles (**9**-**12**) were prepared. In two final analogues (**13** and **14**) the tertiary amine was replaced with a tertiary amide in order to investigate the importance of the positively charged nitrogen on the piperidine ring. Based on the established X-ray crystal structure of *Mtb*TMPK, we performed molecular modeling studies on these piperidinyl thymine analogues in order to better understand the observed SAR and to shed light on further modifications.

Figure 2. Overview of non-nucleoside thymine derivatives synthesized in this study.

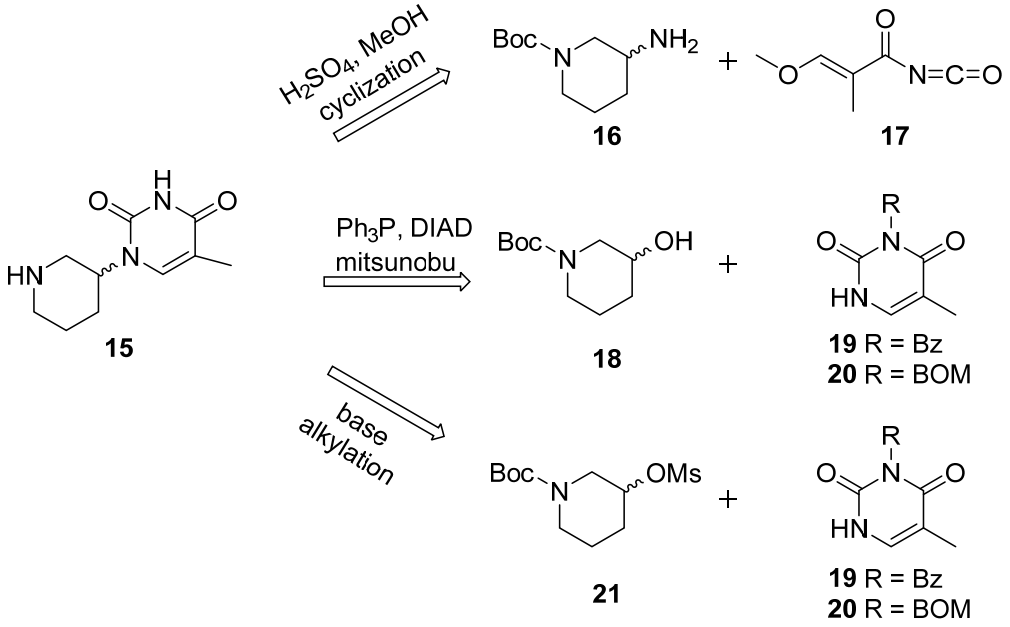


2. Results and Discussion

2.1.Chemistry

In the original synthesis of the hit compound **1**,[14] the key intermediate **15** is prepared using the highly moisture-sensitive isocyanate species **17**, which was obtained from methyl methacrylate using a potentially hazardous multi-step synthesis.[15] In order to access intermediate **15** by an easier and safer means, alternative synthetic routes were explored. According to Rejman and coworkers,[15] there are two alternative methods for the synthesis of the 1-(piperidin-3-yl)thymine **15** in addition to the cyclization between substituted isocyanate **17** and primary amine **16** mentioned above. The first method connects the piperidine and thymine rings using a Mitsunobu reaction, the second one involves an alkylation reaction (Scheme 1).[16]

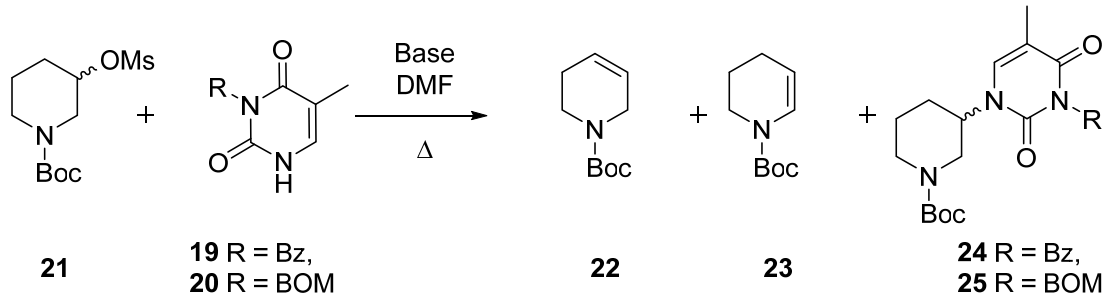
In the Mitsunobu reaction, thymine can react as an ambident nucleophile yielding a mixture of *N*- and *O*-linked isomers. Therefore, the *N3*-position of thymine was protected with a benzoyl- (Bz-, **19**) or a benzyloxymethyl- (BOM-, **20**) as to avoid formation of the *N3*-regioisomer.[17] Despite using the conditions for the Mitsunobu reaction as recommended by Rejman and coworkers,[16] no product was obtained. Further attempts to optimize the reaction conditions proved fruitless.



Scheme 1. Three routes towards piperidinylthymine: isocyanate based pyrimidine synthesis, Mitsunobu reaction and alkylation using a mesylate

The alkylation reaction between thymine and mesylate (**21**)[18] is typically performed at high temperature (e.g., 100 °C or 120 °C) under basic condition (NaH or Cs_2CO_3) with the best obtained yield being 15%.[16] In an attempt to increase the yield of the alkylation, a first trial was conducted using *N3*-Bz-thymine (**19**[17]) and cesium carbonate. Since compound **19** was hydrolyzed back to thymine under the reaction conditions, we switched to *N3*-BOM-protected

thymine (**20**), which gave similar yields with cesium carbonate (21% and 16% at 100 °C and 130 °C, respectively). A commonly observed feature of the alkylation reaction under these conditions was the large amount of unreacted thymine in the reaction mixture. A possible reason was the elimination of mesylate (**21**) into compounds **22** and **23** at high temperature (Scheme 2). Based on these observations we could eventually raise the yield of product **25** substantially (to 51%) by lowering the reaction temperature to 80 °C with portionwise addition of the mesylate (**21**) and base (K_2CO_3) over the course of the reaction (4 days).[19]



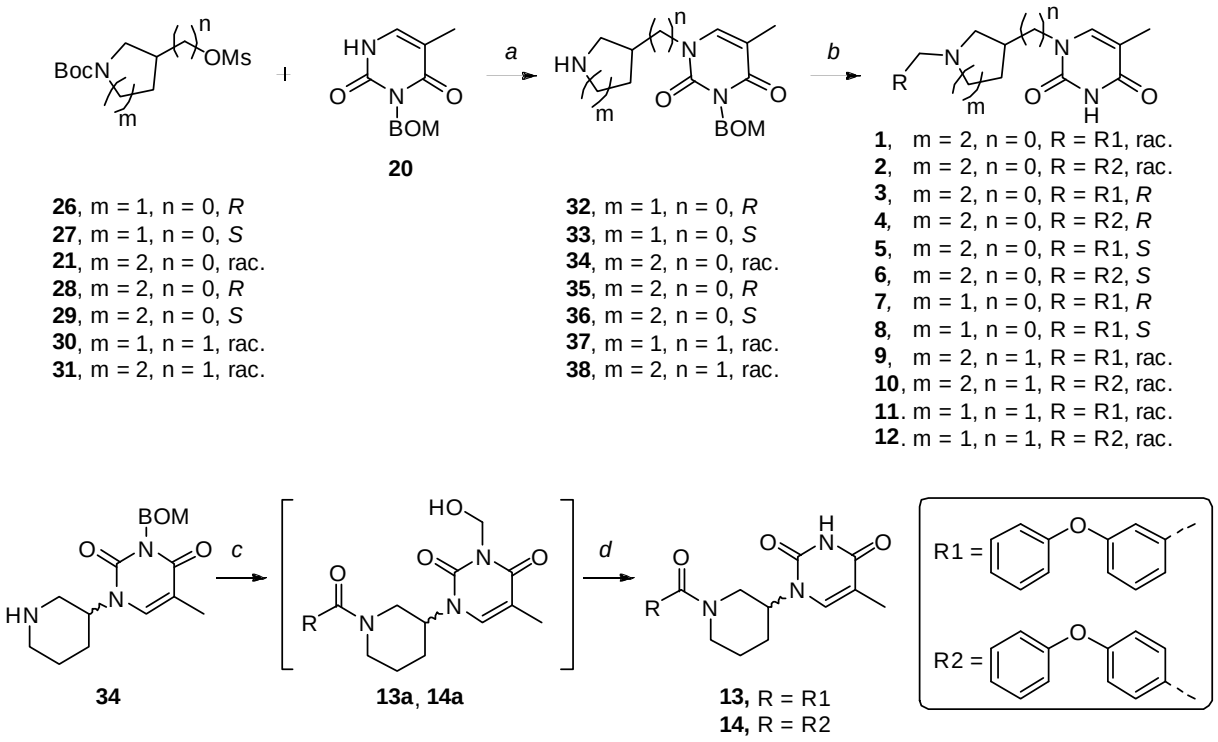
21 | **19** R = Bz, **20** R = BOM | **22** | **23** | **24** R = Bz, **25** R = BOM

Scheme 2: Alkylation of N3-protected thymine using piperidinylmesylates

The alkylation of compound **20** with the azacyclic mesylates (**21** and **26** - **31**) provided the Boc-protected intermediates, which could be deprotected to give the required secondary amine intermediates (**34** and **32-38**). Next, reductive amination with aromatic aldehydes gave the *N3*-BOM-protected products, which were directly deprotected with TFA at 72 °C to afford the final products **1** - **12**.[20] The alkylation reaction was found to occur mostly by the S_N2 nucleophilic substitution reaction, which was demonstrated by chiral purity analysis (cf. experimental part and supplementary information).

For the synthesis of piperidinylamide analogues, corresponding aromatic carboxylic acids were condensed to **34**, followed by hydrogenolysis to provide the final products **13** and **14**

alongside their corresponding hemiaminals **13a** and **14a**, which could be further hydrolyzed to the final products (Scheme 3).

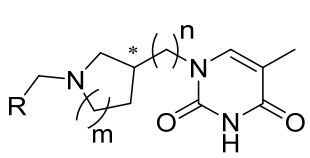


Scheme 3. Synthesis of piperidinyl- and pyrrolidinylthymine *Mtb*TMPK inhibitors. Reagents and conditions: (a) *i*) K_2CO_3, dry DMF, 80 °C, overnight (compound **37** and **38**), 24 h (compound **32** and **33**), 4 days (compound **34** - **36**); *ii*) 10% TFA/ CH_2Cl_2, 2 h - 4 h, rt; (b) *i*) substituted aromatic aldehydes, $NaBH(OAc)_3$, rt, overnight; *ii*) TFA, 72 °C, 30 min – 1 h; (c) *i*) EDC, 4-DMAP, dry CH_2Cl_2, overnight; *ii*) H_2, Pd/C, EtOH, 6 h, rt. (d) THF/H_2O, rt, 4 h.

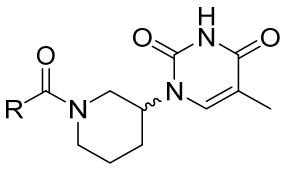
2.2 Biological evaluation and structure-activity relationship (SAR)

The inhibitory potency of compounds (**1** - **14**) was evaluated on *Mtb*TMPK catalytic activity (determination of the inhibitory constant Ki) and the results are summarized in Table 1.

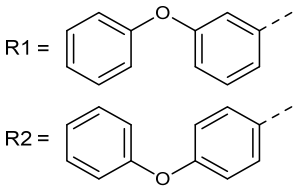
Table 1. Inhibition of *Mtb*TMPK with non-nucleoside thymine derivatives



1-12



13, 14



Compound	m	n	R	Stereochemistry	Ki (μM)
1	2	0	R1	racemic	1.5 ± 0.4
2	2	0	R2	racemic	22.3 ± 0.6
3	2	0	R1	R	8.8 ± 0.3
4	2	0	R2	R	138.5 ± 9.2
5	2	0	R1	S	0.8 ± 0.1
6	2	0	R2	S	21.5 ± 2.4
7	1	0	R1	R	8.2 ± 0.8
8	1	0	R1	S	7.8 ± 1.4
9	2	1	R1	racemic	186.3 ± 24.3
10	2	1	R2	racemic	NI at 0.2 mM
11	1	1	R1	racemic	110.7 ± 9.0
12	1	1	R2	racemic	267.3 ± 25.4
13	2	0	R1	racemic	2.1 ± 0.3
14	2	0	R2	racemic	14.4 ± 2.5

A clear trend emerges in that the potency of the analogues with the meta-biphenyl ether tail is typically approximately 15-fold higher than that of their para-substituted congeners (comparing pairs **1**/**2**, **3**/**4**, **5**/**6**). For the piperidinyl thymine analogues (comparing pairs **3**/**5** and **4**/**6**), the S-enantiomers were about 10-fold more potent than the R-enantiomers, and offered the most effective compound **5** (K_i = 0.8 μM) in this series. This trend was less pronounced in the pyrrolidin-3-ylthymine analogues (compound **7** and **8**). Additionally, these pyrrolidine analogues

are less potent than their piperidinyl counterparts (cf. couples **5**/**8** and **3**/**7**). Introduction of the methylene linker greatly impaired inhibitory potency (cf. pairs **1**/**9**, **2**/**10**), which indicates that the saturated azacycle should be connected directly to the thymine ring. Although there is no benefit from the substitution of the amide moiety (cf. pairs **1**/**13** and **2**/**14)**, bioisosteres of the amide could be probed as a further modification. Compound **5** was found to show promising activity against a *Mtb* H37Ra strain (MIC = 0.9 μM or 0.35 μg/mL) and a selectivity index of 35 versus MRC-5 cells.

To assess the binding mode of these thymine analogues, docking of the piperidinyl thymines **3**, **5** and **6**, the pyrrolidinyl thymine **8** and both enantiomers of amide **13** was performed. The typical interactions found between the nucleobase of dTMP and *Mtb*TMPK are likely to be conserved with compound **5**[5]: (1) π-π stacking between the pyrimidine ring and Phe-70; (2) two hydrogen bonds between the *O4*-thymine and Arg-74; (3) a hydrogen bond between Asn-100 and the *N3*-thymine ring. Moreover, one extra hydrogen bond is formed through the oxygen of the meta-biphenyl ether and Arg-95 (Figure 3), which may contribute to the higher affinity for the enzyme. The docking results indicate that none of the other compounds can form more polar interactions with the enzyme (Figure 4). The thymine ring of compound **3** forms similar interactions as compound **5** (with the Arg-74 and Asn-100 residues), but the meta-biphenyl ether of **3** lacks a hydrogen bond interaction with the enzyme, which may explain the inferior inhibitory potency of this *R*-enantiomer. Similar differences are also observed for compound **6** and **8**, implying that the meta-biphenyl ether tail and piperidine ring are superior to the para-substituted biphenyl ether tail and pyrroline ring, respectively. It is noteworthy that the aromatic tail of **3** is surrounded by residues Ala-35, Phe-36, Pro-37, Tyr 39, Asp-94, Arg-95 and Arg-160, which stabilize binding by hydrophobic interactions. Adding functional groups to this aromatic

tail may further increase interactions with those residues. Docking of the two enantiomers of compound **13** into *Mtb*TMPK, indicates that the *R*-enantiomer may occupy a similar pose as the other inhibitors, while the *S*-enantiomer shows a completely different interaction with the enzyme resulting in a lower docking score (Figure 4). Considering the promising inhibitory activity of the racemate **13**, it may be interesting to investigate the inhibitory potency of the pure enantiomers in the future.

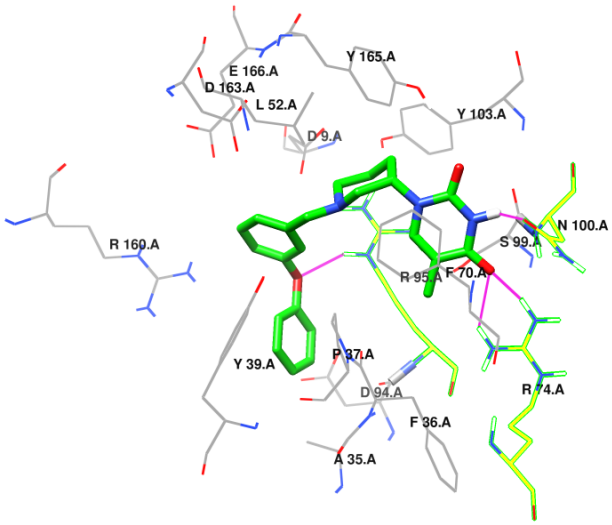


Figure 3. Compound **5** (green stick) docked in the active site of *Mtb*TMPK. All residues interacting with the inhibitors including hydrophobic contact (dark gray wire) and hydrogen-bonding interaction (residues in yellow wire, hydrogen bonds indicated in pink) were calculated using Ligplot[21]. Illustration was created using Chimera[22].

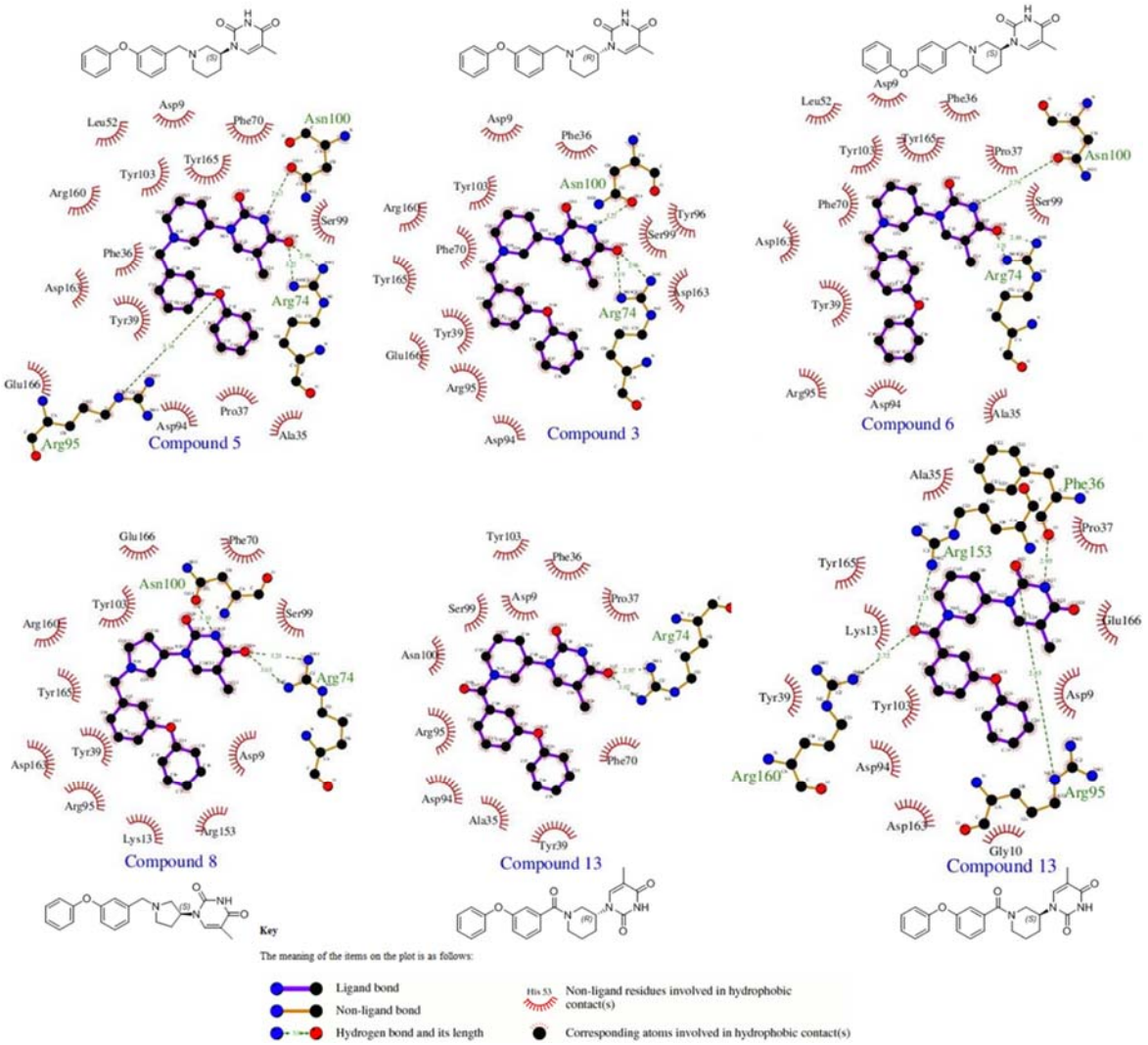


Figure 4. Two-dimensional representation for the interacting mode of compound **3**, **5, 6**, **8** and **13** with *Mtb*TMPK; it is created using the Ligplot[21] program.

Conclusion

Inspired by the published Gram-positive bacterial TMPK inhibitor **1**, we described the design and synthesis of a new series of non-nucleoside *Mtb*TMPK inhibitors. A convenient and less

hazardous synthetic method for 1-substituted azacyclo-thymine analogues has been developed. By exploring different scaffolds, we have demonstrated that there are several spatial requirements with respect to the connection between the thyminyl and piperidinyl ring. First, analogues with *S*-stereochemistry were found to be superior inhibitors of *Mtb*TMPK compared to their enantiomers. Furthermore, piperidinyl analogues have been shown to have a higher potency compared to their pyrrolidinyl counterparts. Additionally, the docking model suggests it is favourable to introduce functional groups to the meta-biphenyl ether tail. For compounds with amide moiety, more attention needs to be paid to the pure enantiomers. Finally we came to the conclusion that the best scaffold of this series is the known *S*-enantiomer (compound **5**)[14a] and future efforts should be focused on piperidinyl thymine analogues with S-stereochemistry combined with a meta-substituted biphenyl ether tail.

4. Experimental section

4.1. Spectrophotometric binding assay

Activity was determined as described in Blondin *et al.*[23] Using a coupled spectrophotometric assay at 334 nm in an Eppendorf ECOM 6122 photometer. The reaction medium (0.5 ml final volume) contained 50 mM Tris-HCl pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate and 2 units each of lactate dehydrogenase, pyruvate kinase and nucleoside diphosphate kinase. One unit of enzyme activity corresponds to 1 mole of the product formed in 1 min. at 30°C. The concentrations of ATP and dTMP were kept constant at 0.5 mM and 0.05 mM respectively, whereas the concentrations of analogues varied between 0.01 and 1 mM.

4.2 Biological Assays on Mycobacterium tuberculosis

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The Minimal Inhibitory Concentration (MIC) against mycobacteria was evaluated by serial dilution method. The in vitro assay was based on a method in which a luminescent Mycobacterium tuberculosis H37Ra strain Lehmann &Neumann (ATCC® 25177™) transformed with pSMTB1 luciferase reporter plasmid is used. The tested compound was solubilized in DMSO (Sigma-Aldrich) at stock concentration of 10 mM. Serial dilutions were made in liquid 7H9 medium [Middlebrook 7H9 broth based (Difco)] with 10% oleic acid, albumin, dextrose, catalase (OADC) enrichment. Volumes of 20 µL of the serial dilutions were added in triplicate to 96 well, flat-bottomed micro well plates. The bacterial suspension was made by thawing and dissolving a frozen Mycobacteria pellet in 7H9-10% OADC. The dissolved pellet was passed through a 5.0 µM filter (Millipore) to eliminate clumps and left for 1 hour to recover at 37 °C, 5% CO_2. Next, the bacterial suspension was diluted in 7H9-10% OADC to obtain 50,000 Relative Light Units (RLU)/mL and a volume of 180 µL of bacteria was added to each well. A bacterial replication was analyzed by luminometry after 7 days of incubation. The bacterial suspension from each well was collected and transferred to a black 96-well plate to evade cross luminescence between wells. The luminescent signal was evoked by addition of the substrate for the bacterial luciferase, 1% n-decanal in ethanol to each well by the Discover multi-plate reader from Promega and the light emission in each well was measured.

4.3 Molecular modeling

All molecular modeling calculations were performed using the software packages AutoDock 4.2 on Windows Cygwin and AutodockTools-1.5.6.[24] The previously reported X-ray structure of the

*Mtb*TMPK (PDB entry 1G3U)[5] was used in all docking experiments. The 2D chemical structures and PDB files of the ligands were drawn and created using ChemBioDraw 13. The PDBQT file of ligands and receptor 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 *Mtb*TMPK (e.g. the typical π-π stacking between Phe-70 and thymine ring of the ligand)[9]. 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. Chemical synthesis

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 (Machery-Nagel). Spots were examined under ultraviolet light at 254 nm. Column chromatography was carried out on a Reveleris X2 (Grace) automated flash unit using the corresponding disposable silica gel cartridges. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 300/75 MHz spectrometer. Chemical shifts are given in parts per million (ppm δ), δ relative to residual solvent peak or TMS for ^{1}H and ^{13}C. Structural assignment was confirmed with COSY,

HSQC and HMBC. Exact mass measurements (HRMS) were performed on a Waters LCT Premier XETM Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/H_2O (1:1) mixture at 100 μL/min. Preparative reversed phase HPLC chromatography was carried out using a Phenomenex Luna C-18 (21.2x250mm) column using an aq. NH_4HCO_3/MeCN gradient. Chiral analysis was carried out with a Daicel Chiralpak-IA HPLC column or a Daicel Chiralcel-ODH HPLC column using hexane/ethanol 80:20 as eluent. Both columns, dimensions 4.6x250mm, 5μm particle size, were used at 35°C.

4.2.1. 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**34**): A suspension of 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**20**, 1.06 g, 4.3 mmol), *tert*-butyl 3-((methylsulfonyl)oxy)piperidine-1-carboxylate (**21**, 1.80 g, 6.45 mmol) and potassium carbonate (1.19 g, 8.6 mmol) in dry DMF (10 mL) was stirred at 80 ℃ for 48 h under argon. Additional **21 (**1.20 g, 4.3 mmol) and potassium carbonate (0.60 g, 4.3 mmol) were added to the reaction mixture and the mixture was stirred at 80 ℃ for 24 h. Additional **21** (1.20 g, 4.3 mmol) and potassium carbonate (0.60 g, 4.3 mmol) were added and the mixture was stirred at 80°C another 24 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL), following by washing with water (100 mL) and brine (100 mL). The organic layer was dried over sodium sulfate. After evaporation, the residue was purified by column chromatography (50% hexane/ethyl acetate) to give the intermediate which was dissolved with CH_2Cl_2 (20 mL), and TFA (2 mL) was added to the solution. The reaction mixture was stirred at room temperature for 3 h, followed by evaporation in vacuo. The residue was dissolved in sat. $NaHCO_3$ solution (30 mL) and extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were washed with brine (50 mL × 2) and dried over sodium sulfate. The water layer can be re-

extracted again if the product is found by TLC. After evaporation, the residue was purified by column chromatography (0.1% Et_3N/4%MeOH/ CH_2Cl_2) to afford the desired product **34** as colorless gel (0.60 g, 42.4%). ^1H NMR (300 MHz, DMSO-d_6) δ: 1.60 - 2.02 (m, 7H, 5-CH_3, piperidin-4-yl and piperidin-5-yl), 2.49 (br. s., 1H, NH), 2.55 - 2.66 (m, 1H, piperidin-6a-yl), 2.73 (dd, *J* = 12.01, 10.25 Hz, 1H, piperidin-2a-yl), 2.99 - 3.10 (m, 1H, piperidin-6b-yl), 3.19 (dd, *J* = 11.86, 2.78 Hz, 1H, piperidin-2b-yl), 4.50 (ddd, *J* = 10.47, 6.52, 4.10 Hz, 1H, piperidin-3-yl), 4.71 (s, 2H), 5.51 (s, 2H), 7.18 (d, *J* = 1.17 Hz, 1H, 3-CH_3), 7.21 - 7.41 (m, 5H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.21 (5-CH_3), 27.00 (piperidin-5-yl), 30.55 (piperidin-4-yl), 46.66 (piperidin-6-yl), 51.06 (piperidin-2-yl), 54.50 (piperidin-3-yl), 71.81 (CH_2, benzyloxy), 73.23 (3-methyl), 110.67 (C-3) 128.53 (Ph), 128.58 (2C, Ph), 129.20 (2C, Ph), 136.76 (C-6), 139.06 (Ph), 152.50 (C-2), 164.25 (C-4). HRMS (ESI): calculated for $[C_{18}H_{23}N_3O_3 + H]^+$, 330.1812; found, 330.1809. The R/S-enantiomers (compound **35** and **36**) were synthesized as described above. The yield of compound **35** and **36** is 38.9% and 41.3% respectively.

*4.2.2 General procedure for synthesis of compound **32, 33, 37** and **38**.*

A suspension of 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**20**, 1 eq), *N*-Boc-protected mesylates (1.1 eq - 2 eq), potassium carbonate (1.5 eq - 2 eq) was stirred at 80 ℃ overnight (compound **37** and **38**) or 24 h (compound **32** and **33**) under argon. The work-up procedure as well as the second step were performed as has been described for the synthesis of compound **34**.

4.2.2.1 (R)-3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**32**): 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4 (1H,3H)-dione (**20**, 0.72g, 2.92 mmol), tert-butyl (S)-3-((methylsulfonyl)oxy)pyrrolidine-1-carboxylate (**26**, 1.55 g, 5.84 mmol) and potassium

carbonate (0.81g, 5.84 mmol) yielded compound **32** as a colorless gel (0.53 g, 57.2%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.77 - 1.98 (m, 4H, 5-CH_3, pyrrolidin-4a-yl), 2.26 – 2.43 (m, 1H, pyrrolidin-4b-yl), 2.89 - 3.12 (m, 2H, pyrrolidin-2a-yl, pyrrolidin-5a-yl), 3.17 (br. s., 1H, NH), 3.23 - 3.38 (m, 2H, pyrrolidin-2b-yl, pyrrolidin-5b-yl), 4.70 (s, 2H, CH_2, 3-methylene), 5.00 - 5.13 (m, 1H, pyrrolidin-3-yl), 5.50 (s, 2H, CH_2, benzyl), 7.13 - 7.44 (m, 6H, Ph, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.14 (5-CH_3), 31.51 (pyrrolidin-4-yl), 46.15 (pyrrolidin-5-yl), 51.21 (pyrrolidin-2-yl), 56.30 (pyrrolidin-3-yl), 70.74 (3-methylene), 72.25 (CH_2, benzyl), 110.63 (C-5), 127.60 (3C, Ph), 128.21 (2C, Ph), 136.74 (C-6), 137.96 (Ph), 151.54 (C-2), 163.33 (C-4). HRMS (ESI): calculated for $[C_{17}H_{21}N_3O_3 + H]^+$, 316.1656; found, 316.1651.

4.2.2.2 (S)-3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**33**): 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4 (1H,3H)-dione (**20**, 1.45g, 6 mmol), tert-butyl (R)-3-((methylsulfonyl)oxy)pyrrolidine-1-carboxylate (**27**, 3.18 g, 12 mmol) and potassium carbonate (1.66 g, 12 mmol) yielded compound **33** as a colorless gel (0.96 g, 50.8%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73 -1.87 (m, 1H, pyrrolidin-4a-yl), 1.92 (d, J = 1.17 Hz, 3H, 5-CH_3), 2.26 - 2.41 (m, 1H, pyrrolidin-4b-yl), 2.89 - 3.09 (m, 2H, pyrrolidin-2a-yl, pyrrolidin-5a-yl), 3.19 - 3.32 (m, 2H, pyrrolidin-2b-yl, pyrrolidin-5b-yl), 4.70 (s, 2H, CH_2, 3-methylene), 5.01 - 5.11 (m, 1H, pyrrolidin-3-yl), 5.50 (s, 2H, CH_2, benzyl), 7.20 - 7.40 (m, 6H, Ph, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.32 (5-CH_3), 31.87 (pyrrolidin-4-yl), 46.37 (pyrrolidin-5-yl), 51.70 (pyrrolidin-2-yl), 56.40 (pyrrolidin-3-yl), 70.89 (3-methylene), 72.38 (CH_2, benzyl), 110.64 (C-5), 127.75 (3C, Ph), 128.36 (2C, Ph), 136.89 (C-6), 138.16 (Ph), 151.70 (C-2), 163.54 (C-4). HRMS (ESI): calculated for $[C_{17}H_{21}N_3O_3 + H]^+$, 316.1656; found, 316.1650.

*4.2.2.3. 3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**37**):* 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4 (1H,3H)-dione (**20**, 1.5 mmol, 0.52 g), tert-

butyl 3-(((methylsulfonyl)oxy)methyl)pyrrolidine-1-carboxylate (**30**, 0.63 g, 2.25 mol,) and potassium carbonate (0.42 g, 3 mmol) yielded compound **37** as a colorless gel (0.29 g, 58.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.54 (dd, *J* = 13.91, 6.59 Hz, 1H, pyrrolidin-4a-yl), 1.88 - 1.99 (m, 4H, 5-CH_3, pyrrolidin-4b-yl), 2.58 - 2.69 (m, 1H, pyrrolidin-3-yl), 2.77 (dd, *J* = 11.13, 5.86 Hz, 1H, pyrrolidin-2a-yl), 2.97 - 3.17 (m, 3H, pyrrolidin-2b-yl, pyrrolidin-5-yl), 3.26 (br. s., 1H, NH), 3.71 (dd, *J* = 7.62, 2.93 Hz, 2H, CH_2, 1-methylene), 4.71 (s, 2H, CH_2, benzyl), 5.50 (s, 1H, CH_2, 3-methylene), 7.08 (d, *J* = 1.17 Hz, 1H, H-6), 7.21 - 7.40 (m, 5H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.14 (5-CH_3), 29.64 (pyrrolidin-4-yl), 38.74 (pyrrolidin-3-yl), 46.10 (pyrrolidin-5-yl), 50.00 (pyrrolidin-2-yl), 52.37 (1-methylene), 70.84 (3-methylene), 72.38 (CH_2, benzyl), 110.12 (C-5), 127.76 (3C, Ph), 128.39 (2C, Ph), 138.16 (Ph), 139.41 (C-6), 151.85 (C-2), 163.82 (C-4). HRMS (ESI): calculated for $[C_{18}H_{23}N_3O_3 + H]^+$, 330.1812; found, 330.1810.

4.2.2.4 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**38**): 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**20**, 0.52 g, 2.1 mmol), tert-butyl 3-(((methylsulfonyl)oxy)methyl)piperidine-1-carboxylate (**31**, 0.66 g, 2.25 mmol) and potassium carbonate (0.44 g, 3.15 mmol) yielded compound **38** as colorless gel (0.26 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12 - 1.30 (m, 1H, piperidin-4a-yl), 1.53 - 1.84 (m, 3H, piperidin-4b-yl, piperidin-5-yl), 1.87 - 1.95 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.11 (dtd, *J* = 13.73, 6.68, 6.68, 3.37 Hz, 1H, piperidin-3-yl), 2.49 (dd, *J* = 12.30, 9.96 Hz, 1H, piperidin-2a-yl), 2.67 (td, *J* = 11.64, 3.08 Hz, 1H, piperidin-6a-yl), 3.03 - 3.17 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.49 (br. s., 1H, NH), 3.52 - 3.72 (m, 2H, 1-methylene), 4.69 (s, 2H, CH_2, benzyl), 5.48 (s, 2H, CH_2, 3-methylene), 6.97 (d, *J* = 1.17 Hz, 1H, H-6), 7.19 - 7.38 (m, 5H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.12 (5-CH_3), 24.01 (piperidin-5-yl), 27.88 (piperidin-4-yl), 35.49 (piperidin-3-yl), 45.90 (piperidin-6-yl), 48.75 (piperidin-2-yl), 52.19 (1-methylene), 70.90 (3-methylene), 72.41

(CH_2, benzyl), 110.16 (C-5), 127.78 (Ph), 127.81 (2C, Ph), 128.40 (2C, Ph), 138.10 (Ph), 139.46 (C-6), 151.91 (C-2), 163.80 (C-4). HRMS (ESI): calculated for $[C_{19}H_{25}N_3O_3 + H]^+$, 344.1969; found, 344.1973.

*4.2.3 General procedure for the synthesis of final compounds **1** - **12**.*

A suspension of compound **32 - 38** (1 eq), substituted aromatic aldehyde (1.5 – 2 eq) and sodium triacetoxyborohydride (1.5 – 3 eq) in 1,2-dichloroethane (~ 0.03 M) was stirred at room temperature under argon overnight. The reaction mixture was evaporated and dried with oil pump vacuum for 0.5 h. The residue was purified by silica chromatography (CH_2Cl_2→90% CH_2Cl_2/MeOH in a linear gradient elution) to afford pure intermediate, which was immediately dissolved with TFA under argon. The reaction mixture was stirred at 72 ℃ for 30 min to 1 h. The reaction was monitored by HRMS. After cooling to room temperature, the reaction mixture was concentrated and dried under oil pump vacuum. The residue was dissolved in an equivolumar mixture of MeCN/t-BuOH/H_2O (1 – 2 mL) and purified by preparative liquid chromatography (Phenomenex Luna C-18 (21.2x250mm), flow rate 17.5 mL/min) using a linear gradient from 10% MeCN-90% aq. 10 mM ammonium bicarbonate → 100% MeCN over 20 min. After lyophilization, products **1** – **12** were obtained as a white powder.

4.2.3.1 5-methyl-1-(1-(3-phenoxybenzyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**1**): Following the general procedure, the use of 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**34**, 85.64 mg, 0.26 mmol), 3-phenoxybenzaldehyde (77.31 mg, 0.39 mmol) and sodium triacetoxyborohydride (110.22 mg, 0.52 mmol) yielded compound **1** (28.9 mg, 28.4%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.45 - 1.78 (m, 7H, 5-CH_3, piperidin-4-yl, piperidin-5-yl), 1.97 - 2.24 (m, 2H, piperidin-2a-yl, piperidin-6a-yl), 2.64 - 2.80 (m, 2H,

piperidin-2b-yl, piperidin-6b-yl), 3.51 (s, 2H, CH_2, 1-benzyl), 4.29 - 4.46 (m, 1H, piperidin-3-yl), 6.89 (d, *J* = 8.20 Hz, 1H, Ph), 6.94 - 7.17 (m, 5H, Ph), 7.29 - 7.43 (m, 3H, Ph), 7.70 (s, 1H, H-6) 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.04 (5-CH_3), 23.87 (piperidin-5-yl), 27.90 (piperidin-4-yl), 50.98 (piperidin-3-yl), 52.13 (piperidin-6-yl), 56.16 (piperidin-2-yl), 61.39 (CH_2, 1-benzyl), 108.61 (C-5), 117.25, 118.55, 118.67, 123.39, 123.84, 129.81, 130.03 (7C, Ph), 137.96 (C-6), 140.34 (Ph), 150.80 (C-2), 156.62 (Ph), 163.62 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found: 392.1972.

4.2.3.2 5-methyl-1-(1-(4-phenoxybenzyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**2**): Following the general procedure the use of 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**34**, 82.70 mg, 0.251 mmol), 4-phenoxybenzaldehyde (74.70 mg, 0.377 mmol) and sodium triacetoxyborohydride (106.4 mg, 0.501 mmol) yielded compound **2** (29.48 mg, 30.0%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.46 - 1.63 (m, 1H, piperidin-5a-yl), 1.63 - 1.80 (m, 6H, 5-CH_3, piperidin-4-yl, piperidin-5b-yl), 1.96 - 2.09 (m, 1H, piperidin-2a-yl), 2.19 (t, *J* = 10.25 Hz, 1H, piperidin-6a-yl), 2.64 - 2.81 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.48 (s, 2H, CH_2, 1-benzyl), 4.33 - 4.47 (m, 1H, piperidin-3-yl), 6.92 - 7.02 (m, 4H, Ph), 7.09 - 7.16 (m, 1H, Ph), 7.27 - 7.33 (m, 2H, Ph), 7.34 - 7.42 (m, 2H, Ph), 7.67 - 7.73 (d, *J* = 3.0 Hz, 1H, H-6), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.06 (5-CH_3), 23.93 (piperidin-5-yl), 28.07 (piperidin-4-yl), 51.04 (piperidin-3-yl), 52.16 (piperidin-6-yl), 56.28 (piperidin-2-yl), 61.30 (CH_2, 1-benzyl), 108.64 (C-5), 118.29 (2C, Ph), 118.62 (2C, Ph), 123.42 (Ph), 130.03 (2C, Ph), 130.42 (2C, Ph), 133.00 (Ph), 138.02 (C-6), 150.83 (C-2), 155.67 (Ph), 156.68 (Ph), 163.65 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found: 392.1970.

4.2.3.3 (R)-5-methyl-1-(1-(3-phenoxybenzyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**3**): Following the general procedure the use of (R)-3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-

yl)pyrimidine-2,4(1H,3H)-dione (**35**, 139.01 mg, 0.422 mmol), 3-phenoxybenzaldehyde (167.30 mg, 0.844 mmol) and sodium triacetoxyborohydride (268.34 mg, 1.266 mmol) yielded compound **3** (45.09 mg, 27.3%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.44 - 1.82 (m, 7H, 5-CH_3, piperidin-4-yl, piperidin-5-yl), 1.96 - 2.11 (m, 1H, piperidin-6a-yl), 2.17 (t, *J* = 9.81 Hz, 1H, piperidin-2a-yl), 2.63 - 2.82 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.51 (s, 2H, CH_2, 1-methylene), 4.38 (br. s., 1H, piperidin-3-yl), 6.89 (d, *J* = 7.91 Hz, 1H, Ph), 6.94 - 7.04 (m, 3H, Ph), 7.04 - 7.17 (m, 2H, Ph), 7.29 - 7.43 (m, 3H, Ph), 7.70 (s, 1H, H-6), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.04 (5-CH_3), 23.89 (piperidin-5-yl), 27.92 (piperidin-4-yl), 50.98 (piperidin-3-yl), 52.13 (piperidin-6-yl), 56.20 (piperidin-2-yl), 61.41 (1-methylene), 108.61 (C-5), 117.22 (Ph), 118.55 (2C, Ph), 118.65 (Ph), 123.39 (Ph), 123.83 (Ph), 129.81 (Ph), 130.01 (2C, Ph), 137.96 (C-6), 140.36 (Ph), 150.80 (C-2), 156.62 (2C, Ph), 163.62 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found: 392.1970. ee: 84.8% (Daicel Chiralpak-IA HPLC column, hexane/ethanol 80/20 as eluent).

4.2.3.4 (R)-5-methyl-1-(1-(4-phenoxybenzyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**4**): Following the general procedure the use of (R)-3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**35**, 139.01 mg, 0.422 mmol), 4-phenoxybenzaldehyde (167.30 mg, 0.844 mmol) and sodium triacetoxyborohydride (268.34 mg, 1.266 mmol) yielded compound **3** (44.10 mg, 26.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.43 - 1.81 (m, 7H, 5-CH_3, piperidin-4-yl, piperidin-5-yl), 1.93 - 2.09 (m, 1H, piperidin-6a-yl), 2.17 (t, *J* = 10.84 Hz, 1H, piperidin-2a-yl), 2.63 - 2.80 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.47 (s, 2H, CH_2, 1-methylene), 4.38 (br. s., 1H, piperidin-3-yl), 6.88 - 7.03 (m, 4H, Ph), 7.08 - 7.16 (m, 1H, Ph), 7.24 - 7.42 (m, 4H, Ph) 7.69 (s, 1H, H-6), 11.18 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.06 (5-CH_3), 23.92 (piperidin-5-yl), 28.06 (piperidin-4-yl), 51.04 (piperidin-3-yl), 52.16

(piperidin-6-yl), 56.27 (piperidin-2-yl), 61.29 (1-methylene), 108.64 (C-5), 118.29 (2C, Ph), 118.64 (2C, Ph), 123.43 (Ph), 130.04 (2C, Ph), 130.44 (2C, Ph), 132.99 (Ph), 138.01 (C-6), 150.83 (C-2), 155.69 (Ph), 156.66 (Ph), 163.65 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found, 392.1972. ee: 90.4% (Daicel Chiralpak-IA HPLC column, hexane/ethanol 80/20 as eluent).

4.2.3.5 (S)-5-methyl-1-(1-(3-phenoxybenzyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**5**)[14a]: Following the general procedure the use of (S)-3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**36**, 139.01 mg, 0.422 mmol), 3-phenoxybenzaldehyde (167.30 mg, 0.844 mmol) and sodium triacetoxyborohydride (268.34 mg, 1.266 mmol) yielded compound **5** (45.40 mg, 27.5%). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ: 1.46 - 1.78 (m, 7H, 5-CH$_3$, piperidin-4-yl, piperidin-5-yl), 1.98 - 2.10 (m, 1H, piperidin-6a-yl), 2.17 (t, *J* = 10.25 Hz, 1H, piperidin-2a-yl), 2.64 - 2.79 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.50 (s, 2H, CH$_2$, 1-methylene), 4.30 - 4.45 (m, 1H, piperidin-3-yl), 6.89 (ddd, *J* = 8.13, 2.56, 1.03 Hz, 1H, Ph), 6.94 - 7.04 (m, 3H, Ph), 7.04 - 7.09 (m, 1H, Ph), 7.10 - 7.17 (m, 1H, Ph), 7.30 - 7.43 (m, 3H, Ph), 7.70 (d, *J* = 1.00 Hz, 1H, H-6), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ: 12.04 (5-CH$_3$), 23.90 (piperidin-5-yl), 27.93 (piperidin-4-yl), 51.00 (piperidin-3-yl), 52.14 (piperidin-6-yl), 56.19 (piperidin-2-yl), 61.43 (1-methylene), 108.59 (C-5), 117.20 (Ph), 118.55 (2C, Ph), 118.64 (Ph), 123.37 (Ph), 123.81 (Ph), 129.80 (Ph), 130.01 (2C, Ph), 137.99 (C-6), 140.36 (Ph), 150.82 (C-2), 156.60 (Ph), 156.63 Ph), 163.62 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found: 392.1973. ee: 84.2% (Daicel Chiralpak-IA HPLC column, hexane/ethanol 80/20 as eluent).

4.2.3.6 (S)-5-methyl-1-(1-(4-phenoxybenzyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**6**): Following the general procedure, (S)-3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-

yl)pyrimidine-2,4(1H,3H)-dione (**36**, 130.11 mg, 0.395 mmol), 4-phenoxybenzaldehyde (156.59 mg, 0.79 mmol) and sodium triacetoxyborohydride (251.17 mg, 1.185 mmol) yielded compound **6** (41.78 mg, 27.2%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.45 - 1.78 (m, 7H, 5-CH_3, piperidin-4-yl, piperidin-5-yl), 1.96 - 2.08 (m, 1H, piperidin-6a-yl), 2.17 (t, *J* = 10.40 Hz, 1H, piperidin-2a-yl), 2.62 - 2.80 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.47 (s, 2H, CH_2, 1-methylene), 4.31 - 4.45 (m, 1H, piperidin-3-yl), 6.89 - 7.01 (m, 4H, Ph), 7.08 - 7.15 (m, 1H, Ph), 7.24 - 7.41 (m, 4H, Ph), 7.65 - 7.71 (m, 1H, H-6), 11.18 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.04 (5-CH_3), 23.92 (piperidin-5-yl), 28.04 (piperidin-4-yl), 51.03 (piperidin-3-yl), 52.14 (piperidin-6-yl), 56.27 (piperidin-2-yl), 61.29 (1-methylene), 108.61 (C-5), 118.27 (2C, Ph), 118.61 (2C, Ph), 123.40 (Ph), 130.03 (2C, Ph), 130.41 (2C, Ph), 132.99 (Ph), 138.01 (C-6), 150.82 (C-2), 155.66 (Ph), 156.65 (Ph), 163.64 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found: 392.1971. ee: 86.4% (Daicel Chiralpak-IA HPLC column, hexane/ethanol 80/20 as eluent).

4.2.3.7 (R)-5-methyl-1-(1-(3-phenoxybenzyl)pyrrolidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**7**): Following the general procedure, (R)-3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**32**, 157.69 mg, 0.50 mmol), 3-phenoxybenzaldehyde (198.22 mg, 1.00 mmol) and sodium triacetoxyborohydride (211.96 mg, 1.00 mmol) yielded compound **8** (53.40 mg, 28.3%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.60-1.80 (m, 4H, 5-CH_3, pyrrolidin-4a-yl), 2.14 - 2.37 (m, 2H, pyrrolidin-4b-yl, pyrrolidin-2a-yl), 2.48 – 2.56 (m, 1H, pyrrolidin-5a-yl), 2.60 - 2.75 (m, 1H, pyrrolidin-5b-yl), 2.89 - 3.03 (m, 1H, pyrrolidin-2b-yl), 3.48 - 3.70 (m, 2H, CH_2, benzyl), 4.87 - 5.01 (m, 1H, pyrrolidin-3-yl), 6.88 (dd, *J* = 7.91, 1.76 Hz, 1H, Ph), 6.93 - 7.01 (m, 3H, Ph), 7.05 - 7.15 (m, 2H, Ph), 7.28 - 7.40 (m, 3H, Ph), 7.62 (d, *J* = 1.17 Hz, 1H, H-6), 11.15 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.29 (5-CH_3), 30.90 (pyrrolidin-4-yl),

52.45 (pyrrolidin-2-yl), 52.74 (pyrrolidin-3-yl), 58.27 (CH_2, benzyl), 58.40 (pyrrolidin-5-yl), 109.16 (C-5), 117.28 (Ph), 118.33 (Ph), 118.44 (2C, Ph), 123.35 (Ph), 123.42 (Ph), 129.86 (Ph), 129.98 (2C, Ph), 137.72 (C-6), 141.23 (Ph), 150.77 (C-2), 156.66 (Ph), 163.67 (C-4). HRMS (ESI): calculated for $[C_{22}H_{23}N_3O_3 + H]^+$, 378.1812; found, 378.1806. ee: 98.8% (Daicel Chiralcel ODH HPLC column, hexane/ethanol 80/20 as eluent).

4.2.3.8 (S)-5-methyl-1-(1-(3-phenoxybenzyl)pyrrolidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**8**): Following the general procedure, (S)-3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**33**, 126.15 mg, 0.40 mmol), 3-phenoxybenzaldehyde (158.58 mg, 0.80 mmol) and sodium triacetoxyborohydride (169.57 mg, 0.80 mmol) yielded compound **8** (42.30 mg, 28.0%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.64 - 1.83 (m, 4H, 5-CH_3, pyrrolidin-4a-yl), 2.17 - 2.38 (m, 2H, pyrrolidin-4b-yl, pyrrolidin-2a-yl), 2.48- 2.56 (m, 1H, pyrrolidin-5a-yl), 2.64 - 2.77 (m, 1H, pyrrolidin-5b-yl), 2.91 - 3.03 (m, 1H, pyrrolidin-2b-yl), 3.52 - 3.72 (m, 2H, CH_2, benzyl), 4.89 - 5.03 (m, 1H, pyrrolidin-3-yl), 6.90 (dd, *J* = 8.20, 1.76 Hz, 1H, Ph), 6.95 - 7.03 (m, 3H, Ph), 7.07 - 7.16 (m, 2H, Ph), 7.31 - 7.42 (m, 3H, Ph), 7.64 (d, *J* = 1.17 Hz, 1H, H-6), 11.17 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.30 (5-CH_3), 30.89 (pyrrolidin-4-yl), 52.44 (pyrrolidin-2-yl), 52.73 (pyrrolidin-3-yl), 58.26 (CH_2, benzyl), 58.40 (pyrrolidin-5-yl), 109.15 (C-5), 117.26 (Ph), 118.31 (Ph), 118.43 (2C, Ph), 123.35 (Ph), 123.39 (Ph), 129.85 (Ph), 129.97 (2C, Ph), 137.71 (C-6), 141.24 (Ph), 150.76 (C-2), 156.66 (Ph), 163.66 (C-4). HRMS (ESI): calculated for $[C_{22}H_{23}N_3O_3 + H]^+$, 378.1812; found, 378.1806. ee: 99.2% (Daicel Chiralcel ODH HPLC column, hexane/ethanol 80/20 as eluent).

4.2.3.9 5-methyl-1-((1-(3-phenoxybenzyl)piperidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**9**): Following the general procedure, 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**38**, 102.00 mg, 0.297 mmol), 3-phenoxybenzaldehyde (88.41 mg, 0.446 mmol) and sodium triacetoxyborohydride (125.9 mg, 0.594 mmol) yielded compound **9** (37.58 mg, 31.2%).^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.88 - 1.07 (m, 1H, piperidin-4a-yl), 1.28 - 1.46 (m, 1H, piperidin-5a-yl), 1.48 - 1.68 (m, 2H, piperidin-4b-yl, piperidin-5b-yl), 1.68 - 2.05 (m, 6H, 5-CH_3, piperidin-2a-yl, piperidin-6a-yl), 2.58 (d, *J* = 9.67 Hz, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.34 - 3.60 (m, 4H, 2CH_2, 1-methyl, phenoxybenzyl-), 6.83 - 7.07 (m, 5H, Ph), 7.09 - 7.17 (m, 1H, Ph), 7.27 - 7.42 (m, 3H, Ph), 7.44 (s, 1H, H-6), 11.19 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.91 (5-CH_3), 23.93 (piperidin-5-yl), 27.22 (piperidin-4-yl), 35.35 (piperidin-3-yl), 50.19 (CH_2, 1-methylene), 53.38 (piperidin-6-yl), 56.51 (piperidin-2-yl), 61.99 (CH_2, phenoxybenzyl-), 108.24 (C-5), 117.00 (Ph), 118.47(Ph), 118.61 (2C, Ph), 123.40 (Ph), 123.72 (Ph), 129.67 (Ph), 130.03 (2C, Ph) 140.92 (Ph), 141.61 (C-6), 151.05 (C-2), 156.62 (Ph), 156.65 (Ph), 164.17 (C-4). HRMS (ESI): calculated for $[C_{24}H_{27}N_3O_3 + H]^+$, 406.2125; 406.2142.

4.2.3.10 5-methyl-1-((1-(4-phenoxybenzyl)piperidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**10**): Following the general procedure, 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**38**, 118.48 mg, 0.345 mmol), 4-phenoxybenzaldehyde (136.77 mg, 0.69 mmol), sodium triacetoxyborohydride (219.38 mg, 1.035 mmol) yielded compound **10** (44.10 mg, 32.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.89 - 1.06 (m, 1H, piperidin-4a-yl), 1.29 - 1.46 (m, 1H, piperidin-5a-yl), 1.48 - 1.67 (m, 2H, piperidin-4b-yl, piperidin-5b-yl), 1.67 - 1.82 (m, 4H, 5-CH_3, piperidin-2a-yl), 1.83 - 2.02 (m, 2H, piperidin-3-yl, piperidin-6a-yl), 2.57 (d, *J* = 8.20 Hz, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.34 - 3.54 (m, 4H, 2CH_2, 1-methyl, phenoxybenzyl-), 6.88 - 6.99 (m, 4H, Ph), 7.07 - 7.14 (m, 1H, Ph), 7.22 - 7.28 (m, 2H, Ph), 7.33

- 7.41 (m, 2H, Ph), 7.44 (d, *J* = 1.17 Hz, 1H, H-6), 11.17 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.91 (5-CH_3), 23.98 (piperidin-5-yl), 27.35 (piperidin-4-yl), 35.32 (piperidin-3-yl), 50.25 (CH_2, 1-methylene), 53.41 (piperidin-6-yl), 56.49 (piperidin-2-yl), 61.87 (CH_2, phenoxybenzyl), 108.21 (C-5), 118.29, 118.47, 123.29, 130.03, 130.35, 133.51 (Ph), 141.64 (C-6), 151.05 (C-2), 155.44 (Ph), 156.78 (Ph), 164.17 (C-4). HRMS (ESI): calculated for $[C_{24}H_{27}N_3O_3 + H]^+$, 406.2125; found, 406.2097.

4.2.3.11 5-methyl-1-((1-(3-phenoxybenzyl)pyrrolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**11**): Following the general procedure, 3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-ylmethyl)pyrimidine-2,4(1H,3H)-dione (**37**, 140.00 mg, 0.425 mmol), 3-phenoxybenzaldehyde (168.49 mg, 0.85 mmol) and sodium triacetoxyborohydride (270.25 mg, 1.275 mmol) yielded compound **11** (72.30 mg, 43.5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.36 - 1.54 (m, 1H, pyrrolidin-4a-yl), 1.69 - 1.89 (m, 4H, 5-CH_3, pyrrolidin-4b-yl), 2.20 - 2.33 (m, 1H, pyrrolidin-2a-yl), 2.46 - 2.61 (m, 4H, pyrrolidin-2b-yl, pyrrolidin-5-yl, pyrrolidin-3-yl),3.48 - 3.68 (m, 4H, 2CH_2, 1-methyl, phenoxybenzyl-), 6.87 (ddd, *J* = 8.05, 2.49, 0.88 Hz, 1H, Ph), 6.93 - 7.03 (m, 3H, Ph), 7.06 - 7.17 (m, 2H, Ph), 7.31 (d, *J* = 7.91 Hz, 1H, Ph), 7.34 - 7.43 (m, 2H, Ph), 7.50 (d, *J* = 1.17 Hz, 1H, H-6), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.92 (5-CH_3), 27.29 (pyrrolidin-4-yl), 36.56 (pyrrolidin-3-yl), 51.00 (1-methylene), 52.91 (pyrrolidin-5-yl), 56.63 (pyrrolidin-2-yl), 58.88 (CH_2, phenoxybenzyl), 108.33 (C-5), 117.00 (Ph), 118.33 (Ph), 118.59 (2C, Ph), 123.39 (Ph), 123.43 (Ph), 129.74 (Ph), 130.03 (2C, Ph), 141.50 (2C, Ph, C-6), 151.06 (C-2), 156.63 (2C, Ph), 164.16 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found, 392.1986.

4.2.3.12 5-methyl-1-((1-(4-phenoxybenzyl)pyrrolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**12**): Following the general procedure, 3-((benzyloxy)methyl)-5-methyl-1-(pyrrolidin-3-

ylmethyl)pyrimidine-2,4(1H,3H)-dione (**37**, 140.00 mg, 0.425 mmol), 4-phenoxybenzaldehyde (173.24 mg, 0.874 mmol) and sodium triacetoxyborohydride (277.88 mg, 1.311 mmol) yielded compound **12** (75.00 mg, 43.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.38 - 1.53 (m, 1H, pyrrolidin-4a-yl), 1.69 - 1.91 (m, 4H, 5-CH_3, pyrrolidin-4b-yl), 2.21 - 2.32 (m, 1H, pyrrolidin-2a-yl), 2.39 - 2.61 (m, 4H, pyrrolidin-2b-yl, pyrrolidin-5-yl, pyrrolidin-3-yl), 3.44 - 3.67 (m, 4H, 2CH_2, 1-methylene, phenoxybenzyl-), 6.91 - 7.03 (m, 4H, Ph), 7.09 - 7.16 (m, 1H, Ph), 7.28 - 7.34 (m, 2H, Ph), 7.34 - 7.42 (m, 2H, Ph), 7.51 (d, J = 1.17 Hz, 1H, H-6), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.92 (5-CH_3), 27.31 (pyrrolidin-4-yl), 36.50 (pyrrolidin-3-yl), 51.08 (1-methylene), 52.92 (pyrrolidin-5-yl), 56.60 (pyrrolidin-2-yl), 58.63 (CH_2, phenoxybenzyl), 108.29 (C-5), 118.38, 118.45, 123.29, 129.93, 129.99, 134.35 (6C, Ph), 141.57 (C-6), 151.06 (C-2), 155.37 (Ph), 156.82 (Ph), 164.16 (C-4). HRMS (ESI): calculated for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found, 392.1962.

4.2.4 5-methyl-1-(1-(3-phenoxybenzoyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**13**): To the reaction mixture of 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**34**, 132.75 mg, 0.403 mmol) and 3-phenoxybenzoic acid (129.49 mg, 0.605 mmol) in dry CH_2Cl_2 (10 mL) was added EDC (125.12 mg, 0.806 mmol) and 4-DMAP (1.32 mg) at room temperature under argon. The reaction mixture was stirred overnight, diluted with CH_2Cl_2 (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over sodium sulfate and concentrated in vacuum. The residue was purified with column chromatography (ethyl acetate/hexane, 25→65% in a linear gradient elution). The resulting amide intermediate was dissolved in EtOH (10 mL) and Pd/C (0.15 g) was added. The reaction was stirred under hydrogen for 6 h and the suspension was filtered. The filtrate was evaporated and dried under high vacuum for 0.5 h. The residue was dissolved in a mixture of THF/H_2O (10

mL, v/v = 2/1) and stirred for 4 h. After evaporation, the residue was purified with column chromatography ($MeOH/CH_2Cl_2$, 1→10% in linear gradient elution). After lyophilization, the desired product was obtained as a white powder (131.10 mg, 80.2%). ^1H NMR (300 MHz, DMSO-d_6, 80 ℃) δ: 1.46 - 1.65 (m, 1H, piperidin-5a-yl), 1.74 - 2.08 (m, 6H, 5-CH_3, piperidin-4-yl, piperidin-5b-yl), 2.91 (t, *J* = 11.72 Hz, 1H, piperidin-6a-yl), 3.15 (t, *J* = 11.86 Hz, 1H, piperidin-2a-yl), 3.80 - 4.21 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 4.33 (tt, *J* = 11.31, 4.36 Hz, 1H, piperidin-3-yl), 6.99 (dd, *J* = 2.34, 1.46 Hz, 1 H) 7.03 - 7.11 (m, 3 H) 7.13 - 7.20 (m, 2 H) 7.37 - 7.48 (m, 3 H) 7.52 (d, *J* = 1.17 Hz, 1 H) 10.95 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.12 (5-CH_3), 24.96 (piperidin-5-yl), 28.21 (piperidin-4-yl), 52.08 (piperidin-3-yl), 109.04 (C-5), 116.50 (Ph), 119.10 (2C, Ph), 119.39 (Ph), 121.46 (Ph), 123.93 (Ph), 130.16 (2C, Ph), 130.27 (Ph), 137.51 (C-6), 137.67 (Ph), 150.72 (C-2), 156.08 (Ph), 156.85 (Ph), 163.59 (C-4), 168.32 (CO, benzoyl). *C (piperidin-2-yl) and C (piperidin-6-yl) cannot be found*. HRMS (ESI): calculated for $[C_{23}H_{23}N_3O_4 + H]^+$, 406.1761; found, 406.1775.

4.2.5 5-methyl-1-(1-(4-phenoxybenzoyl)piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**14**): Applying 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**34**, 105.41 mg, 0.32 mmol), 4-phenoxybenzoic acid (102.82 mg, 0.48 mmol), EDC (99.67 mg, 0.64 mmol) and 4-DMAP (1.05 mg), the procedure described for the synthesis of compound **13** afforded the desired compound **14** as a white powder (96.40 mg, 74.3%). ^1H NMR (300 MHz, DMSO-d_6, 80 ℃) δ: 1.47 - 1.68 (m, 1H, piperidin-5a-yl), 1.71 - 2.10 (m, 6H, 1.74 - 2.08 (m, 6H, 5-CH_3, piperidin-4-yl, piperidin-5b-yl), 2.84 - 2.98 (m, 1H, piperidin-6a-yl), 3.14 (t, *J* = 11.72 Hz, 1H, piperidin-6a-yl), 3.99 (d, *J* = 12.01 Hz, 1H, piperidin-6b-yl), 4.13 (d, *J* = 10.84 Hz, 1H, piperidin-2a-yl), 4.34 (tt, *J* = 11.35, 4.32 Hz, 1H, piperidin-2b-yl), 6.96 - 7.10 (m, 4H, Ph), 7.14 - 7.22 (m, 1H, Ph), 7.35 - 7.47 (m, 4H, Ph), 7.52 (d, *J* = 1.17 Hz, 1H, H-6), 10.93 (br. s., 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6, 80 ℃) δ: 12.13 (5-CH_3), 24.58 (piperidin-5-yl), 28.20 (piperidin-4-yl), 41.70 (piperidin-6-yl), 50.96 (piperidin-3-yl), 109.05 (C-5), 117.53 (2C, Ph), 119.50 (2C, Ph), 124.22 (Ph), 129.23 (2C, Ph), 130.23 (3C, Ph), 137.50 (C-6), 150.78 (C-2), 155.63 (Ph), 158.06 (Ph), 163.60 (C-4), 168.78 (CO, benzoyl). *C (piperidin-2-yl) cannot be found*. HRMS (ESI): calculated for $[C_{23}H_{23}N_3O_4 + H]^+$, 406.1761; found, 406.1757.

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