Journal of
Medicinal Chemistry

Article

Mycobacterium tuberculosis Decaprenylphosphoryl-#-D-ribose Oxidase inhibitors: expeditious reconstruction of sub-optimal hits into a series with potent in vivo activity

Jennifer A Borthwick, Carlos Alemparte, Ian Wall, Benjamin C. Whitehurst, Argyrides Argyrou, Glenn A. Burley, Paco de Dios-Anton, Laura Guijarro, Maria Candida Monteiro, Fatima Ortega, Colin J. Suckling, Julia Castro-Pichel, Monica Cacho, and Robert J Young

J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 10 Jan 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Mycobacterium tuberculosis Decaprenylphosphoryl-β-D-ribose Oxidase inhibitors: expeditious reconstruction of sub-optimal hits into a series with potent in vivo activity

Jennifer A. Borthwick†*, Carlos Alemparte‡, Ian Wall†, Benjamin C. Whitehurst†, Argyrides Argyrou†, Glenn Burley¶, Paco de Dios-Anton‡, Laura Guijarro‡, Maria Candida Monteiro‡, Fatima Ortega‡, Colin J Suckling¶, Julia Castro Pichel‡, Monica Cacho‡*, Robert J. Young†*

† Medicinal Science and Technology, GlaxoSmithKline, Gunnels Wood Road, Stevenage SG1 2NY, UK

‡ Global Health R&D, GlaxoSmithKline, Severo Ochoa 2, Tres Cantos, 28760 Madrid, Spain

¶ Department of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, Scotland

*jennifer.borthwick@blackbelttx.com

*monica.i.cacho@gsk.com

*Rob@blue-burgundy.co.uk

KEYWORDS: DprE1, tuberculosis, H2L, scaffold-hopping, fragment based design.

ABSTRACT

Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) is an essential enzyme in *Mycobacterium tuberculosis* and has recently been studied as a potential drug target, with inhibitors progressing to clinical studies. Here we describe the identification of a novel series of morpholino-pyrimidine DprE1 inhibitors. These were derived from a phenotypic high-throughput screening (HTS) hit with sub-optimal physicochemical properties. Optimization strategies included scaffold-hopping, synthesis and evaluation of fragments of the lead compounds and property-focussed optimization. The resulting optimized compounds had much improved physicochemical properties and maintained enzyme and cellular potency. These molecules demonstrated potent efficacy in an *in vivo* tuberculosis murine infection model.

TEXT

Introduction

Tuberculosis (TB) is the most deadly infectious disease in human history, killing over 1 billion people in the last 200 years and continues to kill at a rate of more than 1 million people per year.[1] Currently treatment options for TB require patients to take a combination of drugs for between 6 months and 3 years.[2] Some of these drugs can cause serious side-effects and many are increasingly becoming ineffective due to evolving resistance patterns,[3] including multidrug resistance (MDR), extremely (XDR) and even totally drug resistant strains (TDR). Therefore, there is an urgent need to develop new drug treatments for TB, especially those with novel mechanisms of action, which would not be expected to suffer from the same resistance mechanisms as marketed drugs.

Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) was identified the target of a series of potent bactericidal molecules, through genetic analysis of resistant strains generated in vitro.[4] DprE1 is the key enzyme in the only known biosynthetic pathway by which mycobacteria produce arabinose, which is an essential component of the cell wall

arabinogalactan scaffold.[5] Furthermore, mycobacteria with reduced expression of functional DprE1 were shown to be non-viable,[6, 7] and amongst 240 clinically isolated strains of *Mycobacterium tuberculosis (Mtb),* [8] no mutations were detected in the gene encoding DprE1. This mechanism of arabinose biosynthesis via Decaprenylphosphoryl-β-D-ribose is unique to mycobacteria, so there is inherent biochemical selectivity over human cells and other bacterial species.[8]

A number of small-molecule inhibitors of DprE1 have now been published, reflecting the high level of interest in this exciting target (Figure 1).[4, 9-23] The covalent binders[24] BTZ043[25] and PBTZ169 (macozinone)[26] have progressed to clinical evaluation, as have the non-covalent compounds AZ7371[27] and OPC-167832.[28]

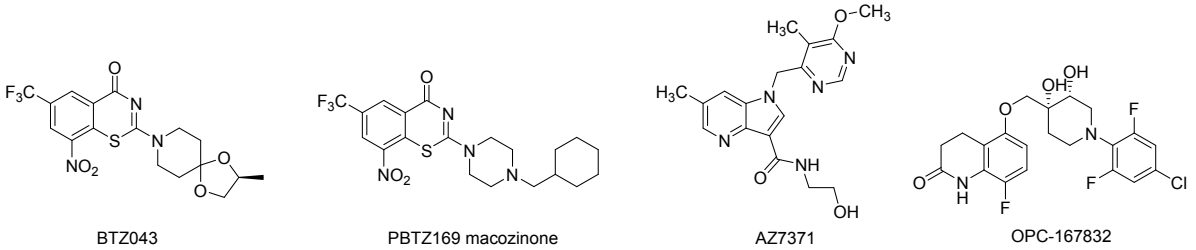


Figure 1. Structures of selected published DprE1 inhibitors.[4, 9, 23, 27, 28]

Pyrrolothiadiazole **1**, one of 177 antitubercular compounds released by GSK[29] was identified as an inhibitor of DprE1 by its reduced activity in a DprE1 over-expressor strain of *M. tuberculosis*.[30] In this paper we describe optimization of 1 into a lead series with an attractive physical profile and highly encouraging *in vivo* efficacy. We demonstrate how the proposed "attention to decreasing lipophilicity and/or minimising aromatic ring count"[29] was achieved through lead hopping. The progression and quality of the molecules produced in the ensuing deconstruction and reconstruction process was monitored using physicochemical measurements and efficiency metrics.[31]

Results and Discussion

To generate some initial structure-activity relationship (SAR) around initial hit, pyrrolothiadiazole **1**was truncated to **2**, retaining reasonable ligand efficiency. Subsequently, a key finding in the move away from the unattractive pyrrolothiadiazole functionality was screening of compounds selected by a similarity search of the GSK collection, based on Tanimoto scores. Of these, piperidinylpyrimidines **3** and **4** retained DprE1 activity and showed a minimum inhibitory concentration (MIC). The most

productive search used the piperidine-acyl-piperidine moiety as the substructure, generating a range of alternatives for the thiadiazole, pyrrole and benzyl groups. These searches identified truncated compounds such as **2**, which retained DprE1 activity in the biochemical assay that translated into an MIC (Figure 2).[30]

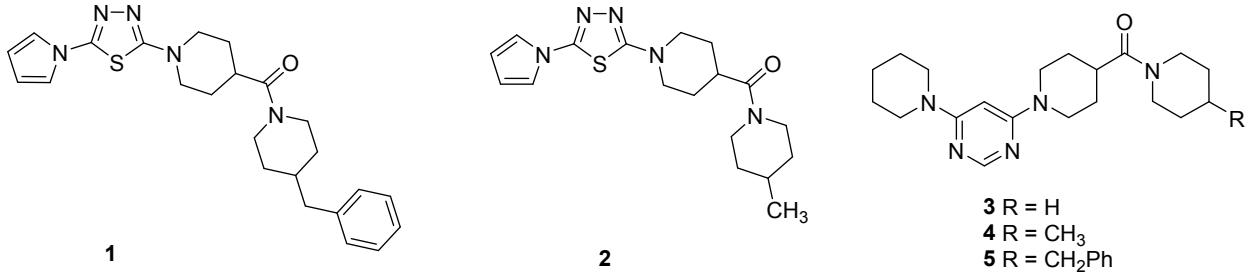


Figure 2. Structure of phenotypic screening hit 1 and analogues identified by substructure and similarity searching.

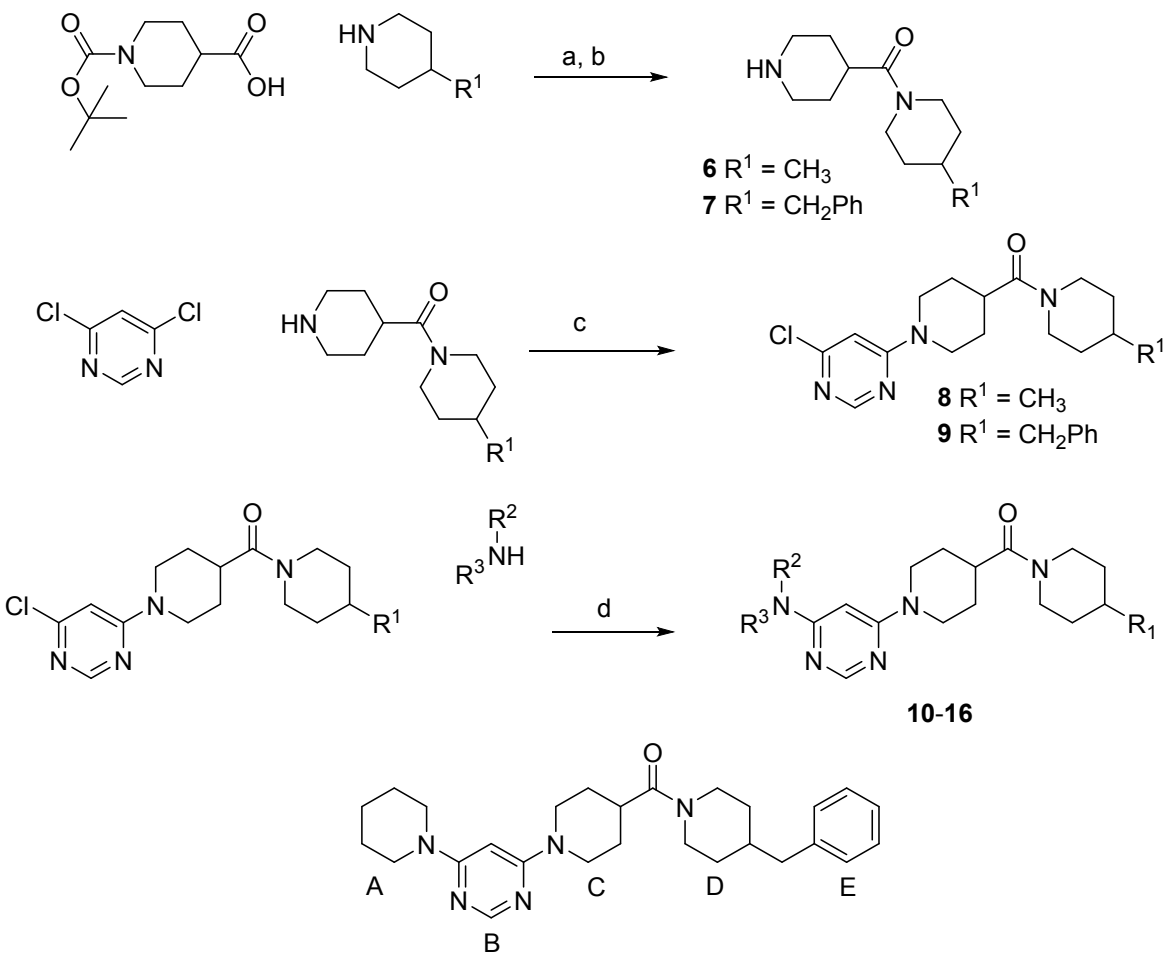
A key point in the hits to leads phase was securing DprE1 activity and an MIC with piperidinopyrimidines **3** and **4** which had markedly improved physical properties (Table 2) and obviated perceived metabolic/toxicological risks with the pyrrole and thiadiazole rings (Figure 2).[32]. The likely common SAR between the thiadiazole- and pyrimidine-containing series was indicated by the benzyl analogues **1** and **5**, but further

physicochemical improvement was a clear priority. These data supported the notion of a systematic exploration of the SAR and structure property relationships (SPR) in the series, facilitated by a relatively straightforward synthetic protocol involving amide formation and S_NAr elaboration of the aromatic heterocycle (Scheme 1). The strategy is best described in terms of the five constituent rings of the structure, labelled A,B,C, D and E for convenience; the execution including fragmentation to <20 heavy atom A-C fragment structures to identify key binding interactions and regrowth along defined vectors to optimize activity, property forecast index (PFI) and ligand efficiency.[31]

Compound	DprE1 pIC_{50}[a]	*Mtb* H37Rv MIC_{90} (μM)	PFI[c]	Solubility CLND (μM)[d]
1	7.3[b]	4.0	9.4	<1
2	6.3	20.8	7.1	328
3	5.8	35.6	5.8	>405
4	6.5	15.6	6.5	>488
5	7.1	2.5	8.9	28

Table 1. Measured properties of hit **1** and analogues **2-5**. [a]DprE1 pIC_{50} is the negative logarithm of the IC_{50}-concentration expressed in molar (M) obtained in the DprE1-

inhibition assay. DprE1 pIC_{50}values above 7.3 are approaching tight binding limit of assay and should be treated with caution.[b] Compound tested inactive (pIC_{50} < 4) on 2 out of 62 test occasions. [c]Property forecast index (PFI), is the sum of the Chrom log $D_{7.4}$ and the number of aromatic rings. [d]Kinetic solubility measured by CLND (see Experimental Section).



Scheme 1. Synthesis of compounds containing replacements for left hand side piperidine. Reagents and conditions: (a) Substituted piperidine (1.2 eq.), COMU (2 eq.), *N,N*-diisopropylethylamine (3 eq.), N,N-dimethylformamide, room temperature, 2 – 20 h; (b) Hydrochloric acid (4 M in dioxane) (5 eq.), room temperature, 0.5 – 17 h, 91-100% over 2 steps; (c) N,N-diisopropylethylamine (2 eq.), ethanol, microwave, 150 °C, 5 min.;

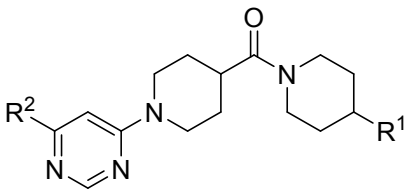
(d) Amine (5 eq.), N,N-diisopropylethylamine (2 eq.), ethanol, microwave, 150 °C, 1 h, 19-43 % over 2 steps.

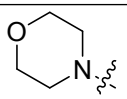
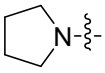
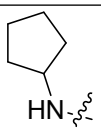
Chemistry

The bis-piperidine amides **6** and **7** were formed by COMU-mediated amide bond formation, followed by BOC-deprotection. Sequential nucleophilic aromatic substitutions onto 4,6-dichloropyrimidine afforded the final products **10-16**. The versatility of this route enabled the three steps to be performed in any order, depending on where any structural diversity was to be introduced. The two nucleophilic aromatic substitutions were carried out in one microwave vial, with the second amine simply pipetted into the vial on completion of the first reaction. These reactions were carried out in an appropriate volume of solvent (0.5-0.9 mL) to allow direct injection of the crude reaction mixture onto mass-directed prep-HPLC at the end of the reactions without the need to carry out any work-up. The designed route allowed rapid exploration of piperidine replacements, affording panel of compounds **10-16**-.

Ring A

A set of small branched and alicyclicamines was selected to explore potential variations in Ring A.



Compound	R1	Ring A = R2	DprE1 pIC_{50}[a]	*Mtb* H37Rv MIC_{90} (μM)	PFI[b]
10	CH_3		6.0	15.6	4.9
11	CH_3	$N(CH_3)_2$	5.0	125	5.0
12	CH_3		5.9	23.4	5.5
13	CH_3		4.7	>125	6.1
14	CH_2Ph	H	4.8	>125	6.8

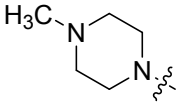
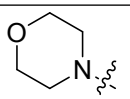
15	CH_2Ph		4.7	125	6.7
16	CH_2Ph		7.6	0.6	7.4

Table 2. Results of replacement of piperidine with alternative rings in compounds **10**-**16**.

[a]DprE1 pIC_{50} is the negative logarithm of the IC_{50}-concentration expressed in molar (M) obtained in the DprE1-inhibition assay. DprE1 pIC_{50}values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b]Property forecast index (PFI), is the sum of the Chrom log $D_{7.4}$ and the number of aromatic rings.

The results for ring A variation (Table 2) showed that SAR was fairly restricted and most of the groups synthesized were less active than the parent piperidine. Only the morpholine group (**10**, **16**) showed a similar level of activity, within assay limits, to piperidine, but with around a 1.5 unit reduction in chrom logD compared with the piperidine compounds (**3**-**5**). In particular, the benzyl compound **16** represented an important milestone in this series, being the first compound to achieve a sub-micromolar

MIC against *Mtb* H37Rv. This compound was thus more fully profiled as shown in Table 3.

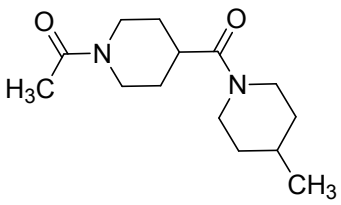
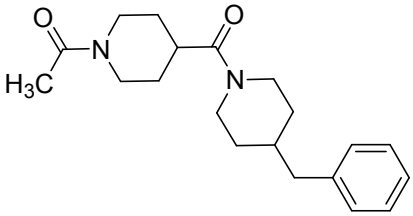
Parameter	Compound 16
DprE1 pIC_{50} [a]	7.6
PFI[b]	7.4
Mtb H37Rv MIC_{90} (μM)	0.6
Intracellular H37Rv MIC_{90} (μM)[c]	0.07
DprE1 OE MIC_{90} (μM)[d]	>16
Antibacterial panel MIC (μg/mL)	>128
CLND solubility (μM)[e]	280
Microsomes Clint h/m (ml/min/g tissue)[f]	4.4/13.5

Table 3. Full profile of compound **16**. [a]DprE1 pIC_{50} is the negative logarithm of the IC_{50}-concentration expressed in molar (M) obtained in the DprE1-inhibition assay. DprE1 pIC_{50}values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b]Property forecast index (PFI), is the sum of the Chrom log $D_{7.4}$ and the number of aromatic rings. [c]MIC against M. tuberculosis (H37Rv) in infected Human THP-1 macrophages. [d]Overexpressor (OE) MICs are for M. tuberculosis transformed with pMV261:dprE1.[30] [e]Kinetic solubility measured by CLND (see Experimental Section). [f]Microsomal fraction stability (see Experimental Section).

The highly potent compound **16** translated its enzyme activity into low MIC values both in the cultured mycobacteria assay, and the intracellular assay in macrophages. Target engagement was demonstrated with **16** in a DprE1 over-expressor strain of *Mtb*; its MIC was shown to increase substantially. Importantly, wider profiling of compound **16** supported the potential of the series, whereby it was inactive against an antibacterial panel of 19 common bacterial species, including *Escherichia coli* and *Staphylococcus aureus*, in line with expectations of this unique mycobacterial process. Encouraged by these findings, further optimization was pursued to address shortcomings such as the high intrinsic clearance in microsomes.

Encouraged by these data a systematic investigation was initiated with tricyclic structures, which, with generally <20 heavy atoms were treated as fragments. These were synthesised to explore the minimum pharmacaphore required for measurable DprE1 inhibition. The ligand efficiency (LE) of the qualified hits suggested that with maintenance of LE, measurable activity would be expected in the DprE1 assay for these fragments. The initial set is exemplified in Table 4. Removal of rings A and B (**18**) or A, B and E (**17**),

by capping **6** or **7** using acetic anhydride, gave no demonstrable activity, unlike the A,B,C analogues **19**-**21** which showed weak activity, but retaining reasonable LE. An oxidised core was also synthesised (**22**), which demonstrated similar activity to pyrimidine, along with good LE.

Compound	DprE1 pIC_{50}[a]	LE[c]
 **17**	<3.0[b]	
 **18**	<4.0	

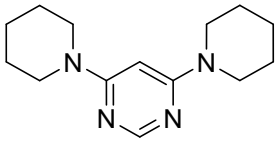
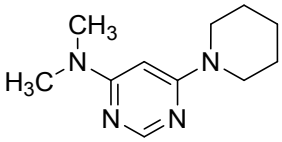
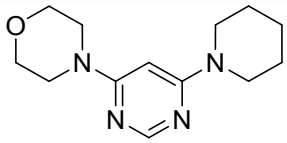
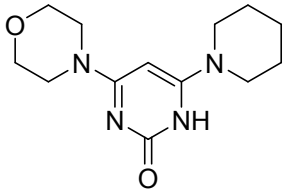
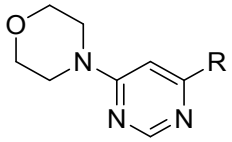
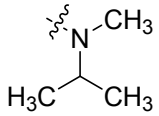
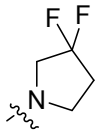
 **19**	4.1	0.31
 **20**	3.9[b]	0.36
 **21**	4.1	0.31
 **22**	4.5	0.32

Table 4. Compounds synthesised to identify efficient binding fragments. [a]DprE1 pIC50 is the negative logarithm of the IC50-concentration expressed in molar (M) obtained in the DprE1-inhibition assay. DprE1 pIC50values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b]FPR assay data (see Experimental section for details). [c]Ligand efficency (LE) = pIC_{50}*1.37/number of heavy atoms.

These results supported growth from the 4-morpholino pyrimidine fragment to find the optimal structure for ring C. The SAR in this region of the molecule appeared to be fairly restricted (Table 5 shows key exemplars), with most of the synthesized compounds tested (44 fragment compounds were synthesized) showing no quantifiable activity in the DprE1 assay, indicating that a piperidine linker was optimal.



Compound	Ring C = R	DprE1 pIC_{50}[a]	LE/LLE_{At}[b]
23		<4.0	
24		4.1	0.30/0.32

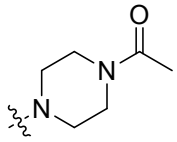
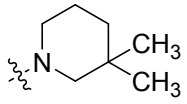
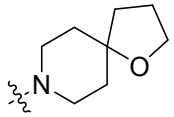
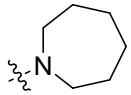
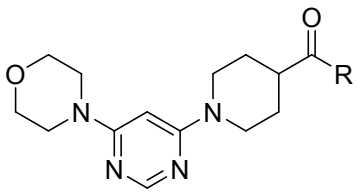
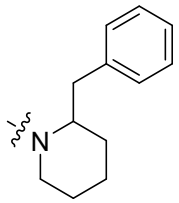
25		<4.0	
26		4.6	0.32/0.25
27		4.1	0.26/0.33
28		<4.0	

Table 5. Results of fragments tested to explore SAR of central piperidine ring. [a]DprE1 pIC50 is the negative logarithm of the IC50-concentration expressed in molar (M) obtained in the DprE1-inhibition assay. DprE1 pIC50values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b]Ligand efficency (LE) = pIC_{50}*1.37/number of heavy atoms. Ligand lipophilicity efficiency Astex (LLE_{AT}) = 0.111 + 1.36*LLE/number of heavy atoms.

4-Piperidinyl was demonstrated to be the optimum C-ring through evaluation of a series of analogues of **16** (data not shown) with pyrrolidine, piperidine and piperazine rings that showed little or no activity.

Alternatives to ring D were then explored. These analogues (Table 6) all showed reduced activity at DprE1 relative to compound **16**, with only the pyrrolidine analogue **36** showing similar potency, again indicating the likely primacy of a 4-substituted, achiral, piperidine connectivity.



Compound	Ring D = R	DprE1 pIC_{50}[a]	*Mtb* H37Rv MIC_{90} (μM)	PFI[b]
29		5.4	125	7.2

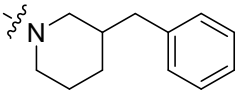
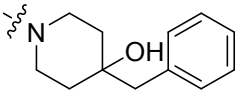
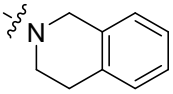
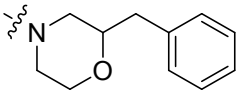
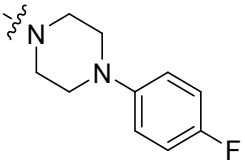
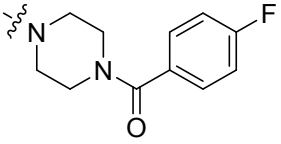
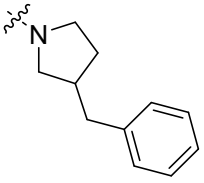
30		6.2	32	7.6
31		5.8	10	5.5
32		6.5	10	6.3
33		5.2	>125	6.3
34		6.2	20	6.3
35		4.6	>80	4.9
36		6.9	4	6.6

Table 6. Results of replacements of distal piperidine ring. [a]DprE1 pIC_{50} is the negative logarithm of the IC_{50}-concentration expressed in molar (M) obtained in the DprE1-

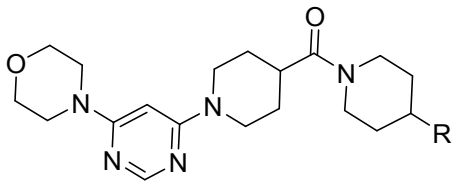
inhibition assay. DprE1 pIC_{50} values above 7.3 are approaching tight binding limit of assay and should be treated with caution.[b]Property forecast index (PFI), is the sum of the Chrom log $D_{7.4}$ and the number of aromatic rings.

Removal or replacement of ring E offered opportunities to further optimize properties, improve potency or alleviate likely metabolic liabilities of an unsubstituted phenyl ring (Table 7).

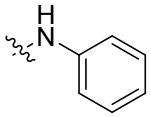
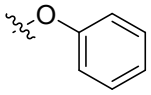
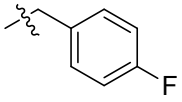
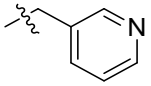
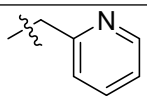
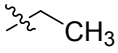
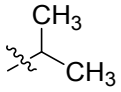
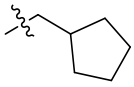
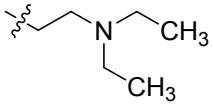
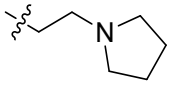
Replacement of the benzylic methylene group with a heteroatom was well-tolerated (Table 7, compounds **37** and **38**) and led to reduction in PFI and improved intrinsic clearance. Blocking likely sites of metabolism with halide substituents, such as in compound **39** retained activity and gave an improved microsomal clearance. Replacement of the phenyl ring with heterocyclic alternatives (compounds **40**-**41**), resulted in fairly restricted SAR, with only pyridine rings retaining any activity against DprE1. Interestingly, the phenyl ring could be replaced entirely with a range of alkyl groups (compounds **42**-**44**). Unfortunately, it was not possible to lower logD by

introducing heteroatoms into alkyl analogues (e.g. with compounds **45**-**46**). All such attempts resulted in complete loss of DprE1 activity.

A further iteration of optimization combined the ether linkage with the improved phenyl replacements. This strategy was very successful in identifying compounds such as **47**-**51** which had excellent DprE1 potencies, low MIC values and very low microsomal clearance values. Within the set available there were highly potent molecules with encouraging profiles worthy of progression into in vivo studies.



Compound	Ring E = R	DprE1 pIC_{50}[a]	*Mtb* H37Rv MIC_{90} (μM)	PFI[b]	Microsomes Clint h/m (ml/min/g tissue)[c]

37		6.4	10	6.3	
38		8.1	2	6.8	7.4/<0.5
39		7.6	0.6	7.4	4.6/3.1
40		7.0	3.3	5.2	3.1/7.2
41		6.6	8.8	5.2	
42		6.8	8.0	5.6	
43		7.1	4.0	6.2	3.3/5.1
44		7.6	3.0	7.9	2.1/3.1
45		<4.0	>125	2.2	
46		4.1	>125	2.1	

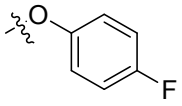
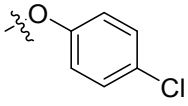
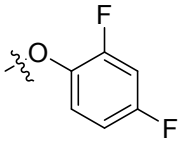
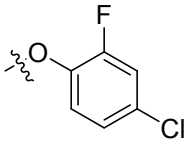
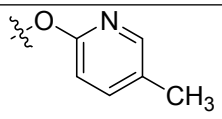
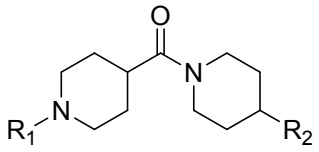
47		7.3	1.7	6.7	<0.5/0.7
48		7.5	1.5	7.6	0.9/1.2
49		7.5	1.0	6.9	0.6/0.5
50		7.7	0.5	7.6	1.8/2.9
51		6.2	16	6.5	

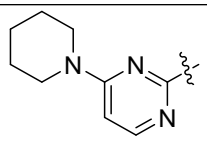
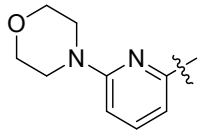
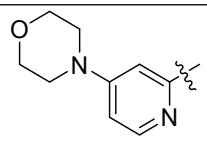
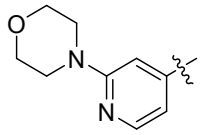
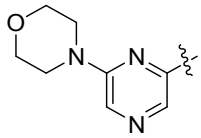
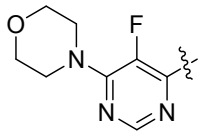
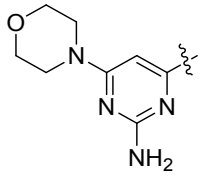
Table 7. Results of replacements of benzyl group.[a]DprE1 pIC_{50} is the negative logarithm of the IC_{50}-concentration expressed in molar (M) obtained in the DprE1-inhibition assay. DprE1 pIC_{50}values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b]Property forecast index (PFI), is the sum of the Chrom log $D_{7.4}$ and the number of aromatic rings. [c]Microsomal fraction stability (see Experimental Section).

To complete the systematic optimization, variation of alternatives to the pyrimidine (ring B) offered means of modulating the physicochemical properties of the molecule, by

varying nitrogen count and basicity. This work followed the A.C,D and E ring optimizations and key substituents from the SAR investigations (Table 8). There were interesting SAR effects observed in these alternative cores: heterocycles containing two nitrogen atoms such as the isomeric pyrimidine **52** or pyrazine **56** retained good potency, but the more basic pyridine compounds **53**-**55** had much reduced potency. Small substituents on the pyrimidine ring in compounds **57**-**59** were well tolerated and offered alternative means of lipophilicity modulation, e.g. the pyrimidinone compound **59**, maintained good activity and MIC levels with lower logD, which may account for its improved metabolic stability.



Compound	R1	R2	DprE1 pIC_{50}[a]	*Mtb* H37Rv MIC_{90} (μM)	PFI[b]	Microsomes Clint h/m (ml/min/g tissue)[c]

52		CH_2Ph	6.8	16.0	10.0	
53		$CH(CH_3)_2$	5.0	>125	8.3	
54		$CH(CH_3)_2$	5.2	>125	5.6	
55		$CH(CH_3)_2$	5.1	>125	5.6	
56		CH_2Ph	7.4	3.0	8.0	5.4/63.7
57		CH_2Ph	7.2	1.3	8.4	2.8/8.3
58		CH_2Ph	7.3	3.5	7.1	3.4/11.6

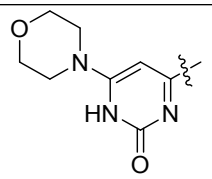
59		CH_2Ph	7.4	3.0	5.6	<0.5/4.9

Table 8. Results of replacements of pyrimidine core. [a]DprE1 pIC_{50} is the negative logarithm of the IC_{50}-concentration expressed in molar (M) obtained in the DprE1-inhibition assay. DprE1 pIC_{50}values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b]Property forecast index (PFI), is the sum of the Chrom log $D_{7.4}$ and the number of aromatic rings. [c]Microsomal fraction stability (see Experimental Section).

As a result of the property-focussed optimization of the series described here, a number of compounds had potent DprE1 inhibition combined with low MIC values and much improved *in vitro* DMPK properties, which together supported progression into *in vivo* efficacy studies. Compounds **39** and **47** (Table 9) were thus progressed into a mouse model of tuberculosis infection.

Compound	**39**	**47**	**60**[f]
DprE1 pIC_{50}[a]	7.6	7.3	7.4
PFI	7.4	6.7	6.6

Mtb H37Rv MIC_{90} (μM)		0.6	1.7	4
Intracellular H37Rv MIC_{90} (μM)		0.21	0.62	0.62
DprE1 OE MIC (μM)[b]		15	62	>125
Cytotoxicity HepG2 pIC_{50}		4.3[c]	4.5[d]	<4
CLND solubility (μM)		160	≥364	26
FaSSIF solubility (μg/mL)		40	74	ND
Microsomes Clint h/m (ml/min/g tissue)		4.6/3.1	<0.5/0.7	<0.5/4.2
In vivo mouse PK[e]	Cl (ml/min/kg)	38.9	23.5	
	Vss (L/kg)	1.3	1.7	
	$t_{1/2}$ (h)	0.45	1.0	
	%F	100%	78.7%	

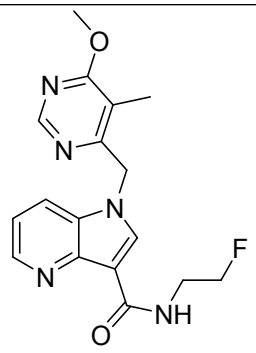
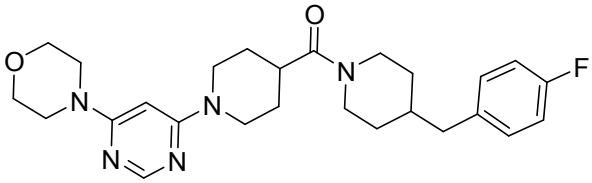
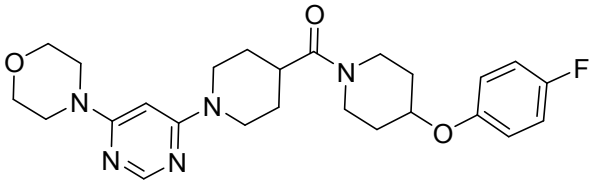
Table 9. Profiles of compounds **39** and **47** in a range of *in vitro* and *in vivo* assays. [a] DprE1 pIC_{50} values above 7.3 are approaching tight binding limit of assay and should be treated with caution. [b] Compound tested inactive (pIC_{50} = 4.0) on 6 out of 10 test occasions. [c] Compound tested inactive (pIC_{50} < 4.0) on 2 out of 4 test occasions. [d] Compound tested

inactive (pIC_{50} < 4.0) on 2 out of 7 test occasions. [e] Clearance (Cl), volume of distribution at steady state (Vss) and half-life ($t_{1/2}$) obtained after intravenous administration to CD-1 male mice at 1 mg/kg; Bioavailability (%F) obtained after oral administration to CD-1 male mice at 5 mg/kg. [f]AZ DprE1 inhibitor benchmark (compound **9** in reference).[18]

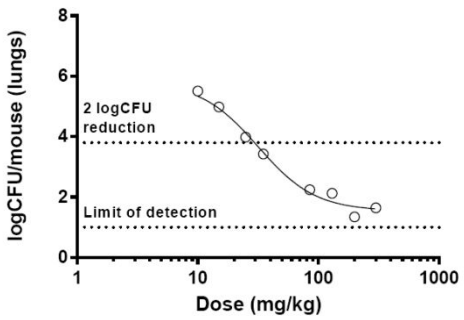
In this acute model of TB infection,[34] mice were subjected to an intratracheal infection of *M. tuberculosis* and then were treated every day for eight days beginning on the day after infection with either compound **39** or **47** at varying doses. AZ DprE1 inhibitor **60** was profiled as a direct comparison, both with and without 1-aminobenzotriazole (ABT), a CYP P450 inhibitor.[18] At the end of the experiment, the animals were sacrificed and the number of colony forming units (CFUs) in the lungs were counted and compared with untreated animals.

a

Compound	Structure	ED_{99} (mg/kg)
60		164

60 + ABT		11
39		30
47		29

b



c

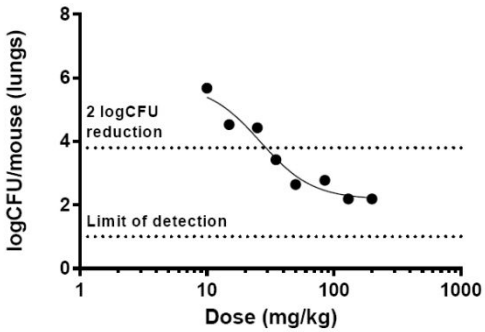


Figure 3: Results from *in vivo* efficacy experiment for compounds **39** and **47**. (a) Calculated ED_{99} values; dose response curves for (b) compound **39** and (c) compound **47**. CFU number in lungs of untreated mice: 5.8 logCFU.

The *in vivo* efficacy study demonstrated that compounds **39** and **47** were highly effective at alleviating acute *M. tuberculosis* infection in mice, with ED_{99} values around 30 mg/kg, similar levels of activity to several marketed anti-tuberculosis drugs in this model.[34]

Conclusions

A highly promising series of DprE1 inhibitors was identified through carrying out judicious property-and efficiency-focused optimization of a phenotypic screening hit with sub-optimal physicochemical properties. This series includes exemplars that are potent inhibitors of DprE1 in vitro, have a promising developability profile and are efficacious in vivo in an acute mouse model of tuberculosis infection.

Experimental

Chemistry

Unless stated otherwise all reagents were used as obtained from commercial sources. All solvents and compounds were of analytical grade and used as supplied.

Liquid Chromatography-Mass Spectroscopy (LCMS) was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm i.d. 1.7 µm packing diameter) at 40 °C, eluting with either 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (solvent A) and acetonitrile (solvent B) or 0.1 % v/v solution of formic acid in water (solvent A) and 0.1 % v/v solution of formic acid in acetonitrile (solvent B). The UV detection is a summed signal from wavelength of 210 nm to 350 nm. This summed wavelength is used for purity measurements quoted. The mass spectra were recorded on a Waters ZQ spectrometer using electrospray positive and negative mode, scanning from 100-1000 AMU. The following elution gradients were used. Ammonium bicarbonate 0-1.5 min. 1-97 % B, 1.5-1.9 min. 97 % B, 1.9-2 min. 97-1% B. Formic acid 0-1.5 min. 3-100 % B, 1.5-1.9 min. 100 % B, 1.9-2 min. 100-3% B. Alternatively, where a 4.5 min run is specified, LCMS was conducted on an Acquity BEH C18 column (50 mm x 2.1 mm i.d. , 1.7µm packing diameter) at 35 °C eluting with 0.1 % v/v solution of formic acid in water (solvent A) and

0.1 % v/v solution of formic acid in acetonitrile (solvent B) using the following gradient. 0-0.4 min. 3 % B, 0.4-3.2 min. 3-98 % B, 3.2-3.8 min. 98 % B, 3.8-4.2 min. 98-3 % B. Preparative mass directed HPLC was conducted on a Waters MassLynx system comprising of a Waters 515 pump with extended pump heads, Waters 2767 autosampler, Waters 996 photodiode array detector and Gilson 202 fraction collector on a XBridge or Sunfire C18 column (30 mm x 150 mm i.d. 5 µm packing diameter) at ambient temperature. The mobile phase was 0.1% formic acid in water or 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (solvent A) and 0.1% formic acid in acetonitrile or acetonitrile (solvent B). The UV detection is a summed signal from wavelength of 210 nm to 350 nm. Mass spectra were recorded on Waters ZQ mass spectrometer using alternate-scan positive and negative electrospray ionization. The software used was MassLynx 3.5 with FractionLynx option or using equivalent alternative systems. The elution gradients used were at a flow rate of 40 mL/min over 10 or 20 min. High resolution mass spectra (HRMS) were obtained on a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface (ESI), over a mass range of 100−1100 Da, with a scan time of 0.9 s and an interscan delay of

0.1 s. Reserpine was used as the external mass calibrant ([M + H]+ = 609.2812 Da). The Q-Tof Ultima mass spectrometer was operated in W reflectron mode to give a resolution (fwhm) of 16000−20000. Ionization was achieved with a spray voltage of 3.2 kV, a cone voltage of 50 V, with cone and desolvation gas flows of 10−20 and 600 L/h, respectively. The source block and desolvation temperatures were maintained at 120 and 250 °C, respectively. The elemental composition was calculated using MassLynx v4.1 for the [M+ H]+ and the mass error quoted as ppm,

^{1}H and ^{13}C NMR spectra were recorded using a Bruker DPX250, DPX400, AV400 or referenced to tetramethylsilane. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) in Hz. The following abbreviations are used for multiplicities: s = singlet; br s = broad singlet; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets; ddd = double double doublet; q = quartet; quin = quintet; sext = sextet. The following abbreviations are used to assign ^{13}C spectra: Ar = aryl; C=O = carbonyl.

All microwave reactions were carried out in a Biotage Initiator® system in specialist microwave vials supplied by Biotage.

The purity of all compounds screened in the biological assays was examined by LC−MS analysis and was found to be ≥95% unless otherwise specified.

(1-(5-(1H-pyrrol-1-yl)-1,3,4-thiadiazol-2-yl)piperidin-4-yl)(4-benzylpiperidin-1-yl)methanone (**1**), (1-(5-(1H-pyrrol-1-yl)-1,3,4-thiadiazol-2-yl)piperidin-4-yl)(4-methylpiperidin-1-yl)methanone (**2**) and piperidin-1-yl(1-(6-(piperidin-1-yl)pyrimidin-4-yl)piperidin-4-yl)methanone (**3**) were purchased from ChemDiv.

General Procedure A: Piperidine Amide Formation

1-(*Tert*-butoxycarbonyl)piperidine-4-carboxylic acid (1 eq.) was dissolved in DMF (2 – 10 mL) and treated with COMU (2 eq.) and DIPEA (3 eq.), followed by the appropriately substituted amine (1.2 eq.). The resulting solutions were stirred at room temperature overnight. The intermediates were worked-up as described and then treated with hydrochloric acid (5-10 eq) and the solutions were stirred at room temperature until reactions were complete. The crude products were worked-up and purified as described.

General Procedure B: Nucleophilic Aromatic Substitutions

4,6-Dichloropyrimidine (1 eq.) was dissolved in ethanol and then treated with an amine (1 eq.) and DIPEA (2.5 eq.). The resulting solutions were heated in a microwave reactor at 150 °C for 5 minutes. A second amine (2 - 5 eq.) was then added and the solutions were heated in a microwave reactor at 150 °C for 1 hour. Unless otherwise stated, the products were purified by direct injection of the reaction solution onto preparative HPLC purification.

(4-Methylpiperidin-1-yl)(1-(6-(piperidin-1-yl)pyrimidin-4-yl)piperidin-4-yl)methanone (4)

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6, 48 mg, 0.23 mmol), followed by piperidine (0.11 mL, 1.14 mmol, 5 eq.) were reacted according to general procedure B to afford the title compound (4) as a colourless oil (53 mg, 0.13 mmol, 56 %). LCMS (formic): Rt = 0.80 min, $[M+H]^+$ = 373. HRMS $C_{21}H_{34}N_5O$ required 372.2758, found 372.2762. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (s, 1 H) 5.86 (s, 1 H) 4.34 (br d, J = 13.2 Hz, 4 H) 3.98 (d, J = 12 Hz, 2 H) 3.94 - 4.09 (m, 6 H) 2.79 - 3.10 (m, 5 H) 1.36 - 1.80 (m, 10 H) 0.98 - 1.15 (m, 1 H) 0.91 (d, J = 6.4 Hz, 3 H).

(4-Benzylpiperidin-1-yl)(1-(6-(piperidin-1-yl)pyrimidin-4-yl)piperidin-4-yl)methanone (5)

(4-Benzylpiperidin-1-yl)(piperidin-4-yl)methanone (7, 77 mg, 0.27 mmol), followed by piperidine (0.13 mL, 1.34 mmol, 5 eq.) were reacted according to general procedure B to afford the title compound (5) as an orange oil (44 mg, 0.10 mmol, 37 %). LCMS (formic): Rt = 0.99 min, $[M+H]^+$ = 448. ^{1}H NMR (DMSO-d_6) δ ppm 8.03 (s, 1 H); 7.25 – 7.31 (m, 2 H); 7.15 – 7.21 (m, 3 H); 5.85 (br. s, 1 H); 4.29 – 4.39 (m, 3 H); 3.95 – 4.02 (m, 1 H); 3.53 (t, J = 5.5 Hz, 4 H); 2.80 - 3.02 (m, 4 H); 1.70 - 1.83 (m, 1 H); 1.54 - 1.67 (m, 7 H); 1.39 - 1.54 (m, 7 H); 1.05 - 1.13 (m, 2 H); 0.91 - 1.02 (m, 1 H).

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6)

4-Methylpiperidine (0.63 mL, 5.3 mmol) was reacted according to general procedure A. The intermediate was worked up by removing the solvent by evaporation and partitioning the residue between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was extracted with additional ethyl acetate (2 X 20 mL) and the combined organic layers were dried by passing through a hydrophobic frit and concentrated to yield a red oil. The residue was treated with hydrochloric acid (4 M in dioxane) (5.56 mL, 22.2 mmol). When the

deprotection was complete, the solution was basified by addition of saturated aqueous sodium bicarbonate (100 mL) and was then extracted with DCM (2 X 100 mL). The combined organic layers were passed through a hydrophobic frit and concentrated. This material was purified on a 150 g C18 GOLD® Aq column, eluting with a gradient of 5 - 85 % acetonitrile in 10 mM ammonium bicarbonate (aq.) to afford the title compound (6) in multiple batches of varying purity, all of which were used as intermediates in following steps. Batch 1 as a brown gum (68 mg, 0.32 mmol, 7 %). LCMS (ammonium bicarbonate): Rt = 0.71 min, $[M+H]^+$ = 211, purity = 100 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.57 (d, *J* = 12.5 Hz, 1 H); 3.87 (d, *J* = 12.5 Hz, 1 H); 3.17 (d, *J* = 11.7 Hz, 2 H); 2.93 – 3.06 (m, 2 H); 2.59 - 2.76 (m, 3 H); 2.46 – 2.61 (m, 2 H); 1.25 (s, 3 H); 1.01 – 1.14 (m, 3 H); 0.95 (d, *J* = 6.5 Hz, 4 H), purity by NMR approx. 90 %. Batch 2 as a brown gum (80 mg, 0.38 mmol, 9 %). LCMS (ammonium bicarbonate): Rt = 0.71 min, $[M+H]^+$ = 211, purity = 33 %. NMR consistent with batch 1, purity by NMR approx. 80 %. Batch 3 as a brown gum (254 mg, 1.21 mmol, 27 %). LCMS (ammonium bicarbonate): Rt = 0.69 min, $[M+H]^+$ = 211, purity = 75 %. NMR consistent with batch 1, purity by NMR approx. 70 %. Batch 4 as a brown gum (540 mg, 2.57 mmol, 58 %). LCMS (ammonium bicarbonate): Rt = 0.67

min, $[M+H]^+$ not visible, purity = 100 %. NMR consistent with batch 1, purity by NMR < 50 %.

(4-Benzylpiperidin-1-yl)(piperidin-4-yl)methanone (7)

4-Benzylpiperidine (0.516 mL, 2.94 mmol) was reacted according to general procedure A. The intermediate was worked up by removing the solvent by evaporation and partitioning the residue between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was extracted with additional ethyl acetate (2 X 20mL) and the combined organic layers were dried by passing through a hydrophobic frit and concentrated. The residue was treated with hydrochloric acid (4 M in dioxane) (3.1 mL, 12.2 mmol). When the deprotection was complete, the solution was basified by addition of saturated aqueous sodium bicarbonate (100 mL) and was then extracted with DCM (2 X 100 mL). The combined organic layers were passed through a hydrophobic frit and concentrated. The crude residue was purified on a 150 g Redisep C18 GOLD® column, eluting with a gradient of 20-60 % acetonitrile in 10 mM ammonium bicarbonate to afford the title compound (7) as an orange oil (637 mg, 2.22 mmol, 91 %). LCMS (ammonium

bicarbonate): Rt = 1.00 min (broad peak), $[M+H]^+$ = 287. (NB. Additional ion in peak for M+ = 316). NMR was broad and could not be assigned, even with heating to 120 °C.

(4-Methylpiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (10)

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6, 40 mg, 0.19 mmol) followed by morpholine (0.08 mL, 0.95 mmol, 5 eq.) were reacted according to general procedure B to afford the title compound (10) as a yellow oil (20 mg, 0.05 mmol, 28 %). LCMS (formic): Rt = 0.67 min, $[M+H]^+$ = 374. HRMS $C_{20}H_{32}N_5O_2$ required 374.2551, found 374.2550. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.23 (s, 1 H); 5.59 (s, 1 H); 4.58 (d, *J* = 13.0 Hz, 1 H); 4.34 (d, *J* = 13.0 Hz, 2 H); 3.90 (d, *J* = 13.2 Hz, 1 H); 3.74 – 3.80 (m, 4 H); 3.50 – 3.56 (m, 4 H); 3.04 (t, *J* = 12.0 Hz, 1 H); 2.89 – 2.99 (m, 2 H); 2.71 - 2.83 (m, 1 H); 2.50 – 2.60 (m, 1 H); 1.56 - 1.93 (m, 7 H); 1.05 - 1.19 (m, 2 H); 0.98 (d, *J* = 6.4 Hz, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 172.0 (C=O); 163.1 (Ar C); 162.4 (Ar C); 156.9 (Ar CH); 81.4 (Ar CH); 65.8 (2 CH_2); 44.9 (CH_2); 44.1 (2 CH_2); 43.3 (2 CH_2); 41.3 (CH_2); 37.3 (CH); 34.6 (CH_2); 33.6 (CH_2); 30.5 (CH); 27.8 (CH_2); 27.7 (CH_2); 21.6 (CH_3).

(1-(6-(Dimethylamino)pyrimidin-4-yl)piperidin-4-yl)(4-methylpiperidin-1-yl)methanone (11)

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6, 68 mg, 0.32 mmol) followed by dimethylamine (2.0 M in THF) (0.54 mL, 1.07 mmol, 5 eq.) were reacted according to general procedure B to afford the title compound (11) as an orange oil (19 mg, 0.06 mmol, 26 %). LCMS (formic): Rt = 0.67 min, $[M+H]^+$ = 332. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.23 (s, 1 H); 5.48 (s, 1 H); 4.59 (d, J = 13.5 Hz, 1 H); 4.35 (d, J = 13.0 Hz, 2 H); 3.91 (d, J = 14.0, 1 H); 3.06 (s, 6 H); 3.00 - 3.05 (m, 1 H); 2.86 – 2.97 (m, 3 H); 2.70 - 2.79 (m, 1 H); 2.55 (t, J = 13.0 Hz, 1 H); 1.56 - 1.91 (m, 6 H); 1.04 - 1.18 (m, 2 H); 0.97 (d, J = 6.5 Hz, 3 H).

4-Methylpiperidin-1-yl)(1-(6-(pyrrolidin-1-yl)pyrimidin-4-yl)piperidin-4-yl)methanone (12)

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6, 40 mg, 0.19 mmol) followed by pyrrolidine (0.079 mL, 0.95 mmol, 5 eq.) were reacted according to general procedure B to afford the title compound (12) as a yellow oil (22 mg, 0.06 mmol, 33 %) . LCMS (formic): Rt = 0.75 min, $[M+H]^+$ = 358. HRMS $C_{20}H_{32}N_5O$ required 358.2601, found 358.2595. ^{1}H

NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (d, J = 0.5 Hz, 1 H); 5.33 (s, 1 H); 4.56 (d, J = 13.0 Hz, 1 H); 4.32 (d, J = 13.0 Hz, 2 H); 3.89 (d, J = 13.0 Hz, 1 H); 3.35 - 3.48 (m, 4 H); 3.02 (t, J = 12.0 Hz, 1 H); 2.89 (t, J = 12.0 Hz, 2 H); 2.68 - 2.78 (m, 1 H); 2.53 (t, J = 12.0 Hz, 1 H); 1.94 - 2.00 (m, 4 H); 1.53 - 1.88 (m, 7 H); 1.01 - 1.17 (m, 2 H); 0.95 (d, J = 6.5 Hz, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 172.0 (C=O); 161.8 (Ar C); 160.7 (Ar C); 156.9 (Ar CH); 81.0 (Ar CH); 45.9 (2 CH_2); 44.9 (CH_2); 43.2 (2 CH_2); 41.3 (CH_2); 37.3 (CH_2); 34.7 (CH_2); 33.5 (CH); 30.5 (CH_2); 27.8 (CH_2); 27.6 (CH); 24.7 (2 CH_2); 21.5 (CH_3).

(1-(6-(Cyclopentylamino)pyrimidin-4-yl)piperidin-4-yl)(4-methylpiperidin-1-yl)methanone (13)

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6, 50 mg, 0.24mmol) followed by cyclopentanamine (0.12 mL, 1.19 mmol, 5 eq.) were reacted according to general procedure B. After the second amine addition the reaction was heated at 150 °C for 9 hours. The title compound (13) was obtained as a yellow oil (29 mg, 0.08 mmol, 33 %). LCMS (formic): Rt = 0.86 min, $[M+H]^+$ = 372, purity = 93 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.12 (s, 1 H); 5.43 (s, 1 H); 4.76 (d, J = 6.5 Hz, 1 H); 4.59 (d, J = 13.0 Hz, 1 H);

4.34 (d, J = 13.0 Hz, 2 H); 3.85 – 3.96 (m, 2 H); 3.04 (t, J = 12.0 Hz, 1 H); 2.93 (t, J = 12.0 Hz, 2 H); 2.70 - 2.80 (m, 1 H); 2.55 (t, J = 12.0 Hz, 1 H); 1.96 - 2.07 (m, 2 H); 1.56 - 1.92 (m, 11 H); 1.44 - 1.55 (m, 2 H); 1.01 - 1.18 (m, 2 H) 0.97 (d, J = 6.5 Hz, 3 H).

General Procedure C: Nucleophilic Aromatic Substitutions

An amine (1 eq.) was dissolved in ethanol (0.5 – 1 mL) and then treated with a chloropyrimidine (0.9 eq.) and DIPEA (2 eq.). The resulting solutions were heated in a microwave reactor at 150 °C until the reaction was complete by LCMS. The products were purified by direct injection of the reaction solution onto preparative HPLC purification.

(4-Benzylpiperidin-1-yl)(1-(pyrimidin-4-yl)piperidin-4-yl)methanone (14)

(4-Benzylpiperidin-1-yl)(piperidin-4-yl)methanone (7, 100 mg, 0.35 mmol) and 4-chloropyrimidine (40 mg, 0.35 mmol) were reacted according to general procedure C with a heating time of 1 h to afford the title compound (14) as a yellow oil (45 mg, 0.12 mmol, 35 %). LCMS (ammonium bicarbonate): Rt = 1.04 min, $[M+H]^+$ = 365. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.59 (s, 1 H); 8.18 (d, J = 6.4 Hz, 1 H); 7.27 - 7.35 (m, 2 H); 7.19 - 7.25 (m,

1 H); 7.11 - 7.18 (m, 2 H); 6.52 (dd, *J* = 6.4, 1.0 Hz, 1 H); 4.62 (d, *J* = 13.2 Hz, 1 H); 4.41 (br. s., 2 H); 3.92 (d, *J* = 13.2 Hz, 1 H); 2.95 - 3.07 (m, 3 H); 2.75 - 2.86 (m, 1 H); 2.45 - 2.65 (m, 4 H); 1.63 - 1.91 (m, 6 H); 1.06 - 1.29 (m, 2 H).

(4-Benzylpiperidin-1-yl)(1-(6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)piperidin-4-yl)methanone (15)

(4-Benzylpiperidin-1-yl)(piperidin-4-yl)methanone (7, 100 mg, 0.35 mmol) followed by 1-methylpiperazine (0.19 mL, 1.75 mmol, 5 eq.) were reacted according to general procedure B to afford the title compound (15) as a yellow oil (70 mg, 0.15 mmol, 43 %). LCMS (formic): Rt = 0.75 min, $[M+H]^+$ = 463. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.05 (s, 1 H); 7.28 (t, *J* = 7.0 Hz, 2 H); 7.15 – 7.21 (m, 3 H); 5.88 (s, 1 H); 4.34 (d, *J* = 12.0 Hz, 3 H); 3.98 (d, *J* = 13.0 Hz, 1 H); 3.49 - 3.55 (m, 4 H) 2.79 – 3.01 (m, 4 H); 2.40 - 2.54 (m, 3 H); 2.33 (t, *J* = 4.9 Hz, 4 H); 2.19 (s, 3 H); 1.69 - 1.82 (m, 1 H); 1.53 – 1.66 (m, 4 H); 1.37 - 1.54 (m, 2 H); 0.88 - 1.18 (m, 2 H).

(4-Benzylpiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (16)

(4-Benzylpiperidin-1-yl)(piperidin-4-yl)methanone (7, 1.0 g, 3.5 mmol) followed by morpholine (1.5 mL, 17.5 mmol, 5 eq.) were reacted according to general procedure B. The solvents were removed by evaporation and then purified on a 80g Redisep Gold® silica column, eluting with a gradient of 10 - 95 % ethyl acetate in cyclohexane, followed by 95 – 100 % (10 % methanol in ethyl acetate) in cyclohexane. The appropriate fractions were combined and concentrated to yield the title compound (16) as an off-white solid (1.32 g, 2.94 mmol, 84 %). Mp 153-155 °C. LCMS (formic): Rt = 0.84 min, $[M+H]^+$ = 450. HRMS $C_{26}H_{36}N_5O_2$ required 450.2864, found 450.2855. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.07 (s, 1 H); 7.24 - 7.30 (m, 2 H); 7.14 - 7.20 (m, 3 H); 5.88 (s, 1 H); 4.34 (br. d, J = 13.0 Hz, 3 H); 3.98 (d, J = 13.0 Hz, 1 H); 3.61 - 3.66 (m, 4 H); 3.45 - 3.51 (m, 4 H); 2.81 - 3.00 (m, 4 H); 2.41 – 2.54 (m, 3 H); 1.69 - 1.82 (m, 1 H); 1.53 – 1.66 (m, 4 H); 1.38 - 1.51 (m, 2 H); 0.88 - 1.15 (m, 2 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 178.8 (C=O); 171.9 (Ar C); 163.0 (Ar C); 162.4 (Ar CH); 156.9 (Ar CH); 140.0 (Ar C); 128.9 (2 Ar CH); 128.0 (Ar CH); 125.7 (Ar CH); 81.4 (Ar CH); 65.8 (2 CH_2); 44.8 (CH_2); 44.1 (2 CH_2); 43.2 (CH_2); 42.0 (CH_2); 41.2 (CH_2); 37.5 (CH_2); 37.3 (CH); 32.6 (CH_2); 31.4 (CH_2); 30.3 (CH);

27.8 (CH_2); 27.7 (CH_2). IR (ATR) cm^{-1} 3024; 2920; 2852; 1628; 1578; 1446; 1200; 1121; 973; 800; 695.

General Procedure D: Acetylations

The appropriate amine (20 mg) was dissolved in acetic anhydride (250 µL) and the solution was stirred at room temperature for 17 h. The solvent was removed by evaporation and the crude products were purified.

1-(4-(4-Methylpiperidine-1-carbonyl)piperidin-1-yl)ethanone (17)

(4-Methylpiperidin-1-yl)(piperidin-4-yl)methanone (6, 20mg, 0.10 mmol) was reacted according to general procedure D. The product was purified by preparative HPLC and then repurified by on a Redisep® silica 4g column, eluting with a gradient of 0 - 95 % (0.1 % triethylamine in 10 % methanol/DCM) in 0.1 % triethylamine in DCM. The appropriate fractions were combined and concentrated to afford the title compound (17) as a colourless gum (9 mg, 0.03 mmol, 36 %). LCMS (formic): Rt = 0.70 min, $[M+H]^+$ = 253. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 4.58 (d, *J* = 13.0 Hz, 2 H); 3.88 (d, *J* = 13.0 Hz, 2 H);

2.99 - 3.16 (m, 2 H); 2.65 - 2.77 (m, 2 H); 2.55 (t, J = 13.0 Hz, 1 H); 2.10 (s, 3 H); 1.79 - 1.90 (m, 1 H); 1.59 - 1.78 (m, 6 H); 1.02 - 1.16 (m, 2 H); 0.97 (d, J = 6.5 Hz, 3 H).

1-(4-(4-Benzylpiperidine-1-carbonyl)piperidin-1-yl)ethanone (18)

(4-Benzylpiperidin-1-yl)(piperidin-4-yl)methanone (7, 20 mg, 0.07 mmol) was reacted according to general procedure D. The product was purified by preparative HPLC to afford the title compound (18) as a colourless oil (7 mg, 0.02 mmol, 24 %). LCMS (ammonium bicarbonate): Rt = 0.97 min, $[M+H]^+$ = 329. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.23 - 7.39 (m, 2 H); 7.13 - 7.20 (m, 3 H); 4.45 - 4.58 (m, 2 H); 4.06 (d, J = 13.0 Hz, 1 H); 3.89 – 3.99 (m, 1 H); 3.12 - 3.24 (m, 1 H); 3.01 - 3.10 (m, 1 H); 2.91 - 3.01 (m, 1 H); 2.66 - 2.78 (m, 1 H); 2.59 - 2.62 (m, 1 H); 2.56 (dd, J = 7.0, 3.0 Hz, 3 H); 2.09 (d, J = 1.5 Hz, 3 H); 1.80 - 1.90 (m, 1 H); 1.64 - 1.79 (m, 4 H); 1.45 - 1.64 (m, 1 H); 1.04 - 1.25 (m, 2 H).

4,6-Di(piperidin-1-yl)pyrimidine (19)

4,6-Dichloropyrimidine (100 mg, 0.67 mmol) was dissolved in Ethanol (0.5 mL) and added to piperidine (0.66 mL, 6.7 mmol). The resulting solution was heated in a microwave reactor at 150 ºC for 1 h. A few drops of DMSO was added to the solution to ensure

complete dissolution then the solution was directly injected onto preparative HPLC purification in 2 injections. The appropriate fraction from each run was combined and concentrated to afford the title compound (19) as a white solid (130 mg, 0.53 mmol, 79 %). Mp 103 - 106 °C. LCMS (ammonium bicarbonate): Rt = 1.13 min, $[M+H]^+$ = 247. HRMS $C_{14}H_{23}N_4$ required 247.1917, found 247.1921. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.01 (d, J = 0.5 Hz, 1 H); 5.82 (s, 1 H); 3.52 (t, J = 6.0 Hz, 8 H); 1.56 - 1.63 (m, 4 H); 1.44 - 1.51 (m, 8 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 163.5 (Ar CH); 156.9 (2 Ar C); 80.7 (Ar CH); 44.6 (4 CH_2); 25.0 (4 CH_2); 24.3 (2 CH_2). IR (ATR) cm^{-1} 2923; 2849; 1577; 1490; 1437; 1348; 1215; 1018; 970; 800.

***N,N*-Dimethyl-6-(piperidin-1-yl)pyrimidin-4-amine (20)**

Dimethylamine (2.0 M in THF) (0.34 mL, 0.67 mmol) followed by piperidine (0.33 mL, 3.4 mmol, 5 eq.) were reacted according to general procedure B. A few drops of DMSO was added to the solution to ensure complete dissolution then purification by mass-directed preparative HPLC in 2 injections was carried out. Only one injection collected product. The appropriate fraction from this run was concentrated to afford the title compound (20)

as a yellow oil (36 mg, 0.18 mmol, 26 %). LCMS (formic): Rt = 0.58 min, $[M+H]^+$ = 207. HRMS $C_{11}H_{19}N_4$ required 207.1604, found 207.1608. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.02 (d, J = 0.5 Hz, 1 H); 5.64 (s, 1 H); 3.52 (t, J = 5.0 Hz, 4 H); 2.98 (s, 6 H); 1.56 - 1.64 (m, 2 H); 1.44 - 1.52 (m, 4 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 162.9 (Ar C); 162.2 (Ar C); 156.7 (Ar CH); 80.1 (Ar CH)I 44.6 (2 CH_2); 36.8 (2 CH_3); 25.0 (2 CH_2); 24.3 (CH_2). IR (ATR) cm^{-1} 3375; 2929; 2852; 1581; 1489; 1428; 1335; 1247; 975; 798.

4-(6-(Piperidin-1-yl)pyrimidin-4-yl)morpholine (21)

Morpholine (0.059 mL, 0.67 mmol) followed by piperidine (0.332 mL, 3.36 mmol, 5 eq.) were reacted according to general procedure B. Methanol (2 mL) was added to the solution to ensure complete dissolution then purification by preparative HPLC in 3 injections was carried out. The appropriate fractions from each run were combined and concentrated to afford the title compound (21) as a white solid (96 mg, 0.39 mmol, 58 %). Mp 100 - 102 °C. LCMS (formic): Rt = 0.52 min, $[M+H]^+$ = 249. HRMS $C_{13}H_{21}N_4O$ required 249.1710, found 249.1712. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.25 (s, 1 H); 5.58 (s, 1 H); 3.77 – 3.81 (m, 4 H); 3.51 - 3.59 (m, 8 H); 1.59 - 1.72 (m, 6 H). ^{13}C NMR (101 MHz,

DMSO-d_6) δ ppm 163.1 (Ar C); 162.4 (Ar C); 156.7 (Ar CH); 81.1 (Ar CH); 65.8 (2 CH_2); 44.6 (2 CH_2); 44.1 (2 CH_2); 25.0 (2 CH_2); 24.3 (CH_2). IR (ATR) cm^{-1} 2938; 2856; 2826; 1574; 1466; 1440; 1211; 1119; 991; 971; 804; 796.

4-(2-Chloro-6-((4-methoxybenzyl)oxy)pyrimidin-4-yl)morpholine

To a solution of 2,4,6-trichloropyrimidine (2.51 mL, 21.8 mmol) and caesium carbonate (21.3 g, 65.4 mmol) in acetonitrile (50 mL) was added (4-methoxyphenyl)methanol (2.71 mL, 21.8 mmol). This mixture was stirred at room temperature for 72 hours, after which morpholine (2.28 mL, 26.2 mmol) was added. The resulting mixture was stirred for 16 hours, after which the solvent was removed under reduced pressure. The resulting solid was suspended in ethyl acetate (200 mL). Water (200 mL) was added and the mixture was separated. The organic layer was washed with water (2 x 200 mL), brine (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was adsorbed onto diatomaceous earth and purified by flash chromatography, eluting under a gradient of TBME (0-25%) in cyclohexane to afford the three regioisomers, which eluted in the following order: 4-(4-chloro-6-((4-methoxybenzyl)oxy)pyrimidin-2-yl)morpholine

(2.95 g, 8.79 mmol, 40 %, white solid), 4-(2-chloro-6-((4-methoxybenzyl)oxy)pyrimidin-4-yl)morpholine (412 mg, 1.23 mmol, 6 %, opaque gum), 4-(6-chloro-2-((4-methoxybenzyl)oxy)pyrimidin-4-yl)morpholine (1.52 g, 4.53 mmol, 21 %, transparent gum). LCMS (ammonium bicarbonate): Rt = 1.29 min, $[M+H]^+$ = 336. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.36-7.38 (m, 2H); 6.93-6.96 (m, 2H); 6.13 (s, 1H); 5.23 (s, 2H); 3.76 (s, 3H); 3.63-3.64 (m, 4H); 3.53-3.55 (m, 4H).

4-(2-((4-Methoxybenzyl)oxy)-6-(piperidin-1-yl)pyrimidin-4-yl)morpholine

To a solution of 4-(6-chloro-2-((4-methoxybenzyl)oxy)pyrimidin-4-yl)morpholine (75 mg, 0.22 mmol) and DIPEA (0.117 mL, 0.67 mmol) in ethanol (0.9 mL) was added piperidine (66 µL, 0.67 mmol). The mixture was heated to 150 ^{o}C in the microwave for 5 hours, after which time direct purification of the reaction mixture by preparative HPLC afforded the title compound (61 mg, 0.16 mmol, 71 %) as a red glass. LCMS (ammonium bicarbonate): Rt = 1.35 min, $[M+H]^+$ = 385. ^{1}H NMR (400 MHz, CD_3OD): δ ppm 7.34-7.38 (m, 2H); 6.88-6.91 (m, 2H); 5.48 (s, 1H); 5.42 (s, 2H); 3.79 (s, 3H); 3.71-3.73 (m, 4H); 3.55-3.58 (m, 4H); 3.49-3.51 (m, 4H); 1.65-1.72 (m, 2H); 1.55-1.61 (m, 4H).

4-Morpholino-6-(piperidin-1-yl)pyrimidin-2(1*H*)-one (22)

4-(2-((4-Methoxybenzyl)oxy)-6-(piperidin-1-yl)pyrimidin-4-yl)morpholine (60 mg, 0.16 mmol) was dissolved in a mixture of dichloromethane and trifluoroacetic acid (3 mL, 1:1). This mixture was stirred for 1 hour, after which time it was concentrated to dryness under reduced pressure. The residue was dissolved in DMF (1 mL) and subjected to preparative HPLC purification to give the title compound (22) (37 mg, 0.14 mmol, 89 %) as a white solid. M.p. 259-270 ^{o}C (decomp.). LCMS (ammonium bicarbonate): Rt = 0.68 min, $[M+H]^+$ = 265. HR-MS (ESI): $C_{13}H_{21}N_4O_2$ $[M+H^+]$ required 265.1659, found 265.1665. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.39 (s, 1H); 5.18 (s, 1H); 3.60-3.63 (m, 4H); 3.41-3.45 (m, 8H); 1.56-1.62 (m, 2H); 1.47-1.51 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 162.3; 160.6; 157.9; 70.8; 65.8 (2C); 45.7 (2C); 44.9 (2C); 25.1 (2C); 24.0. IR (neat) cm^{-1} 2927; 2840; 1596; 1534; 1498; 1427; 1382; 1360; 1265; 1228; 1207; 1115; 1028; 1000; 896; 861; 828; 778; 747.

4-(6-Chloropyrimidin-4-yl)morpholine

To a stirred solution of 4,6-dichloropyrimidine (10 g, 67.1 mmol) in ethanol (100 mL) cooled to 5 ºC was added triethylamine (9.36 mL, 67.1 mmol) followed by morpholine (7.02 g, 81 mmol) and the reaction was stirred for 16 hrs at room temperature. During the reaction solid was formed, which was filtered off and dried over vacuo to obtain 4-(6-chloropyrimidin-4-yl)morpholine (11.74 g, 58.7 mmol, 87 %) as a white solid. LCMS (formic 4.5 min. run): Rt = 1.50 min, $[M+H]^+$ = 200. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.40 (d, *J* = 0.7 Hz, 1 H) 6.49 (d, *J* = 0.9 Hz, 1 H) 3.73 - 3.85 (m, 4 H) 3.53 - 3.69 (m, 4 H).

N-isopropyl-N-methyl-6-morpholinopyrimidin-4-amine (23)

A solution of 4-(6-chloropyrimidin-4-yl)morpholine (100 mg, 0.50 mmol), *N*-methylpropan-2-amine (44. mg, 0.60 mmol) and DIPEA (0.262 mL, 1.50 mmol) in DMSO were irradiated with microwaves at 150 °C for 1 hr. The reaction mixture was diluted with water, extracted with ethyl acetate (2 x 10 mL), washed with brine and dried over anhydrous Na_2SO4. The organic layer was evaporated under reduced pressure and the crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluted with 80% ethyl acetate in hexane. The product was repurified by preparative HPLC using the following

conditions: Column: XTERRA RP18 (150x19) mm, 5µm. Mobile Phase: A: 10 mM ammonium bicarbonate (Aq) B: Acetonitrile Flow: 15 ml/min. Method: 0/55, 12/55, 13/100, 18/100. The title compound (23) was obtained as an off-white solid (81 mg, 0.34 mmol, 68 %). LCMS (formic): Rt = 0.46 min, $[M+H]^+$ = 237. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.05 (s, 1 H) 5.66 (s, 1 H) 4.73 - 5.06 (m, 1 H) 3.58 - 3.75 (m, 4 H) 3.38 - 3.49 (m, 4 H) 2.76 (s, 3 H) 1.08 (d, J=6.8 Hz, 6 H).

4-(6-(3,3-Difluoropyrrolidin-1-yl)pyrimidin-4-yl)morpholine (24)

Morpholine (0.029 mL, 0.34 mmol), followed by 3,3-difluoropyrrolidine (96 mg, 0.67 mmol, 2 eq.) were reacted according to general procedure B to afford the title compound (24) as an off-white solid (51 mg, 0.19 mmol, 56 %). Mp 140 - 142 °C. LCMS (ammonium bicarbonate): Rt = 0.77 min, $[M+H]^+$ = 271. HRMS $C_{12}H_{17}F_2N_4O$ required 271.1365, found 271.1370. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.10 (d, J = 0.5 Hz, 1 H); 5.67 (s, 1 H); 3.81 (t, J = 13.5 Hz, 2 H); 3.58 - 3.67 (m, 6 H); 3.48 – 3.53 (m, 4 H); additional 2H masked by DMSO peak. ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 162.4 (Ar C); 160.7 (Ar C); 156.9 (Ar CH); 128.2 (t, J = 247 Hz, CF_2); 81.5 (Ar CH); 65.8 (2 CH_2); 52.8 (t, J = 32 Hz, CH_2);

44.1 (2 CH_2); 43.6 (t, J = 4.0 Hz, CH_2); 32.8 (t, J = 24 Hz, CH_2). IR (ATR) cm^{-1} 2960; 2874; 2838; 1582; 1481; 1310; 1237; 1114; 974; 927; 800.

1-(4-(6-Morpholinopyrimidin-4-yl)piperazin-1-yl)ethanone (25)

Morpholine (0.029 mL, 0.34 mmol), followed by 1-(piperazin-1-yl)ethanone (86 mg, 0.67 mmol, 2 eq.) were reacted according to general procedure B to afford the title compound (25) as a white solid (59 mg, 0.20 mmol, 60 %). Mp = 165-166 °C. LCMS (ammonium bicarbonate): Rt = 0.58 min, $[M+H]^+$ = 292. HRMS $C_{14}H_{22}N_5O_2$ required 292.1768 found 292.1780. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.10 (s, 1 H); 5.92 (s, 1 H); 3.59 - 3.67 (m, 6 H); 3.46 - 3.55 (m, 10 H); 2.03 (s, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 178.7 (C=O); 163.0 (Ar C); 162.5 (Ar C); 156.9 (Ar CH); 81.6 (Ar CH); 65.8 (2 CH_2); 45.1 (CH_2); 44.1 (2 CH_2); 43.6 (CH_2); 43.4(CH_2); 40.4 (CH_2); 21.0 (CH_3). IR (ATR) cm^{-1} 2967; 2859; 1645; 1591; 1491; 1446; 1420; 1246; 1227; 1206; 1117; 992; 976; 806.

4-(6-(3,3-Dimethylpiperidin-1-yl)pyrimidin-4-yl)morpholine (26)

To a solution of 4-(6-chloropyrimidin-4-yl)morpholine (200 mg, 1.00 mmol) and 3,3-dimethylpiperidine (170 mg, 1.50 mmol) in acetonitrile (3 mL) stirred at room temp was

added DIPEA (1.05 mL, 6.01 mmol). The reaction mixture was stirred at 130 °C for 48 hr. The reaction mixture was concentrated and dissolved in ethyl acetate (40 mL) and the organic layer was washed with water (2 X 20 mL), and brine solution (10 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified on silica gel (100-200 mesh) column chromatography using 40% ethyl acetate in petroleum ether as an eluent and lyophilised to the title compound (26) as a solid (40 mg, 0.14 mmol, 14 %). LCMS (formic): Rt = 0.62 min, $[M+H]^+$ = 277. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.22 (d, J = 0.7 Hz, 1 H) 5.56 (s, 1 H) 3.71 - 3.87 (m, 4 H) 3.45 - 3.59 (m, 6 H) 3.27 (s, 2 H) 1.64 (ddd, J = 6.0, 3.6, 2.2 Hz, 2 H) 1.37 - 1.49 (m, 2 H) 0.94 (s, 6 H).

8-(6-Morpholinopyrimidin-4-yl)-1-oxa-8-azaspiro[4.5]decane (27)

Morpholine (0.029 mL, 0.34 mmol), followed by 1-oxa-8-azaspiro[4.5]decane hydrochloride (obtained from the GSK compound collection, 119 mg, 0.67 mmol, 2 eq.) were reacted according to general procedure B to afford the title compound (27) as a white gum (48 mg, 0.16 mmol, 46 %). LCMS (ammonium bicarbonate): Rt = 0.82 min, $[M+H]^+$ = 305. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 8.06 (s, 1 H); 5.90 (s, 1 H); 3.71 -

3.79 (m, 4 H); 3.61 - 3.66 (m, 4 H); 3.39 - 3.51 (m, 6 H); 1.83 - 1.93 (m, 2 H); 1.64 - 1.71 (m, 2 H); 1.44 - 1.57 (m, 4 H).

4-(6-(Azepan-1-yl)pyrimidin-4-yl)morpholine (28)

A solution of 4-(6-chloropyrimidin-4-yl)morpholine (100 mg, 0.50 mmol), azepane (60 mg, 0.60 mmol) and DIPEA (0.262 mL, 1.50 mmol) in DMSO (5 mL) was irradiated with microwaves at 150 °C for 1 hr. The reaction mixture was diluted with water, extracted with ethyl acetate (2 x 10mL), washed with brine and dried over anhydrous Na_2SO_4. The organic layer was evaporated under reduced pressure. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluted with 80% ethyl acetate in hexane, as an eluent to afford the title compound (28) as an orange solid (72 mg, 0.27 mmol, 55 %). LCMS (formic): Rt = 0.54 min, $[M+H]^+$ = 263. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (d, J = 0.7 Hz, 1 H) 5.66 (s, 1 H) 3.61 - 3.72 (m, 4 H) 3.53 - 3.60 (m, 4 H) 3.39 - 3.49 (m, 4 H) 1.68 (br s, 4 H) 1.37 - 1.52 (m, 4 H).

Ethyl 1-(6-chloropyrimidin-4-yl)piperidine-4-carboxylate

To a solution of 4,6-dichloropyrimidine (15.00 g, 101 mmol) in ethanol (150 mL), ethyl piperidine-4-carboxylate (15.83 g, 101 mmol) was added at room temperature, then triethylamine (21.05 mL,

151 mmol) was added at 0 °C and the reaction mixture was stirred at RT for 4 h. The reaction mixture was directly evaporated under reduced pressure and the residue was diluted with water, extracted with ethyl acetate (2 x 250 mL), washed with brine and dried over anhydrous Na_2SO_4. The organic layer was evaporated under reduced pressure. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 12% - 15% ethyl acetate in hexane. The appropriate fractions were concentrated to yield the title compound as a colourless liquid (19.6 g, 72 mmol, 72 %). LCMS (formic 4.5 min. run): Rt = 2.07 min., $[M+H]^+$ = 256.

Ethyl 1-(6-morpholinopyrimidin-4-yl)piperidine-4-carboxylate

A solution of ethyl 1-(6-chloropyrimidin-4-yl)piperidine-4-carboxylate (19.00 g, 70.4 mmol) and morpholine (18.41 mL, 211 mmol) in N,N-Dimethylformamide (200 mL) was stirred at 100 °C for 18 hr. The reaction mixture was diluted with ice-cold water, extracted with ethyl acetate (2 x 500 mL), washed with brine and dried over anhydrous Na_2SO_4. The organic layer was evaporated under reduced pressure. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 60% ethyl acetate in hexane. The product was repurified by combi-flash column chromatography, eluting with 50% ethyl acetate in hexane. The appropriate fractions were concentrated to yield the

title compound as a white solid (12.3 g, 38.4 mmol, 55 %). LCMS (formic 4.5 min. run): Rt = 1.58 min., $[M+H]^+$ = 321.

1-(6-Morpholinopyrimidin-4-yl)piperidine-4-carboxylic acid

To a solution of ethyl 1-(6-morpholinopyrimidin-4-yl)piperidine-4-carboxylate (12.00 g, 37.5 mmol) in tetrahydrofuran (60 mL), lithium hydroxide (1.79 g, 75 mmol) in Water (60 mL) solution was added at RT and the reaction mixture was stirred at RT for 3 hr. The reaction mixture was acidified with 1N aqueous HCl solution to pH = 6, extracted with ethyl acetate (2 x 150 mL), washed with brine and dried over anhydrous Na_2SO_4. The organic layer was evaporated under reduced pressure. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 4% methanol in DCM. The appropriate fractions were concentrated to yield the title compound as a white solid (8.9 g, 30.4 mmol, 81 %).

General Procedure E: Amide formations

1-(6-Morpholinopyrimidin-4-yl)piperidine-4-carboxylic acid (1 eq.) was dissolved in DMF (1 – 2 mL) and treated with COMU (3 eq.), DIPEA (2 – 4 eq.) and then the appropriate

amine (1.2 – 1.5 eq.). The solutions were stirred at room temperature until reactions were complete by LCMS analysis. The solutions were partitioned between DCM and water and the layers were separated using hydrophobic frits and the aqueous layers were re-extracted with additional DCM. The combined organic layers were concentrated to yield crude products, which were purified by preparative HPLC.

General Procedure F: Amide formations

1-(6-Morpholinopyrimidin-4-yl)piperidine-4-carboxylic acid (1 eq.) was dissolved in DMF and treated with HATU (1.5 - 3 eq.), DIPEA (3 - 5 eq.) and then the appropriate amine (1 - 2 eq.). The solutions were stirred at room temperature until complete. The solutions were partitioned between ethyl acetate and water and the layers were separated, dried over Na_2SO_4 and concentrated to yield crude products.

(2-Benzylpiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (29)

2-Benzylpiperidine (36 mg, 0.21 mmol, 1.2 eq.) was reacted according to general procedure E to afford the title compound (29) as a yellow gum (20 mg, 0.04 mmol, 26 %). LCMS (formic): Rt = 1.04 min, $[M+H]^+$ = 450. HRMS $C_{26}H_{36}N_5O_2$ required 450.2864 found

450.2860. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.99 – 8.11 (m, 1 H); 7.12 - 7.33 (m, 5 H); 5.79 – 5.92 (m, 1 H); 4.09 - 4.43 (m, 3 H); 3.59 – 3.67 (m, 4 H); 3.44 – 3.51 (m, 4 H); 3.09 - 3.26 (m, 1 H); 2.66 - 2.92 (m, 4 H); 2.39 – 2.48 (m, 1 H); 2.24 – 2.34 (m, 1 H); 1.63 - 1.83 (m, 2 H); 1.43 - 1.63 (m, 4 H); 1.30 - 1.43 (m, 2 H); 1.13 - 1.29 (m, 2 H).

(3-Benzylpiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (30)

3-Benzyl piperidine (36 mg, 0.21 mmol, 1.2 eq.) was reacted according to general procedure E to afford the title compound (30) as an orange solid (18 mg, 0.04 mmol, 24 %). Mp 65 – 68 °C. LCMS (ammonium bicarbonate): Rt = 1.18 min, $[M+H]^+$ = 450. HRMS $C_{26}H_{36}N_5O_2$ required 450.2864 found 450.2858. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.07 (s, 1 H); 7.25 - 7.35 (m, 2 H); 7.14 - 7.24 (m, 3 H); 5.88 (d, J = 3.5 Hz, 1 H); 4.25 - 4.38 (m, 2 H); 4.13 - 4.24 (m, 1 H); 3.69 – 3.92 (m, 1 H); 3.61 – 3.66 (m, 4 H); 3.46 – 3.51 (m, 4 H); 2.97 - 3.10 (m, 1 H); 2.82 - 2.95 (m, 2 H); 2.64 - 2.82 (m, 1 H); 2.52 - 2.63 (m, 1 H); 2.45 - 2.48 (m, 1 H); 2.34 - 2.44 (m, 1 H); 1.66 – 1.76 (m, 2 H); 1.53 - 1.65 (m, 2 H); 1.38 - 1.52 (m, 3 H); 1.28 – 1.38 (m, 1 H); 1.14 - 1.26 (m, 1 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 171.7 (C=O); 163.0 (Ar C); 162.4 (Ar C); 156.9 (Ar CH); 139.8 (Ar C);

128.9 (2 Ar CH); 128.2 (2 Ar CH); 125.9 (Ar CH); 81.4 (Ar CH); 65.8 (CH_2); 50.2 (CH_2); 46.6 (CH_2); 45.4 (CH_2); 44.1 (CH_2); 43.3 (CH_2); 41.8 (CH_2); 37.6 (CH_2); 37.4 (CH); 37.3 (CH); 30.4 (CH_2); 27.7 (CH_2); 27.5 (CH_2); 25.6 (CH_2); 24.5 (CH_2).

(4-Benzyl-4-hydroxypiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (31)

4-Benzylpiperidin-4-ol (196 mg, 1.03 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column chromatography eluting with 8% methanol in DCM. The product-containing fractions were evaporated under reduced pressure, and the product was repurified by preparative HPLC under the following conditions. Column: X Bridge C18 (19mm × 100mm) 5µm. Mobile Phase: A: 10 mM ammonium bicarbonate in Water B: acetonitrile. Isocratic: 60:40 (A:B). Flow rate: 17 mL/min. The appropriate fractions were concentrated to yield the title compound (31) as a white solid (87 mg, 0.19 mmol, 18%). LCMS (formic): Rt = 0.61 min., $[M+H]^+$ = 466. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.1 (s, 1H); 7.2-7.3 (m, 5H); 5.9 (s,

1H); 4.4 (s, 1H); 4.3 (d, *J* = 16 Hz, 2H); 4.1 (d, *J* = 8 Hz, 1H); 3.8 (d, *J* = 8 Hz); 3.6-3.7 (m, 4H); 3.6-3.6 (m, 4H); 2.8 – 2.9 (m, 4H); 2.7 (s, 2H); 1.5 – 1.7 (m, 2H); 1.2 – 1.5 (m, 6H).

(3,4-Dihydroisoquinolin-2(1H)-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (32)

1,2,3,4-Tetrahydroisoquinoline (0.067 mL, 0.54 mmol, 1.5 eq.) was reacted according to general procedure E to afford the title compound (32) as a yellow gum (113 mg, 0.28 mmol, 77 %). LCMS (formic): Rt = 0.63 min, $[M+H]^+$ = 408.14. HRMS $C_{23}H_{30}N_5O_2$ required 408.2394 found 408.2390. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (s, 1 H); 7.13 - 7.26 (m, 4 H); 5.90 (s, 1 H); 4.76 (s, 1 H); 4.60 (s, 1 H); 4.32 – 4.42 (m, 2 H); 3.78 (t, *J* = 5.5 Hz, 1 H); 3.60 - 3.70 (m, 5 H); 3.45 - 3.54 (m, 4 H); 3.04 (br. s., 1 H); 2.84 - 2.98 (m, 3 H); 2.72 – 2.78 (m, 1 H); 1.60 – 1.72 (m, 2 H); 1.42 - 1.58 (m, 2 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 178.7 (C=O); 163.0 (Ar C); 162.4 (Ar C); 156.8 (Ar CH); 133.6 (Ar C); 128.5 (Ar CH); 128.3 (Ar CH); 126.4 (Ar CH); 126.2 (Ar CH); 126.1 (Ar C); 81.4 (Ar CH); 65.8 (2 CH_2); 44.1 (2 CH_2); 43.7 (CH_2); 43.3 (CH_2); 42.4 (CH_2); 37.6 (CH); 29.1 (CH_2);

27.8 (CH_2); 27.7 (CH_2); 27.5 (CH_2). IR (ATR) cm^{-1} 2922; 2851; 1632; 1578; 1440; 1197; 1114; 986; 974; 799; 749.

(2-Benzylmorpholino)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (33)

2-Benzylmorpholine (36 mg, 0.21 mmol,1.2 eq.) was reacted according to general procedure E to afford the title compound (33) as an orange solid (42 mg, 0.09 mmol, 55 %). Mp 82 – 84 °C. LCMS (ammonium bicarbonate): Rt = 1.03 min, $[M+H]^+$ = 452. HRMS $C_{25}H_{34}N_5O_3$ required 452.2656 found 452.2668. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.07 (s, 1 H); 7.16 - 7.37 (m, 5 H); 5.89 (s, 1 H); 4.34 (d, J = 13.0 Hz, 2 H); 4.11 - 4.23 (m, 1 H); 3.78 - 3.94 (m, 2 H); 3.60 - 3.66 (m, 4 H); 3.38 - 3.55 (m, 5 H); 3.10 – 3.22 (m, 1 H); 2.62 - 3.00 (m, 6 H); 2.39 - 2.48 (m, 1 H); 1.56 – 1.66 (m, 2 H); 1.36 - 1.55 (m, 2 H). IR (ATR) cm^{-1} 2853; 1635; 1579; 1487; 1441; 1199; 1113; 987; 973; 799; 752; 670.

(4-(4-Fluorophenyl)piperazin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (34)

1-(4-Fluorophenyl)piperazine (74 mg, 0.41 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column

chromatography, eluting with 5% methanol in DCM, and was then repurified by preparative TLC to yield the title compound (34) as an off-white solid (85 mg, 0.19 mmol, 54 %). LCMS (formic): Rt = 0.66 min., $[M+H]^+$ = 455. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.1 (s, 1H); 7.0 – 7.1 (m, 2H); 6.9 – 7.0 (m, 2H); 5.9 (s, 1H); 4.3 – 4.4 (d, J = 16 Hz, 2H); 3.6 – 3.7 (m, 8H); 3.5 – 3.6 (m, 4H); 3.0 – 3.2 (m, 5H); 2.8 – 3.0 (m, 2H); 1.6 – 1.7 (m, 2H); 1.4 – 1.6 (m, 2H); 1.2 – 1.3 (m, 1H).

(4-(4-Fluorobenzoyl)piperazin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (35)

(4-Fluorophenyl)(piperazin-1-yl)methanone (71 mg, 0.34 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 8% methanol in DCM, and then re-purified by preparative HPLC using the following conditions. Column: X Bridge C18 (19mm × 100mm) 5µm. Mobile Phase: A: 10 mM ammonium bicarbonate in Water B: acetonitrile Isocratic: 60:40 (A:B) Flow rate : 17 mL/min. The appropriate fractions were concentrated to yield the title compound (35) as a white solid (80 mg, 0.17 mmol, 48 %). LCMS (formic):

Rt = 0.58 min., $[M+H]^+$ = 483. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.1 (s, 1H); 7.5 – 7.6 (m, 2H); 7.2 – 7.3 (m, 2H); 5.9 (s, 1H); 4.4 (d, J = 16 Hz, 2 H); 3.4 – 3.7 (m, 16 H); 2.8 – 3.0 (m, 3H); 1.6 – 1.7 (m, 2H); 1.4 – 1.6 (m, 2H).

(3-Benzylpyrrolidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (36)

3-Benzylpyrrolidine (33 mg, 0.21 mmol, 1.2 eq.) was reacted according to general procedure E to afford the title compound (36) as a yellow gum (34 mg, 0.08 mmol, 45 %). LCMS (formic): Rt = 0.98 min, $[M+H]^+$ = 436. HRMS $C_{25}H_{34}N_5O_2$ required 436.2707 found 436.2704. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.07 (s, 1 H); 7.26 - 7.33 (m, 2 H); 7.17-7.25 (m, 3 H); 5.89 (s, 1 H); 4.31 – 4.41 (m, 2 H); 3.61 - 3.69 (m, 5 H); 3.46 - 3.51 (m, 4 H); 3.35 – 3.46 (m, 1 H); 3.14 - 3.22 (m, 1 H); 2.80 - 2.95 (m, 3 H); 2.61 - 2.76 (m, 3 H); 1.78 – 1.99 (m, 1 H); 1.59 - 1.70 (m, 3 H); 1.38 - 1.56 (m, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 172.7 (C=O); 163.6 (Ar C); 162.9 (Ar C); 157.4 (Ar CH); 140.9 (Ar C); 129.1 (Ar CH); 128.8 (Ar CH); 128.8 (Ar CH); 126.5 (Ar CH); 126.4 (Ar CH); 81.9 (Ar CH); 66.4 (CH_2); 51.4 (CH_2); 51.0 (CH_2); 45.8 (CH_2); 45.4 (CH_2); 44.6 (CH_2); 43.8 (CH_2); 43.8

(CH_2); 39.0 (CH); 38.7 (CH); 31.6 (CH_2); 29.8 (CH_2); 27.7 (CH_2); 27.7 (CH_2). IR (ATR) cm^{-1} 3459; 2949; 2852; 1628; 1577; 1494; 1437; 1337; 1201; 1115; 972; 799; 751; 700.

(1-(6-Morpholinopyrimidin-4-yl)piperidin-4-yl)(4-(phenylamino)piperidin-1-yl)methanone (37)

N-phenylpiperidin-4-amine (72 mg, 0.41 mmol) was reacted according to general procedure F. The crude product was purified on a silica gel (4 g) column eluting with a gradient of 8-10 % methanol in dichloromethane. The appropriate fractions were concentrated and the solid was then washed with diethyl ether (20 mL) and lypholized to yield the title compound (37) as an off-white solid (64 mg, 0.14 mmol, 41 %). LCMS (formic): Rt = 0.55 min., $[M+H]^+$ = 451. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 8.1 (s, 1H); 7.0 – 7.1 (m, 2H); 6.6 – 6.7 (m, 2H); 6.5 – 6.6 (m, 2H); 5.9 (s, 1H); 5.4 – 5.5 (m, 1H); 4.3 (d, J = 8 Hz, 2H); 4.2 (d, J = 8 Hz, 1H); 4.0 (d, J = 8 Hz, 1H); 3.6 – 3.7 (m, 4H); 3.4 – 3.5 (m, 5H); 3.2 – 3.3 (m, 1H); 2.7 – 3.0 (m, 4H); 1.9 – 2.0 (m, 2H); 1.6 – 1.7 (m, 2H); 1.4 – 1.6 (m, 2H); 1.1 – 1.4 (m, 2H).

(1-(6-Morpholinopyrimidin-4-yl)piperidin-4-yl)(4-phenoxypiperidin-1-yl)methanone (38)

4-Phenoxypiperidine (72 mg, 0.406 mmol) was reacted according to general procedure F. The reaction mixture was diluted with water (5 mL) and extracted with dichloromethane (3 X 10 mL) and then dried under high vacuo. The crude product was triturated with diethyl ether (2 X 30 mL), and then dried under high vacuo to yield the title compound (38) as an off-white solid (102 mg, 0.22 mmol, 65 %). LCMS (formic): Rt = 0.71 min., $[M+H]^+$ = 452. NMR (400 MHz, DMSO-d_6) δ ppm 8.1 (s, 1H); 7.2 – 7.3 (m, 2H); 6.9 – 7.0 (m, 3H); 5.9 (s, 1H); 4.6 – 4.7 (m, 1H); 4.4 (d. J = 8 Hz, 2H); 3.9 (br.s, 2H); 3.8 – 3.9 (m, 4H); 3.5 – 3.6 (m, 4H); 3.4 – 3.5 (m, 1H); 3.2 – 3.3 (m, 1H); 2.8 – 3.0 (m, 3 H); 1.8 – 2.0 (m, 2H); 1.6 – 1.7 (m, 3H); 1.4 – 1.6 (m, 3H).

(4-(4-Fluorobenzyl)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (39)

4-(4-Fluorobenzyl)piperidine hydrochloride (0.40 g, 1.73 mmol) was reacted according to general procedure F. The crude product was purified by HPLC, using a x-brigde column, in basic media, method: 40-100% 10 mM NH_4HCO_3 in acetonitrile. Appropriate fractions were collected and concentrated to afford the title compound (39) as a white solid (616

mg, 1.25 mmol, 73 %). LCMS (formic): Rt = 0.81 min., $[M+H]^+$ = 468. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.14 (d, *J* = 0.8 Hz, 1 H); 7.27 (br d, *J* = 5.6 Hz, 2 H); 7.10 - 7.21 (m, 2 H); 5.97 (s, 1 H); 4.34 - 4.49 (m, 3 H); 4.00 - 4.12 (m, 1 H); 3.64 - 3.77 (m, 4 H); 3.56 (br d, *J* = 5.1 Hz, 4 H); 2.88 - 3.09 (m, 4 H); 2.47 - 2.55 (m, 1 H); 1.80 (br d, *J* = 3.8 Hz, 1 H); 1.65 (br s, 6 H); 0.94 - 1.18 (m, 2 H).

(1-(6-Morpholinopyrimidin-4-yl)piperidin-4-yl)(4-(pyridin-3-ylmethyl)piperidin-1-yl)methanone (40)

1-(6-Morpholinopyrimidin-4-yl)piperidine-4-carboxylic acid (352 mg, 1.20 mmol) was dissolved at 0 ºC in DMF (10 mL). (Z)-2-(5-(ethylideneamino)-1H-1,2,3-triazol-1-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate (V) (669 mg, 1.81 mmol) was added at 0 ºC followed by DIPEA (1.08 mL, 6.0 mmol). After 20 min 3-(piperidin-4-ylmethyl)pyridine, 2hydrochloride (300 mg, 1.20 mmol) was added to the mixture and reaction stirred for 8 h. DMF was removed under vacuum and the residue was dissolved in ethyl acetate (40 mL) and treated with 1 N NH_4Cl (20 mL), and the layers were separated. The organic solution was washed with brine and dried over Na_2SO_4. The solvent was removed and the residue was purified by chromatography using a SILYCEL

cartridge and as mobile phase a gradient cyclohexane/ethyl acetate:ethanol 3:1 from 100/0 to 10/90. Appropriate fractions were combined and solvent removed under vacuum to afford the title compound (40) as a white solid (240 mg, 0.53 mmol, 44 %). LCMS (formic): Rt = 0.39 min., $[M+H]^+$ = 451. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.52 (dd, *J* = 4.8, 1.8 Hz, 1 H); 8.45 - 8.48 (m, 1 H); 8.28 (d, *J* = 0.8 Hz, 1 H); 7.50 (dt, *J* = 7 .7, 2.1 Hz, 1 H); 7.24 - 7.29 (m, 1 H); 5.63 (s, 1 H); 4.61 - 4.73 (m, 1 H); 4.39 (br s, 2 H); 3.97 (br d, *J* = 12.9 Hz, 1 H); 3.78 - 3.85 (m, 4 H); 3.52 - 3.62 (m, 4 H); 2.90 - 3.10 (m, 3 H); 2.72 - 2.83 (m, 1 H); 2.61 (s, 3 H); 1.70 - 1.95 (m, 7 H); 1.47 (s, 2 H); 1.12 - 1.33 (m, 3 H).

(1-(6-Morpholinopyrimidin-4-yl)piperidin-4-yl)(4-(pyridin-2-ylmethyl)piperidin-1-yl)methanone (41)

2-(Piperidin-4-ylmethyl)pyridine dihydrochloride (102 mg, 0.41 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 8% methanol in DCM. The product was repurified by preparative HPLC using the following conditions Column: X Bridge C18 (19mm × 100mm) 5µm, Mobile Phase: A: 10mM ammonium bicarbonate in water B: acetonitrile, Isocratic: 60:40 (A:B) Flow rate: 17 mL/min. The appropriate fractions were

concentrated to afford the title compound (41) as a white solid (45 mg, 0.10 mmol, 29 %). LCMS (formic): Rt = 0.37 min., $[M+H]^+$ = 451. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.5 (m, 1H); 8.1 (s, 1H); 7.6 – 7.7 (m, 1H); 7.2 – 7.3 (m, 2H); 5.9 (s, 1H); 4.4 (d, J = 16 Hz, 3H); 4.0 (d, J = 16 Hz, 1H); 3.6- 3.7 (m, 4H); 3.5 – 3.6 (m, 4H); 2.8 – 3.1 (m, 4H); 2.7 (d, J = 8 Hz, 2H); 2.5 (m, 1H); 1.9 – 2.1 (m, 1H); 1.5 – 1.7 (m, 4 H); 1.4 – 1.5 (m, 1H); 0.9 – 1.2 (m, 2 H).

(4-Ethylpiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (42)

4-Ethylpiperidine hydrochloride (35 mg, 0.23 mmol) was reacted according to general procedure F. The product was purified by preparative HPLC to afford the title compound (42) (21 mg, 0.05 mmol, 23 %). LCMS (formic): Rt = 0.69 min., $[M+H]^+$ = 388. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.0 (s, 1H); 5.8 (s, 1H); 4.4 – 4.5 (m, 1H); 4.3 – 4.4 (m, 2H); 4.0 – 4.1 (m, 1H); 3.7 (m, 4H); 3.5 – 3.6 (m, 4H); 3.3 (m, 1H); 2.9 – 3.2 (m, 4H); 2.5 – 2.7 (m, 1H); 1.4 – 1.9 (m, 6 H); 1.2 – 1.4 (m, 3H); 0.8 – 1.2 (m, 4 H).

(1-(6-Chloropyrimidin-4-yl)piperidin-4-yl)(4-isopropylpiperidin-1-yl)methanone

To a solution of (4-isopropylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (6.5 g, 23.65 mmol) in ethanol (70 mL) was added triethylamine (6.59 mL, 47.3 mmol) dropwise over 5 min, followed by 4,6-dichloropyrimidine (3.52 g, 23.65 mmol) portion wise over 2 min. The reaction was stirred at room temperature for 24 h. The reaction mixture was

concentrated under reduced pressure and then diluted with water (150 mL) and extracted into ethyl acetate (2 x 100 mL). The combined organic layers were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude compound was purified on silica flash chromatography, eluting with 55 % ethyl acetate in n-hexane. The product containing fractions were combined and concentrated under reduced pressure to afford (1-(6-chloropyrimidin-4-yl)piperidin-4-yl)(4-isopropylpiperidin-1-yl)methanone (5.83 g, 16.34 mmol, 69 %) as an off-white solid. LCMS (formic 4.5 min. run): Rt = 2.42 min., $[M+H]^+$ = 351, 353.

(4-Isopropylpiperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (43)

To a suspension of (1-(6-chloropyrimidin-4-yl)piperidin-4-yl)(4-isopropylpiperidin-1-yl)methanone (180 mg, 0.513 mmol) in ethanol (2 mL) in a 2-5 mL microwave vial DIPEA (0.108 mL, 0.616 mmol) was added and the reaction mixture heated to dissolve the reagent. Then morpholine (0.054 mL, 0.616 mmol) was added. The vial was heated 6 hours at 150 ºC with stirring. The solution was concentrated in vacuo and the residue was purified by chromatography, using a 12 g SILICYCLE cartridge, eluting with cyclohexane

/ ethyl acetate:ethanol 3:1 from 100/0 to 30 / 70 gradient. The appropriate fractions were combined and evaporated in vacuo and then was repurified by chromatography, using a 12 g SILICYCLE cartridge, eluting with DCM/methanol 97/3. The appropriate fractions were combined and evaporated in vacuo to afford the title compound (43) as a gum (178 mg, 0.44 mmol, 86 %). LCMS (formic): Rt = 0.75 min., $[M+H]^+$ = 402. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.28 (d, *J* = 0.8 Hz, 1 H); 5.63 (s, 1 H); 5.34 (s, 1 H); 4.71 (br d, *J* = 12.9 Hz, 1 H); 4.39 (s, 1 H); 4.28 - 4.50 (m, 2 H); 4.00 (br d, *J* = 13.1 Hz, 1 H); 3.72 - 3.84 (m, 4 H); 3.47 - 3.64 (m, 4 H); 2.88 - 3.18 (m, 3 H); 2.79 (br d, *J* = 4.5 Hz, 1 H); 2.39 - 2.62 (m, 1 H); 1.72 - 1.99 (m, 6 H); 1.42 - 1.56 (m, 1 H); 1.26 - 1.37 (m, 1 H); 1.09 - 1.26 (m, 2 H); 0.93 (d, *J* = 6.8 Hz, 6 H).

4-(Cyclopentylmethyl)pyridine

A solution of 4-methylpyridine (5.0 g, 53.7 mmol) in tetrahydrofuran (5 mL) was cooled to -78 ºC. LDA (53.7 mL, 107 mmol) was added dropwise to the cooled solution over a period of 4 min. The mixture was then stirred at -40 ºC for 20 min and was cooled to -78 ºC. Bromocyclopentane (8.00 g, 53.7 mmol) was added dropwise to the reaction solution over a period of 4 min. The reaction mixture was stirred at -78 ºC for 1 h. The reaction mixture was warmed to room temperature and stirred for 3 h. Saturated aqueous ammonium

chloride solution (500 mL) was then added and the product was extracted with ethyl acetate (250 mL). The ethyl acetate layer was washed with brine solution (225 mL). The ethyl acetate layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography silica gel (100-200 mesh), eluting with 12% ethyl acetate in petroleum ether. The solvents were removed to yield the title compound as a yellow liquid (3.5 g, 21.7 mmol, 40 %). LCMS (formic 4.5 min. run): Rt = 3.69 min., $[M+H]^+$ = 162. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.47 (d, J = 6.1 Hz, 2 H); 7.09 (d, J = 5.9 Hz, 2 H); 2.60 (d, J = 7.5 Hz, 2 H); 2.01 - 2.22 (m, 1 H); 1.44 - 1.79 (m, 6 H); 1.01 - 1.28 (m, 2 H).

4-(Cyclopentylmethyl)piperidine hydrochloride

To a solution of 4-(cyclopentylmethyl)pyridine (1.0 g, 6.20 mmol) in methanol (20 mL) at RT were added HCl (0.75 mL, 4.50 mmol) and platinum (IV) oxide (130 mg, 0.57 mmol). The reaction mixture was stirred at RT for 24 h under hydrogen (60 psi). The reaction mixture was filtered through a celite pad and washed with methanol (10 mL). The methanol was concentrated under reduced pressure and the crude product was then triturated with diethyl ether (10 mL). The obtained solid was filtered and washed with

diethyl ether (5 mL). The solid was dried under high vacuum to yield the title compound (700 mg, 4.2 mmol, 55 %) as an off white solid. LCMS (formic 4.5 min. run): Rt = 1.88 min., $[M+H]^+$ = 168.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.69 - 9.00 (m, 2 H); 3.10 - 3.25 (m, 2 H); 2.72 - 2.93 (m, 2 H); 1.70 - 1.93 (m, 5 H); 1.42 - 1.63 (m, 5 H); 1.12 - 1.38 (m, 4 H); 0.88 - 1.11 (m, 2 H).

(4-(Cyclopentylmethyl)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (44)

To a solution of 1-(6-morpholinopyrimidin-4-yl)piperidine-4-carboxylic acid (200 mg, 0.684 mmol), 4-(cyclopentylmethyl)piperidine hydrochloride (139 mg, 0.68 mmol) and HATU (390 mg, 1.03 mmol) in N,N-Dimethylformamide (DMF) (8 mL), DIPEA (0.358 ml, 2.05 mmol) was added at RT and stirred at RT for 12 hr. Reaction mixture was diluted with water (20 mL), extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica-gel (100-200 mesh) column chromatography, eluting with 4% methanol in DCM to yield the title compound (44) as a white solid (100 mg, 0.22 mmol, 33 %). LCMS (formic): Rt = 0.90 min., $[M+H]^+$ = 442. ^{1}H

NMR (400 MHz, DMSO-d_6) δ ppm 8.07 (d, J = 0.7 Hz, 1 H); 5.86 - 5.92 (m, 1 H); 4.29 - 4.43 (m, 3 H); 3.93 - 4.04 (m, 1 H); 3.60 - 3.68 (m, 4 H); 3.45 - 3.52 (m, 4 H); 2.83 - 3.07 (m, 4 H); 1.77 - 1.96 (m, 2 H); 1.38 - 1.77 (m, 13 H); 1.18 - 1.28 (m, 2 H); 0.97 - 1.14 (m, 3 H); 0.79 - 0.94 (m, 1 H).

(4-(2-(Diethylamino)ethyl)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (45)

N,N-diethyl-2-(piperidin-4-yl)ethan-1-amine dihydrochloride (77 mg, 0.30 mmol) was reacted according to general procedure F. The product was purified by preparative HPLC to afford the title compound (45) (589 mg, 0.13 mmol, 23 %). LCMS (formic): Rt = 0.38 min., $[M+H]^+$ = 459. ^{1}H 1H NMR (300 MHz, CD_3OD) δ ppm 8.0 (s, 1H); 5.8 ppm (s, 1H); 4.6-4.5 ppm (br.d, ½ CH_2, 1H, [Eq.]); 4.4-4.3 ppm (br.d, CH_2, 2H, [Eq.]); 4.1-4.0 ppm (br.d, ½ CH_2, 1H [Eq.]); 3.8-3.7 ppm, (m, 4H, 2 x CH_2); 3.6-3.5 ppm (m, 4H, 2 x CH_2); 3.2-2.9 ppm, (2 x br. m, 4H, 2 x CH_2 [Ax.]); 2.7-2.5 ppm, (7H, m + q, 3 x CH_2 + CH [Ax.]); 1.9-1.0 ppm (17H, br. m + t, CH + 5 x CH_2 + 2 x CH_3).

(1-(6-Morpholinopyrimidin-4-yl)piperidin-4-yl)(4-(2-(pyrrolidin-1-yl)ethyl)piperidin-1-yl)methanone (46)

4-(2-(Pyrrolidin-1-yl)ethyl)piperidine dihydrochloride (70 mg, 0.27 mmol) was reacted according to general procedure F. The product was purified by preparative HPLC to afford

the title compound (46) (59 mg, 0.13 mmol, 23 %). LCMS (formic): Rt = 0.38 min., $[M+H]^+$ = 459. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.0 (s, 1H); 5.8 (s, 1H); 4.4 – 4.6 (m, 1H); 4.3 – 4.4 (m, 2H); 4.0 – 4.2 (m, 1H); 3.7 – 3.8 (m, 4H); 3.4 – 3.6 (m, 4H); 2.9 – 3.2 (m, 4H); 2.5 – 2.7 (m, 7H); 1.4 – 1.9 (m, 13H); 1.0 – 1.3 (m, 2H).

(4-(4-Fluorophenoxy)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (47)

4-(4-Fluorophenoxy)piperidine hydrochloride (0.4 g, 1.73 mmol) was reacted according to general procedure F. The crude product was purified by preparative HPLC, using a x-brigde column, in basic media, method: 40-100% 10 mM NH_4HCO_3-acetonitrile. Appropriate fractions were collected, affording the title compound (47) as a white solid: (583 mg, 1.24 mmol, 73 %). LCMS (formic): Rt = 1.15 min., $[M+H]^+$ = 470. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.15 (s, 1 H); 7.18 (d, J = 8.6 Hz, 2 H); 7.03 - 7.12 (m, 2 H); 5.98 (s, 1 H); 4.57 - 4.70 (m, 1 H); 4.33 - 4.50 (m, 2 H); 3.85 - 4.00 (m, 2 H); 3.66 - 3.78 (m, 4 H); 3.53 - 3.61 (m, 4 H); 3.44 - 3.52 (m, 1 H); 3.24 - 3.34 (m, 1 H); 2.90 - 3.09 (m, 3 H); 1.89 - 2.11 (m, 2 H); 1.61 - 1.80 (m, 3 H); 1.45 - 1.61 (m, 3 H).

(4-(4-Chlorophenoxy)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (48)

4-(4-Chlorophenoxy)piperidine (87 mg, 0.410 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column

chromatography, eluting with 4% methanol in DCM and then repurified by HPLC using the following conditions. Column: XTERRA RP18 (150 x 19) mm, 5µm, Mobile Phase : A: 10 mM ammonium bicarbonate (Aq) B: Acetonitrile, Flow : 15 ml/min, Method : 0/55, 12/55, 13/100, 18/100. The appropriate fractions were concentrated to yield the title compound (48) as an off-white solid (84 mg, 0.17 mmol, 51 %). LCMS (formic): Rt = 0.81 min., $[M+H]^+$ = 486. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.02 - 8.14 (m, 1 H); 7.26 - 7.38 (m, 2 H); 6.93 - 7.08 (m, 2 H); 5.84 - 5.93 (m, 1 H); 4.56 - 4.69 (m, 1 H); 4.30 - 4.42 (m, 2 H); 3.75 - 3.92 (m, 2 H); 3.58 - 3.70 (m, 4 H); 3.45 - 3.54 (m, 4 H); 3.36 - 3.45 (m, 1 H); 3.16 - 3.26 (m, 1 H); 2.83 - 3.02 (m, 3 H); 1.84 - 2.03 (m, 2 H); 1.40 - 1.71 (m, 6 H).

Tert-butyl 4-(2,4-difluorophenoxy)piperidine-1-carboxylate

To tert-butyl 4-hydroxypiperidine-1-carboxylate (1.55 g, 7.7 mmol), triphenylphosphine (3.02 g, 11.5 mmol), and 2,4-difluorophenol (0.734 mL, 7.7 mmol) in THF (50 mL) was added diisopropyl diazene-1,2-dicarboxylate (1.52 mL, 7.7 mmol) dropwise at room temperature. The mixture was stirred overnight. Ethyl acetate was added (50 mL). The organic layer was then washed with 2 N aq. NaOH and brine and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified by silica gel flash

chromatography, eluting with a gradient of 0 - 50 % [ethyl acetate:ethanol (3:1)] in cyclohexane to provide the title compound tert-butyl 4-(2,4-difluorophenoxy)piperidine-1-carboxylate (1.57 g, 5.0 mmol, 65 %) as a colourless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.21 - 7.35 (m, 2 H); 7.01 (td, J = 2.0, 0.9 Hz, 1 H); 4.48 (br t, J = 3.9 Hz, 1 H); 3.58 - 3.71 (m, 2 H); 3.16 (br t, J = 9.7 Hz, 2 H); 1.81 - 1.95 (m, 2 H); 1.52 (dtd, J = 12.9, 8.7, 8.7, 3.9 Hz, 2 H); 1.36 - 1.43 (s, 9 H).

4-(2,4-Difluorophenoxy)piperidine, hydrochloride

In a round-bottom flask, tert-butyl 4-(2,4-difluorophenoxy)piperidine-1-carboxylate (1.57 g, 5.0 mmol) was dissolved in dichloromethane (10 mL) under stirring. 3M HCl in methanol (20 mL, 60.0 mmol) was added at room temperature and the mixture was stirred at room temperature for 8 h. The solvents were concentrated to yield the title compound 4-(2,4-difluorophenoxy)piperidine, hydrochloride (1.20 g, 4.8 mmol, 96 %), as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.77 - 9.12 (m, 2 H); 7.22 - 7.41 (m, 2 H); 6.95 - 7.11 (m, 1 H); 4.57 (dt, J = 7.4, 3.8 Hz, 1 H); 3.20 (br s, 2 H); 2.99 - 3.10 (m, 2 H); 2.07 (br d, J = 4.0 Hz, 2 H); 1.86 (br s, 2 H).

(4-(2,4-Difluorophenoxy)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (49)

Under nitrogen atmosphere, in a 100 mL round-bottom flask, 1-(6-morpholinopyrimidin-4-yl)piperidine-4-carboxylic acid (1.48 g, 5.1 mmol) was dissolved in anhydrous dichloromethane (25 mL) and di(1H-imidazol-1-yl)methanone (0.98 g, 6.1 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 hours. 4-(2,4-difluorophenoxy)piperidine (1.08 g, 5.1 mmol) was added and the reaction mixture stirred at room temperature overnight. Water was added and the reaction mixture was stirred for 10 min. The phases were separated and the organic phase was washed with water and then brine, filtered and evaporated under reduced pressure. The crude obtained was purified by column chromatography (80 g silicycle cartridge), using as eluents a mixture of cyclohexane/ethyl acetate:ethanol (3:1), from 0 % to 50 % ethyl acetate:ethanol (3:1). The appropriate fractions were collected and evaporated under reduced pressure. This product was then combined with a batch from an identical synthesis by dissolving the solids in DCM and evaporating the solvent under reduced pressure. The solid obtained

was triturated mechanically and kept in the oven at 40 ºC under high vacuum overnight to afford the title compound (49) as a white solid (3.48 g, 7.1 mmol). LCMS (formic): Rt = 0.76 min., $[M+H]^+$ = 488. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.28 (s, 1 H); 7.01 (td, *J* = 9.1, 5.6 Hz, 1 H); 6.91 (ddd, *J* = 11.1, 8.3, 3.0 Hz, 1 H); 6.80 - 6.87 (m, 1 H); 5.64 (s, 1 H); 4.32 - 4.50 (m, 3 H); 3.77 - 3.90 (m, 7 H); 3.62 - 3.71 (m, 1 H); 3.58 (d, *J* = 5.1 Hz, 4 H); 3.45 - 3.54 (m, 1 H); 2.99 (br s, 2 H); 2.77 - 2.87 (m, 1 H); 1.80 - 2.03 (m, 8 H); 1.75 - 2.01 (m, 1 H).

4-(4-Chloro-2-fluorophenoxy)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (50)

4-(4-Chloro-2-fluorophenoxy)piperidine hydrochloride (137 mg, 0.51 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 8% methanol in DCM. The product was re-purified by HPLC using the following conditions: MP-A: 10mM ammonium bicarbonate (Aq) MP-B: acetonitrile, Column: x BRIDGE C18 (250 X30 mm) 5um, Method - T/%B 0/10,1/10, 16/89,16.1/100,20.5/100,20.6/10,24/10. Flow: 30 mL/min. The appropriate

fractions were combined and concentrated to yield the title compound (50) as a white solid (45 mg, 0.09 mmol, 17 % yield). LCMS (formic): Rt = 0.82 min., $[M+H]^+$ = 504. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (s, 1 H); 7.44 (dd, J = 11.1, 2.5 Hz, 1 H); 7.26 - 7.37 (m, 1 H); 7.15 - 7.25 (m, 1 H); 5.90 (s, 1 H); 4.58 - 4.71 (m, 1 H); 4.36 (br d, J = 12.7 Hz, 2 H); 3.83 (br s, 2 H); 3.59 - 3.70 (m, 4 H); 3.45 - 3.52 (m, 4 H); 3.35 - 3.48 (m, 1 H); 3.18 - 3.28 (m, 1 H); 2.81 - 3.04 (m, 3 H); 1.84 - 2.06 (m, 2 H); 1.58 - 1.72 (m, 3 H); 1.41 - 1.57 (m, 3 H).

4-((5-Methylpyridin-2-yl)oxy)piperidin-1-yl)(1-(6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (51)

5-Methyl-2-(piperidin-4-yloxy)pyridine (79 mg, 0.41 mmol) was reacted according to general procedure F. The crude compound was purified by silica-gel (100-200 mesh) column chromatography, eluting with 4% methanol in DCM and then repurified by HPLC using the following conditions: Column: XTERRA RP18 (150x19) mm, 5µ, Mobile Phase: A: 10 mM ammonium bicarbonate (Aq) B: acetonitrile Flow: 15 ml/min Method: 0/55, 12/55, 13/100, 18/10. The appropriate fractions were combined and concentrated to yield the title compound (51) as a white solid (74 mg, 0.16 mmol, 46 %). LCMS (formic): Rt = 0.65 min., $[M+H]^+$ = 467. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (s, 1 H); 7.96 (s, 1 H); 7.88 - 8.00 (m, 1 H); 7.45 - 7.61 (m, 1 H); 6.70 (d, J = 8.3 Hz, 1 H); 5.90 (s, 1 H); 5.04 - 5.30 (m, 1 H);

4.36 (br d, *J* = 12.9 Hz, 2 H); 3.74 - 3.98 (m, 2 H); 3.63 (br d, *J* = 5.0 Hz, 4 H); 3.36 - 3.54 (m, 5 H); 3.19 (br t, *J* = 9.9 Hz, 1 H); 2.81 - 3.04 (m, 3 H); 2.20 (s, 3 H); 1.88 - 2.08 (m, 2 H); 1.63 (br s, 3 H); 1.48 (br d, *J* = 10.7 Hz, 3 H).

(4-Benzylpiperidin-1-yl)(1-(4-(piperidin-1-yl)pyrimidin-2-yl)piperidin-4-yl)methanone (52)

To a solution of 1-(4-piperidin-1-yl-pyrimidin-2-yl)-piperidine-4-carboxylic acid (100 mg, 0.34 mmol) in DMF (3 mL) was added HATU (157 mg, 0.41 mmol). The mixture was stirred at room temperature for 1 h. 4-Benzylpiperidine (0.073 mL, 0.41 mmol) and DIPEA (0.120 mL, 0.69 mmol) were added and it was stirred at room temperature overnight. To the reaction mixture were added sat $NaHCO_3$ and ethyl acetate. The layers were separated and the organic layer was washed with 1N NH_4Cl. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified on silica gel column and was eluted with Cyclohexane/ ethyl acetate : ethanol 3:1 gradient 0 - 30 %. Collected fractions were evaporated under reduced pressure to give a pale yellow solid, which was dissolved in ethyl acetate and washed with sat. $NaHCO_3$ and with 1 N NH_4Cl. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford the title compound (52) as a white solid (91 mg, 0.20 mmol,

59 %). LCMS (formic): Rt = 0.90 min., $[M+H]^+$ = 448, purity = 92 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.89 (d, *J* = 6.3 Hz, 1 H); 7.29 (d, *J* = 7.6 Hz, 2 H); 7.18 - 7.26 (m, 1 H); 7.15 (d, *J* = 7.3 Hz, 2 H); 5.88 (br d, *J* = 6.1 Hz, 1 H); 4.70 - 4.82 (m, 2 H); 4.63 (br d, *J* = 13.6 Hz, 1 H); 3.93 (br d, *J* = 12.6 Hz, 1 H); 3.71 - 3.78 (m, 1 H); 3.58 (br d, *J* = 0.8 Hz, 3 H); 2.99 (br s, 3 H); 2.66 - 2.79 (m, 1 H); 2.41 - 2.63 (m, 3 H); 1.65 - 1.89 (m, 13 H); 1.08 - 1.24 (m, 2 H).

Tert-butyl 4-(4-isopropylpiperidine-1-carbonyl)piperidine-1-carboxylate

To a solution on N-Boc-isonipecotic acid (1.29 g, 5.6 mmol) in THF (40 mL) was added carbonyl diimidazole (1 g, 6.2 mmol) and the mixture was stirred at 50 ˚C for 30 minutes. The solution was cooled to room temperature and 4-isopropylpiperidine (1 g, 7.9 mmol) and triethylamine (0.78 mL, 5.6 mmol) were added and the mixture was stirred at room temperature for 2 hours. The solvent was removed in vacuo and the residue taken up in ethyl acetate and washed with water (☐ 4) and dried over Na_2SO_4. The crude product was purified by flash chromatography eluting with 50 % heptane in ethyl acetate to yield the title compound (tert-butyl 4-(4-isopropylpiperidine-1-carbonyl)piperidine-1-carboxylate) as a white solid (1.88 g, 5.6 mmol, 99 %).

(4-Isopropylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride

To a solution of tert-butyl 4-(4-isopropylpiperidine-1-carbonyl)piperidine-1-carboxylate (1.88 g, 5.6 mmol) in dioxane (10 mL) was added 4 M HCl in dioxane (approx. 10 mL) and the mixture was stirred at room temperature for 2 hours. A white precipitate formed. The solid was collected by filtration but was hygroscopic and liquified during filtration. The remaining solid was dissolved in methanol and concentrated in vacuo to yield the title compound ((4-isopropylpiperidin-1-yl)(piperidin-4-yl)methanone) as a white solid (1.44 g, 5.2 mmol, 94 %).

(4-Isopropylpiperidin-1-yl)(1-(6-morpholinopyridin-2-yl)piperidin-4-yl)methanone (53)

To a solution of (4-isopropylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (136 mg, 0.49 mmol) in toluene (1 mL) was added 4-(6-bromo-2-yl)morpholine (100 mg, 0.41 mmol), Cs_2CO_3 (321 mg, 0.98 mmol), $Pd(OAc)_2$ (1 mg) and BINAP (7.6 mg). The mixture was degassed with argon for 15 minutes and then heated to reflux overnight. The mixture was cooled to room temperature, filtered through a pad of celite, washed with methanol and concentrated. The crude product was purified by flash column chromatography, eluting with 10 % ethyl acetate in heptane to yield the title compound (53) as a white solid

(138 mg, 0.35 mmol, 85 %). LCMS (formic): Rt = 1.14 min., $[M+H]^+$ = 401. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.3 – 7.4 (m, 1H); 6.0 – 6.1 (m, 1H); 5.9 – 6.0 (m, 1H); 4.6 – 4.8 (m, 1H); 4.2 – 4.4 (m, 2H); 3.9 – 4.1 (m, 1H); 3.7 – 3.9 (m, 4H); 3.4 – 3.5 (m, 4H); 2.9 – 3.1(m, 1H); 2.8 – 2.9 (m, 2H); 2.6 – 2.8 (m, 1H); 2.4 – 2.5 (m, 1H); 1.7 – 2.0 (m, 6H); 1.4 – 1.5 (m, 1H); 1.0 – 1.4 (m, 3H); 0.8 – 1.0 (m, 6H).

(4-Isopropylpiperidin-1-yl)(1-(4-morpholinopyridin-2-yl)piperidin-4-yl)methanone (54)

To a solution of (4-isopropylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (136 mg, 0.49 mmol) and 2-chloro-4-morpholinopyridine (97 mg, 0.49 mmol) in IMS (5 mL) was added DIPEA (0.21 mL, 1.23 mmol) and the mixture was heated in a microwave reactor at 160 ˚C for 20 h. The solvent was removed in vacuo and the residue purified by flash column chromatography eluting with 10 % methanol in ethyl acetate. The appropriate fractions were combined and concentrated to afford the title compound (54) as a white solid (55 mg, 0.14 mmol, 28 %). LCMS (formic): Rt = 0.75 min., $[M+H]^+$ = 401. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.9 – 8.0 (m, 1H); 6.1 – 6.2 (m, 1H); 6.0 (s, 1H); 4.6 – 4.8 (m, 1H); 4.2 – 4.4 (m, 2H); 3.9 – 4.0 (m, 1H); 3.8 – 3.9 (m, 4H); 3.2 – 3.3 (m, 4H); 2.9 – 3.1 (m, 1H); 2.8 – 2.9 (m, 2H); 2.6 – 2.8 (m, 1H); 2.4 – 2.6 (m, 1H); 1.6 – 2.0 (m, 5H); 1.5 – 1.6 (m, 1H); 1.0 – 1.3 (m, 4H); 0.8 – 0.9 (m, 6 H).

(4-Isopropylpiperidin-1-yl)(1-(2-morpholinopyridin-4-yl)piperidin-4-yl)methanone (55)

To a solution of (4-isopropylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (136 mg, 0.49 mmol) and 4-(4-chloropyridin-2-yl)morpholine (97 mg, 0.49 mmol) in IMS (5 mL) was added DIPEA (0.21 mL, 1.23 mmol) and the mixture was heated in a microwave reactor at 160 ˚C for 24 h. The solvent was removed in vacuo and the residue purified by flash column chromatography eluting with 10 % methanol in ethyl acetate. The appropriate fractions were combined and concentrated to afford the title compound (55) as a pale brown solid (31 mg, 0.078 mmol, 16 %). LCMS (formic): Rt = 0.75 min., $[M+H]^+$ = 401. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.9 – 8.0 (m, 1H); 6.1 – 6.2 (m, 1H); 6.0 (s, 1H); 4.6 – 4.8 (m, 1H); 4.2 – 4.4 (m, 2H); 3.9 – 4.1 (m, 1H); 3.8 – 3.9 (m, 4H); 3.2 – 3.3 (m, 4H); 2.9 – 3.1 (m, 1H); 2.8 – 2.9 (m, 2H); 2.6 – 2.8 (m, 1H); 2.4 – 2.6 (m, 1 H); 1.7 – 2.0 (m, 5 H); 1.4 – 1.6 (m, 1H); 1.2 – 1.3 (m, 2 H); 1.0 – 1.2 (m, 2H); 0.8 – 1.0 (m, 6H).

(4-Benzylpiperidin-1-yl)(1-(6-morpholinopyrazin-2-yl)piperidin-4-yl)methanone (56)

To a solution of (4-benzylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (87 mg, 0.27 mmol) in Ethanol (1 mL) was added 2,6-dichloropyrazine (40 mg, 0.27 mmol) and DIPEA (0.094 mL, 0.54 mmol) and the mixture was heated at 150 °C in a microwave for

5 minutes. Morpholine (0.094 mL, 1.07 mmol) was then added and heating continued at 150 °C for a further 4 h. The solution was purified by preparative HPLC using formic acid modifier and then repurified by preparative HPLC using ammonium bicarbonate modifier. The appropriate fractions were concentrated to afford the title compound (56) as a black gum. LCMS (formic): Rt = 0.98 min., $[M+H]^+$ = 450. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.52 (s, 1 H); 7.41 (s, 1 H); 7.29 (d, *J* = 7.3 Hz, 2 H); 7.19 - 7.25 (m, 1 H); 7.16 (m, 1 H); 7.14 (m, 1 H); 4.63 (br d, *J* = 13.7 Hz, 1 H); 4.30 (br d, *J* = 11.7 Hz, 2 H); 3.92 (br d, *J* = 13.2 Hz, 1 H); 3.78 - 3.85 (m, 4 H); 3.46 - 3.53 (m, 4 H); 3.00 (br. m, 1 H); 2.85 - 2.95 (m, 2 H); 2.67 - 2.77 (m, 1 H); 2.46 - 2.64 (m, 3 H); 1.69 - 1.94 (m, 7 H); 1.09 - 1.25 (m, 2 H).

(4-Benzylpiperidin-1-yl)(1-(5-fluoro-6-morpholinopyrimidin-4-yl)piperidin-4-yl)methanone (57)

To a solution of (4-benzylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (77 mg, 0.24 mmol) in Ethanol (1 mL) was added 4,6-dichloro-5-fluoropyrimidine (40 mg, 0.24 mmol) and DIPEA (0.084 mL, 0.48 mmol) and the mixture was stirred at room temperature overnight. Additional morpholine (0.084 mL, 0.96 mmol) was added and the reaction was stirred at 140 °C in a microwave reactor for 30 minutes. A further portion of morpholine

(0.084 mL, 0.96 mmol) was added and heating continued at 140 °C for a further 1 h. The solution was then purified by preparative HPLC and the appropriate fractions combined and concentrated to afford the title compound (57) as a white solid (75 mg, 0.15 mmol, 66 %). LCMS (formic): Rt = 1.18 min., $[M+H]^+$ = 468. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.95 (d, J = 2.0 Hz, 1 H); 7.28 (m, 2 H); 7.11 - 7.20 (m, 3 H); 4.36 (br d, J = 12.6 Hz, 1 H); 4.18 (br d, J = 12.6 Hz, 2 H); 3.92 - 4.13 (m, 1 H); 3.62 - 3.75 (m, 4 H); 3.47 - 3.57 (m, 4 H); 2.81 - 3.11 (m, 5 H); 2.52 - 2.57 (m, 1 H); 2.38 - 2.48 (m, 1 H); 1.76 (br dd, J = 7.3, 3.8 Hz, 1 H); 1.62 (br s, 6H); 0.91 - 1.17 (m, 2 H).

(1-(2-Amino-6-morpholinopyrimidin-4-yl)piperidin-4-yl)(4-benzylpiperidin-1-yl)methanone (58)

To a solution of (4-benzylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (120 mg, 0.37 mmol) in EtOH (0.9 mL) was added 4-chloro-6-morpholinopyrimidin-2-amine (40 mg, 0.19 mmol) and DIPEA (98 μL, 0.56 mmol). The mixture was heated at 150 °C in the microwave for 3 hours, after which time direct purification of the mixture by preparative HPLC afforded the title compound (58) as a straw-coloured foam (64 mg, 0.14 mmol, 74 %). HR-MS (ESI): $C_{26}H_{37}N_6O_2$ [M+H$^+$] requires 465.2973, found 465.2980. ^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 7.27-7.29 (m, 2H); 7.17-7.20 (m, 3H); 5.56 (s, 2H); 5.30 (s, 1H); 4.35 (d, J = 12.6 Hz, 1H); 4.27 (d, J = 12.6 Hz, 2H); 3.97 (d, J = 12.6 Hz, 1H); 3.61-3.62 (m, 4H); 3.40-3.42 (m, 4H); 2.95 (t, J = 12.2 Hz, 1H); 2.85 (tt, J = 3.7, 11.3 Hz, 1H); 2.75-2.80 (m, 2H); 2.52 (s, 2H); 2.45 (t, J = 12.2 Hz, 1H); 1.72-1.77 (m, 1H); 1.56-1.64 (m, 4H); 1.40-1.48 (m, 2H); 1.04-1.12 (m, 1H); 0.93-1.01 (m, 1H).

^{13}C NMR (125 MHz, DMSO-d_6): δ ppm172.1; 164.6; 164.0; 162.2; 140.0; 129.0 (2C); 128.1 (2C); 125.8; 73.1; 66.0 (2C); 44.8; 44.3 (2C); 43.3 (2C); 42.0; 41.2; 37.5 (2C); 32.6; 31.4; 28.0; 27.8, IR (neat) cm^{-1} 3342; 2917; 2848; 1628; 1565; 1418; 1370; 1252; 1210; 1188; 1115; 987; 967; 898; 789; 745; 700 ..

4-(4-(4-Benzylpiperidine-1-carbonyl)piperidin-1-yl)-6-morpholinopyrimidin-2(1*H*)-one (59)

To a solution of 4-(6-chloro-2-((4-methoxybenzyl)oxy)pyrimidin-4-yl)morpholine (75 mg, 0.22 mmol) and DIPEA (0.117 mL, 0.67 mmol) in ethanol (0.9 mL) was added (4-benzylpiperidin-1-yl)(piperidin-4-yl)methanone hydrochloride (144 mg, 0.45 mmol). The mixture was heated to 150 °C in the microwave for 8 hours, after which time direct purification of the reaction mixture by preparative HPLC afforded the title compound (59) as a straw coloured glass that was scratched to give an amorphous white solid (84 mg, 0.18 mmol, 81 %). M.pt. 117-123 °C. HR-MS (ESI): $C_{26}H_{36}N_5O_3$ [M+H$^+$] requires 466.2813, found 466.2814.^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.41 (br.s, 1H); 7.27-7.31 (m, 2H); 7.17-7.21 (m, 3H); 5.21 (s, 1H); 4.36 (d, *J* = 12.6 Hz, 1H); 4.12-4.17 (m, 2H); 3.98 (d, *J* = 12.6 Hz, 1H); 3.60-3.63 (m, 4H); 3.42-3.45 (m, 4H); 2.86-2.99 (m, 4H);

2.52-2.54 (m, 2H); 2.42-2.49 (m, 1H); 1.70-1.81 (m, 1H); 1.44-1.65 (m, 6H); 1.05-1.12 (m, 1H); 0.95-1.01 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 171.9; 162.2; 160.6; 158.0; 140.0; 129.0 (2C); 128.1 (2C); 125.8; 71.1; 65.8 (2C); 48.6; 44.9 (2C); 44.8; 44.3; 42.0; 41.2; 37.5; 37.0; 32.6; 31.5; 28.0; 27.8. IR (neat) cm^{-1} 2918; 2851; 1596; 1507; 1441; 1366; 1255; 1205; 1115; 967; 747; 701.

N-(2-fluoroethyl)-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1H-pyrrolo[3,2-b]pyridine-3-carboxamide (60) was synthesized by the procedure described previously in Shirude et al. [18] (no. 9 in the paper).

Strain and Growth Conditions. M. tuberculosis H37Rv (ATC25618) wild type was grown in Middlebrook 7H9-ADC broth (Difco) supplemented with 0.05% Tween 80 and on 7H10-OADC or 7H11-OADC agar (Difco) at 37 °C. Isoniazid and hygromycin were purchased from Sigma-Aldrich. When required, hygromycin (50 μg/mL) was added to the culture medium.

MIC Determination. MIC determination assay was performed using a resazurin reduction assay with fluorescent readout as described previously.[35] Isoniazid was used

as a positive control (MIC = 1.8 μM), and rifampicin was used as a no-growth control. MIC determination against bacterial strains ewas performed as descrivied in Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A6,Vol 23 No.2. Intracellular MIC Determination. The assays were performed as described previously[35] using human THP-1macrophages differentiated with phorbol-12-myristate-13-acetate.The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol."

Microsomal Fraction Stability. The assays were performed as described previously.[35] The human biological samples were sourced ethically and their research use was in accordance with the terms of the informed consents.

The classification of the clearance values (Clint) from mouse microsomes is:

<1 ml/min/g tisse: low clearance.

1-5 ml/min/g tissue: moderate clearance.

>5 ml/min/g tissue: high clearance.

The classification of the clearance values (Clint) from human microsomes is:

<0.4 ml/min/g tissue: low clearance.

0.4-2 ml/min/g tissue: moderate clearance.

>2 ml/min/g tissue: high clearance.

DprE1 Enzymatic Inhibition, Time-Dependent DprE1 Inhibition, and DprE1-Overexpressing Strain. Expression and purification of Mt-DprE1 and cloning of Mt-DprE1 were performed as described by Batt et al.[36] Enzymatic data referred to as FPR in this paper (farnesylphosphoryl-β-D-ribose as substrate) were generated as described. The remaining DprE1 assay data was generated using a modified version of the assay described in that report using geranylgeranyllphosphoryl-β-D-ribose as substrate. The new protocol is in the process of being submitted for publication.

HepG2 Cytotoxicity Assay; Artificial Membrane Permeability, Kinetic Aqueous Solubility (CLND), and Hydrophobicity (Chrom log DpH7.4). These assays were performed as described previously.[29, 35]

DprE1 Enzymatic Inhibition and DprE1-Overexpressing Strain. Expression and purification of Mt-DprE1 and cloning of Mt-DprE1 were performed as described by Batt et al.[30] Enzymatic data referred to as FPR in this paper (farnesylphosphoryl-β-D-ribose as substrate) were generated as described. The remaining DprE1 assay data was generated using a modified version of the assay described in that report using geranylgeranyllphosphoryl-β-D-ribose as substrate. The new protocol is in the process of being submitted for publication.

HepG2 Cytotoxicity Assay; Artificial Membrane Permeability, Kinetic Aqueous Solubility (CLND), and Hydrophobicity (Chrom log DpH7.4). These assays were performed as described previously.[29, 356]

Phamacokinetics studies. For pharmacokinetic studies, CD-1 male mice (22-25 g) were used and compound concentrations were determined using peripheral whole blood. All

animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.

Compounds (39 and 47) were administered by intravenous route at 1 mg/kg single dose in 5%DMSO/20% Encapsin and by oral gavage at 5 mg/kg single dose in 1% methyl cellulose (1% MC). Aliquots of 20 µL of blood were taken from the lateral tail vein by puncture from each mouse (n =3 per route) at 5, 15 and 30 minutes, 1, 2, 4, 6, 8 and 24 hours post-dose for intravenous route and at 15, 30, and 45 minutes, 1, 2, 4, 6, 8 and 24 hours post-dose for oral route. LC-MS/MS was used as the analytical method for the establishment of compound concentration in blood. Pharmacokinetic analysis was performed by non-compartmental data analysis (NCA) with Phoenix WinNonlin 6.3 (Pharsight, Certara L.P) and supplementary analysis was performed with GraphPad Prism 6 (GraphPad Software, Inc).

Therapeutic efficacy. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care,

Welfare and Treatment of Animals. Specific pathogen-free, 8-10 week-old female C57BL/6 mice were purchased from Harlan Laboratories and were allowed to acclimate for one week. Mice were infected intratracheally with 100.000 CFU/mouse (M. tuberculosis H37Rv strain). Compounds were orally administered for eight consecutive days, starting from day 1 after infection. Lungs were harvested on day 9, 24 hours after the last compound administration. All lung lobes were aseptically removed, homogenized and frozen. Homogenates were plated in 10% OADC-7H11 medium supplemented with activated charcoal (0.4%) to avoid product carry over, and incubated for 18 days at 37 °C. No adverse clinical signs were observed in any animal.

The number of CFU/mouse measured for each mouse and the differences in the lung microorganism burden (log10 CFUs/lungs) obtained in the treated mice with respect to untreated controls (Day 9 after infection) were calculated. CFU number in lungs of untreated mice: 5.8 logCFU. This value is included in the interval mean ± 2 SD of the values of the last experiments. Quality controls: In this experiment, Moxifloxacin (100 mg/kg) was administered for eight consecutive days starting from day 1 after infection as

an inter-assay control. It reduced 2.7 logCFU the bacterial lung number in comparison with the untreated mice (5.8 logCFU).

This quality control value is included in the accepted interval.

The mouse treated with compound 47 at 300 mg/kg was sacrificed due to bad clinical conditions after the second dosification.

Some mice were pretreated with ABT to inhibit their cytochrome activity in order to increase the exposure of compound 60. ABT was administered by oral route 2 hours before the compound.

Data analysis: Non linear fitting to logistic equation of log10 (logCFU at day 9 after infection).

Parameter of efficacy: Effective dose 99 % (ED99), defined as the dose in mg/Kg that reduced bacterial burden at day 9 after infection by 99 % with respect to untreated mice.

AUTHOR INFORMATION

Corresponding Author

*J.A.B. jennifer.borthwick@blackbelttx.com

*M.C. monica.i.cacho@gsk.com

*R.J.Y. Rob@blue-burgundy.co.uk

Present affiliations:

J.A.B. Black Belt Tx Ltd, Stevenage Bioscience Catalyst, Accelerator Building, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2FX

R.J.Y. Blue Burgundy (Drug Discovery Consulting) Ltd

B.C.W. Medicinal Chemistry, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge CB4 0FZ, UK

A.A. Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge CB4 0FZ, UK

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. J.A.B., M.C. and R.J.Y. and wrote the manuscript with input from all authors.

ACKNOWLEDGEMENTS

We thank Global Alliance for TB Drug Development for helpful discussions and financial support and all the people at GSK who have contributed to this work.

Supporting Information

Molecular formula strings for all compounds in this paper are available as supplementary information.

Abbreviations

ABT 1-Aminobenzotriazole

ADC Albumin–dextrose–catalase

AMU Atomic Mass Units

AZ AstraZeneca

BEH Ethylene Bridged Hybrid

BINAP 2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene

CFU Colony Forming Unit

Clint Intrinsic Clearance

CL Clearance

CLND Chemiluminescent Nitrogen Detection

COMU 1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate

DIPEA Diisopropylethylamine

DprE1 Decaprenylphosphoryl-β-D-ribose 2'-epimerase

EtOH Ethanol

FPR Farnesylphosphoryl-β-D-ribose

GSK GlaxoSmithKline

HATU 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, N-[(Dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide

IMS Industrial methylated spririt

OADC 10% Oleic acid–albumin–dextrose–catalase

OE Overexpressor

PFI Property Forecast Index

SPR Structure Property Relationship

TBME *tert*-Butyl methyl ether

TDR Totally Drug Resistant

Vss Volume of distribution at steady state

XDR Extremely Drug Resistant

REFERENCES

1. WHO Global Tuberculosis Report 2018.

http://www.who.int/tb/publications/global_report/en/ (accessed Nov 14, 2018).

2. Zumla, A.; Raviglione, M.; Hafner, R.; Fordham von Reyn, C. Tuberculosis. *New England Journal of Medicine* **2013,** 368, 745-755.

3. Zumla, A.; Chakaya, J.; Centis, R.; D'Ambrosio, L.; Mwaba, P.; Bates, M.; Kapata, N.; Nyirenda, T.; Chanda, D.; Mfinanga, S.; Hoelscher, M.; Maeurer, M.; Migliori, G. B. Tuberculosis Treatment and Management—an Update on Treatment Regimens, Trials, New Drugs, and Adjunct Therapies. *The Lancet Respiratory Medicine* **2015,** 3, 220-234.

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

5. (a) Crellin, P. K.; Brammananth, R.; Coppel, R. L. Decaprenylphosphoryl-β–D-Ribose 2'-Epimerase, the Target of Benzothiazinones and Dinitrobenzamides, Is an Essential Enzyme in Mycobacterium smegmatis. *PLoS ONE* **2011**, 6, e16869. (b)

Jankute, M.; Grover, S.; Rana, A.K.; Besra, G.S. Arabinogalactan and Lipoarabinomannan Biosynthesis: Structure, Biogenesis and their Potential as Drug Targets. Future Microbiol. **2012**, 7, 129-147.

6. Carroll, P.; Faray-Kele, M. C.; Parish, T. Identifying Vulnerable Pathways in *Mycobacterium tuberculosis* by Using a Knockdown Approach. *Applied and Environmental Microbiology* **2011,** 77, 5040-5043.

7. Kolly, G. S.; Boldrin, F.; Sala, C.; Dhar, N.; Hartkoorn, R. C.; Ventura, M.; Serafini, A.; McKinney, J. D.; Manganelli, R.; Cole, S. T. Assessing the Essentiality of the Decaprenyl-phospho-d-arabinofuranose Pathway in *Mycobacterium tuberculosis* Using Conditional Mutants. *Molecular Microbiology* **2014,** 92, 194-211.

8. Manina, G.; Pasca, R.; Buroni, S.; De Rossi, E.; Riccardi, G. Decaprenylphosphoryl-β-D-Ribose 2'-Epimerase from *Mycobacterium tuberculosis* is a Magic Drug Target. *Current Medicinal Chemistry* **2010,** 17, 3099-3108.

9. Chatterji, M.; Shandil, R.; Manjunatha, M. R.; Solapure, S.; Ramachandran, V.; Kumar, N.; Saralaya, R.; Panduga, V.; Reddy, J.; KR, P.; Sharma, S.; Sadler, C.; Cooper, C. B.; Mdluli, K.; Iyer, P. S.; Narayanan, S.; Shirude, P. S. 1,4-Azaindole, a

Potential Drug Candidate for Treatment of Tuberculosis. *Antimicrobial Agents and Chemotherapy* **2014,** 58, 5325-5331.

10. Christophe, T.; Jackson, M.; Jeon, H. K.; Fenistein, D.; Contreras-Dominguez, M.; Kim, J.; Genovesio, A.; Carralot, J. P.; Ewann, F.; Kim, E. H.; Lee, S. Y.; Kang, S.; Seo, M. J.; Park, E. J.; Škovierová, H.; Pham, H.; Riccardi, G.; Nam, J. Y.; Marsollier, L.; Kempf, M.; Joly-Guillou, M. L.; Oh, T.; Shin, W. K.; No, Z.; Nehrbass, U.; Brosch, R.; Cole, S. T.; Brodin, P. High Content Screening Identifies Decaprenyl-Phosphoribose 2' Epimerase as a Target for Intracellular Antimycobacterial Inhibitors. *PLoS Pathog* **2009,** 5, e1000645.

11. Gao, C.; Ye, T. H.; Wang, N. Y.; Zeng, X. X.; Zhang, L. D.; Xiong, Y.; You, X. Y.; Xia, Y.; Xu, Y.; Peng, C. T.; Zuo, W. Q.; Wei, Y.; Yu, L. T. Synthesis and Structure Activity Relationships Evaluation of Benzothiazinone Derivatives as Potential Anti-tubercular Agents. *Bioorganic & Medicinal Chemistry Letters* **2013,** 23, 4919-4922.

12. Landge, S.; Mullick, A. B.; Nagalapur, K.; Neres, J.; Subbulakshmi, V.; Murugan, K.; Ghosh, A.; Sadler, C.; Fellows, M. D.; Humnabadkar, V.; Mahadevaswamy, J.; Vachaspati, P.; Sharma, S.; Kaur, P.; Mallya, M.; Rudrapatna, S.; Awasthy, D.;

Sambandamurthy, V. K.; Pojer, F.; Cole, S. T.; Balganesh, T. S.; Ugarkar, B. G.; Balasubramanian, V.; Bandodkar, B. S.; Panda, M.; Ramachandran, V. Discovery of Benzothiazoles as Antimycobacterial Agents: Synthesis, Structure–activity Relationships and Binding Studies with Mycobacterium tuberculosis Decaprenylphosphoryl-β-d-ribose 2′-oxidase. *Bioorganic & Medicinal Chemistry* **2015,** 23, 7694-7710.

13. Makarov, V.; Lechartier, B.; Zhang, M.; Neres, J.; van der Sar, A. M.; Raadsen, S. A.; Hartkoorn, R. C.; Ryabova, O. B.; Vocat, A.; Decosterd, L. A.; Widmer, N.; Buclin, T.; Bitter, W.; Andries, K.; Pojer, F.; Dyson, P. J.; Cole, S. T. Towards a New Combination Therapy for Tuberculosis with Next Generation Benzothiazinones. *EMBO Molecular Medicine* **2014,** 6, 372-283.

14. Naik, M.; Humnabadkar, V.; Tantry, S. J.; Panda, M.; Narayan, A.; Guptha, S.; Panduga, V.; Manjrekar, P.; Jena, L. k.; Koushik, K.; Shanbhag, G.; Jatheendranath, S.; Manjunatha, M. R.; Gorai, G.; Bathula, C.; Rudrapatna, S.; Achar, V.; Sharma, S.; Ambady, A.; Hegde, N.; Mahadevaswamy, J.; Kaur, P.; Sambandamurthy, V. K.; Awasthy, D.; Narayan, C.; Ravishankar, S.; Madhavapeddi, P.; Reddy, J.; Prabhakar, K.

R.; Saralaya, R.; Chatterji, M.; Whiteaker, J.; McLaughlin, B.; Chiarelli, L. R.; Riccardi, G.; Pasca, M. R.; Binda, C.; Neres, J.; Dhar, N.; Signorino-Gelo, F.; McKinney, J. D.; Ramachandran, V.; Shandil, R.; Tommasi, R.; Iyer, P. S.; Narayanan, S.; Hosagrahara, V.; Kavanagh, S.; Dinesh, N.; Ghorpade, S. R. 4-Aminoquinolone Piperidine Amides: Noncovalent Inhibitors of DprE1 with Long Residence Time and Potent Antimycobacterial Activity. *Journal of Medicinal Chemistry* **2014,** 57, 5419-5434.

15. Neres, J.; Hartkoorn, R. C.; Chiarelli, L. R.; Gadupudi, R.; Pasca, M. R.; Mori, G.; Venturelli, A.; Savina, S.; Makarov, V.; Kolly, G. S.; Molteni, E.; Binda, C.; Dhar, N.; Ferrari, S.; Brodin, P.; Delorme, V.; Landry, V.; de Jesus Lopes Ribeiro, A. L.; Farina, D.; Saxena, P.; Pojer, F.; Carta, A.; Luciani, R.; Porta, A.; Zanoni, G.; De Rossi, E.; Costi, M. P.; Riccardi, G.; Cole, S. T. 2-Carboxyquinoxalines Kill Mycobacterium tuberculosis through Noncovalent Inhibition of DprE1. *ACS Chemical Biology* **2015,** 10, 705-714.

16. Panda, M.; Ramachandran, S.; Ramachandran, V.; Shirude, P. S.; Humnabadkar, V.; Nagalapur, K.; Sharma, S.; Kaur, P.; Guptha, S.; Narayan, A.; Mahadevaswamy, J.; Ambady, A.; Hegde, N.; Rudrapatna, S. S.; Hosagrahara, V. P.;

Sambandamurthy, V. K.; Raichurkar, A. Discovery of Pyrazolopyridones as a Novel Class of Noncovalent DprE1 Inhibitor with Potent Anti-Mycobacterial Activity. *Journal of Medicinal Chemistry* **2014,** 57, 4761-4771.

17. Shirude, P. S.; Shandil, R.; Sadler, C.; Naik, M.; Hosagrahara, V.; Hameed, S.; Shinde, V.; Bathula, C.; Humnabadkar, V.; Kumar, N.; Reddy, J.; Panduga, V.; Sharma, S.; Ambady, A.; Hegde, N.; Whiteaker, J.; McLaughlin, R. E.; Gardner, H.; Madhavapeddi, P.; Ramachandran, V.; Kaur, P.; Narayan, A.; Guptha, S.; Awasthy, D.; Narayan, C.; Mahadevaswamy, J.; Vishwas, K. G.; Ahuja, V.; Srivastava, A.; Prabhakar, K. R.; Bharath, S.; Kale, R.; Ramaiah, M.; Choudhury, N. R.; Sambandamurthy, V. K.; Solapure, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Azaindoles: Noncovalent DprE1 Inhibitors from Scaffold Morphing Efforts, Kill Mycobacterium tuberculosis and Are Efficacious in Vivo. *Journal of Medicinal Chemistry* **2013,** 56, 9701-9708.

18. Shirude, P. S.; Shandil, R. K.; Manjunatha, M. R.; Sadler, C.; Panda, M.; Panduga, V.; Reddy, J.; Saralaya, R.; Nanduri, R.; Ambady, A.; Ravishankar, S.; Sambandamurthy, V. K.; Humnabadkar, V.; Jena, L. K.; Suresh, R. S.; Srivastava, A.; Prabhakar, K. R.; Whiteaker, J.; McLaughlin, R. E.; Sharma, S.; Cooper, C. B.; Mdluli,

K.; Butler, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Lead Optimization of 1,4-Azaindoles as Antimycobacterial Agents. *Journal of Medicinal Chemistry* **2014,** 57, 5728-5737.

19. Stanley, S. A.; Grant, S. S.; Kawate, T.; Iwase, N.; Shimizu, M.; Wivagg, C.; Silvis, M.; Kazyanskaya, E.; Aquadro, J.; Golas, A.; Fitzgerald, M.; Dai, H.; Zhang, L.; Hung, D. T. Identification of Novel Inhibitors of *M. tuberculosis* Growth Using Whole Cell Based High-Throughput Screening. *ACS Chemical Biology* **2012,** 7, 1377-1384.

20. Wang, F.; Sambandan, D.; Halder, R.; Wang, J.; Batt, S. M.; Weinrick, B.; Ahmad, I.; Yang, P.; Zhang, Y.; Kim, J.; Hassani, M.; Huszar, S.; Trefzer, C.; Ma, Z.; Kaneko, T.; Mdluli, K. E.; Franzblau, S.; Chatterjee, A. K.; Johnsson, K.; Mikusova, K.; Besra, G. S.; Fütterer, K.; Robbins, S. H.; Barnes, S. W.; Walker, J. R.; Jacobs, W. R.; Schultz, P. G. Identification of a Small Molecule with Activity Against Drug-resistant and Persistent Tuberculosis. *Proceedings of the National Academy of Sciences* **2013,** 110, E2510-E2517.

21. Chikhale, R. V.; Barmade, M. A.; Murumkar, P. R.; Yadav, M. R. Overview of the Development of DprE1 Inhibitors for Combating the Menace of Tuberculosis. *Journal of Medicinal Chemistry* **2018,** 61, 8563-8593.

22. Rogacki, M. K.; Pitta, E.; Balabon, O.; Huss, S.; Lopez-Roman, E. M.; Argyrou, A.; Blanco-Ruano, D.; Cacho, M.; Vande Velde, C. M. L.; Augustyns, K.; Ballell, L.; Barros, D.; Bates, R. H.; Cunningham, F.; Van der Veken, P. Identification and Profiling of Hydantoins—A Novel Class of Potent Antimycobacterial DprE1 Inhibitors. *Journal of Medicinal Chemistry* **2018,** 61, 11221-11249.

23. Oh, S.; Park, Y.; Engelhart, C. A.; Wallach, J. B.; Schnappinger, D.; Arora, K.; Manikkam, M.; Gac, B.; Wang, H.; Murgolo, N.; Olsen, D. B.; Goodwin, M.; Sutphin, M.; Weiner, D. M.; Via, L. E.; Boshoff, H. I. M.; Barry, C. E. Discovery and Structure–Activity-Relationship Study of N-Alkyl-5-hydroxypyrimidinone Carboxamides as Novel Antitubercular Agents Targeting Decaprenylphosphoryl-β-d-ribose 2′-Oxidase. *Journal of Medicinal Chemistry* **2018,** 61, 9952-9965.

24. Tiwari, R.; Moraski, G. C.; Krchňák, V.; Miller, P. A.; Colon-Martinez, M.; Herrero, E.; Oliver, A. G.; Miller, M. J. Thiolates Chemically Induce Redox Activation of BTZ043

and Related Potent Nitroaromatic Anti-Tuberculosis Agents. *Journal of the American Chemical Society* **2013,** 135, 3539-3549.

25. A Single Ascending Dose Study of BTZ043. *ClinicalTrials.gov (accessed Dec. 9, 2019)*.

26. Lupien, A.; Vocat, A.; Foo, C. S.-Y.; Blattes, E.; Gillon, J.-Y.; Makarov, V.; Cole, S. T. Optimized Background Regimen for Treatment of Active Tuberculosis with the Next-Generation Benzothiazinone Macozinone (PBTZ169). *Antimicrobial Agents and Chemotherapy* **2018,** 62, e00840-18.

27. A Phase 1 Study to Evaluate Safety, Tolerability, PK, and PK Interactions of TBA-7371. *ClinicalTrials.gov (accessed Dec. 9, 2019)*.

28. A Phase 1/2 Trial of Multiple Oral Doses of OPC-167832 for Uncomplicated Pulmonary Tuberculosis. *ClinicalTrials.gov (accessed Dec. 9, 2019)* .

29. Ballell, L.; Bates, R. H.; Young, R. J.; Alvarez-Gomez, D.; Alvarez-Ruiz, E.; Barroso, V.; Blanco, D.; Crespo, B.; Escribano, J.; González, R.; Lozano, S.; Huss, S.; Santos-Villarejo, A.; Martín-Plaza, J. J.; Mendoza, A.; Rebollo-Lopez, M. J.; Remuiñan-Blanco, M.; Lavandera, J. L.; Pérez-Herran, E.; Gamo-Benito, F. J.; García-Bustos, J.

F.; Barros, D.; Castro, J. P.; Cammack, N. Fueling Open-Source Drug Discovery: 177 Small-Molecule Leads against Tuberculosis. *ChemMedChem* **2013,** 8, 313-321.

30. Batt, S. M.; Cacho Izquierdo, M.; Castro Pichel, J.; Stubbs, C. J.; Vela-Glez Del Peral, L.; Pérez-Herrán, E.; Dhar, N.; Mouzon, B.; Rees, M.; Hutchinson, J. P.; Young, R. J.; McKinney, J. D.; Barros Aguirre, D.; Ballell, L.; Besra, G. S.; Argyrou, A. Whole Cell Target Engagement Identifies Novel Inhibitors of Mycobacterium tuberculosis Decaprenylphosphoryl-β-d-ribose Oxidase. *ACS Infectious Diseases* **2015,** 1, 615-626.

31. Young, R. J.; Leeson, P. D. Mapping the Efficiency and Physicochemical Trajectories of Successful Optimizations. *Journal of Medicinal Chemistry* **2018**, 61, 15, 6421-6467.

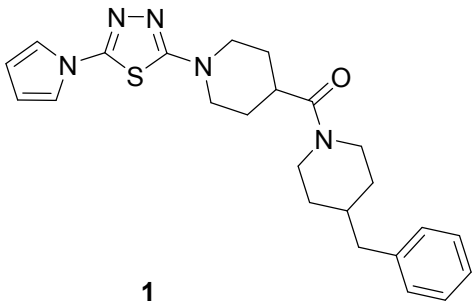
32. St. Jean, D. J.; Fotsch, C. Mitigating Heterocycle Metabolism in Drug Discovery. *Journal of Medicinal Chemistry* **2012,** 55, 6002-6020.

33. Rastogi, N.; Labrousse, V. r.; Goh, K. S. In Vitro Activities of Fourteen Antimicrobial Agents Against Drug Susceptible and Resistant Clinical Isolates of Mycobacterium tuberculosis and Comparative Intracellular Activities Against the Virulent H37Rv Strain in Human Macrophages. *Curr Microbiol* **1996,** 33, 167-175.

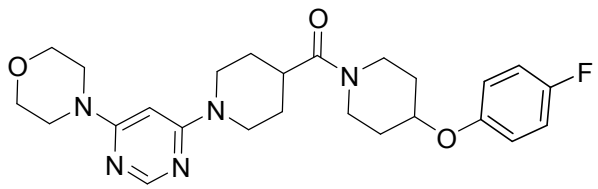
34. Rullas, J.; García, J. I.; Beltrán, M.; Cardona, P. J.; Cáceres, N.; García-Bustos, J. F.; Angulo-Barturen, I. Fast Standardized Therapeutic-Efficacy Assay for Drug Discovery against Tuberculosis. *Antimicrobial Agents and Chemotherapy* **2010,** 54, 2262-2264.

35. Pitta, E.; Rogacki, M. K.; Balabon, O.; Huss, S.; Cunningham, F.; Lopez-Roman, E. M.; Joossens, J.; Augustyns, K.; Ballell, L.; Bates, R. H.; Van der Veken, P. Searching for New Leads for Tuberculosis: Design, Synthesis, and Biological Evaluation of Novel 2-Quinolin-4-yloxyacetamides. *Journal of Medicinal Chemistry* **2016,** 59, 6709-6728.

36. Batt, S. M.; Cacho Izquierdo, M.; Castro Pichel, J.; Stubbs, C. J.; Vela-Glez Del Peral, L.; Pérez-Herrán, E.; Dhar, N.; Mouzon, B.; Rees, M.; Hutchinson, J. P.; Young, R. J.; McKinney, J. D.; Barros Aguirre, D.; Ballell, L.; Besra, G. S.; Argyrou, A. Whole Cell Target Engagement Identifies Novel Inhibitors of Mycobacterium tuberculosis Decaprenylphosphoryl-β-d-ribose Oxidase. *ACS Infectious Diseases* **2015,** 1, 615-626.



1



47

7.3	**DprE1pIC$_{50}$**	7.3
4.0	***Mtb* H37Rv MIC$_{90}$ (μM)**	1.7
9.4	**PFI**	6.7
<1	**CLND solubility (μM)**	≥364