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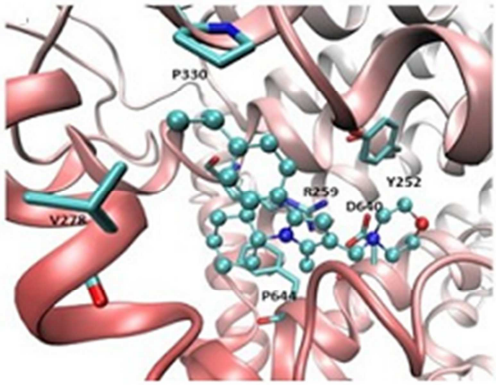

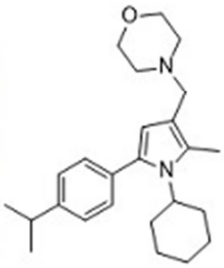

21

MIC H37Rv = 0.3 uM
Cytotox HepG2 IC_{50} = 80 uM
hERG IC_{50} = 50 uM
Cli mouse = 3.98 ml/min/g

In vivo potent BM635 analogue with improved drug-like properties

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Abstract

BM635 is the hit compound of a promising anti-TB compound class. Herein we report systematic variations around the central pyrrole core of **BM635** and we describe the design, synthesis, biological evaluation, pharmacokinetic analysis, as well as *in vivo* TB mouse efficacy studies of novel **BM635** analogues that show improved physicochemical properties. This hit-to-lead campaign led to the identification of a new analogue, 4-((1-isopropyl-5-(4-isopropylphenyl)-2-methyl-1H-pyrrol-3-yl)methyl)morpholine (**17**), that shows excellent activity (MIC = 0.15 μM; SI = 133) against drug-sensitive *Mycobacterium tuberculosis* strains, as well as efficacy in a murine model of TB infection.

Keywords

Tuberculosis, pyrroles, MmpL3, drug discovery, anti-mycobacterials.

[1]

Abbreviations: TB, tuberculosis; WHO, World Health Organization; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant TB; Mtb, *M. tuberculosis*; WGS, whole genome sequencing; MmpL3, mycobacterium membrane protein Large 3; RND, resistance, nodulation and division; TLC, thin layer chromatography; MIC, minimum inhibitory concentration; TFA, trifluoro acetic acid; TEA, trimethylamine; DCM, dichloromethane; DCE, dichloroethane; $TOX_{50,}$ concentration of compound resulting in 50% inhibition; HSA, human serum albumin; CLND, chemiluminescent nitrogen detection; FaSSIF, fasted state simulated intestinal fluid; hERG, human ether-a-go-go-related gene; SAR, structure activity relationship; Vss, volume of distribution in blood.

1. Introduction

Despite the fact that tuberculosis (TB) mortality has fallen by 47% since 1990, TB remains one of the world's deadliest diseases. The World Health Organization (WHO) estimated over 10.4 million new cases and 1.8 million deaths by 2015 [1]. Drug-susceptible TB is treated with a standard 6-months course therapy comprised of a combination of four drugs: rifampicin, isoniazid, pyrazinamide and ethambutol. Unfortunately, standard anti-TB drugs have been used for decades, and resistance to the medicines is widespread. Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid and rifampicin, the two most powerful, first-line (or standard) anti-TB drugs [1,2]. In some cases, more severe drug resistance can develop. Extensively drug-resistant TB (XDR-TB) is a form of multi-drug resistant tuberculosis that responds to even fewer available medicines, including the most effective second-line anti-TB drugs. About 480 000 people developed MDR-TB in the world in 2014, and it is estimated that about 9.7% of MDR-TB cases had XDR-TB [1].

Over the past decades, drug discovery and development efforts have yielded a few new interesting anti-mycobacterial agents, including the imidazo pyridine amide Q203, the nitroimidazole PA-824, the 1,2-diamine SQ-109, and the benzothiazinone BTZ-043. Despite that, only bedaquiline and delamanid have advanced to approval for pulmonary MDR-TB infections when other effective treatment options are not useful [3]. Hence, there is an urgent need to develop more effective and tolerable treatments for both drug-susceptible and drug-resistant TB.

We have previously identified a series of 1,5-diphenyl pyrroles as a highly potent compound class against drug-susceptible *M. tuberculosis* (*Mtb*) strains through phenotypic screening [4]. A standard medicinal chemistry approach led to the identification of **BM635** (Fig. 1), active against both replicating and non-replicating bacilli and proving to be efficacious in a murine model of tuberculosis infection [5–10].

Using whole genome sequencing (WGS) methodology, we also determined the possible target of this class of compounds; point mutations in the *Rv0206c* gene, which encodes for Mycobacterium membrane protein Large 3 (MmpL3), were found [11]. MmpL3 is a member of the Mycobacterial membrane protein Large (MmpL) family that belongs to the Resistance, Nodulation and Division (RND) superfamily and is highly conserved in all the species of mycobacteria and essential for *Mtb* [12–14].

Having proved the potentiality of this compound class, we started a medicinal chemistry campaign in order to improve **BM635**' liabilities: low water solubility and high proportion of

sp^2 centers. Herein we report systematic variations around the central pyrrole core of **BM635** (Fig. 1) and we describe the design, synthesis, biological evaluation, pharmacokinetic analysis, as well as *in vivo* TB mouse efficacy studies of novel **BM635** analogues (compounds **1-45,** Figs 2 and 3) that show improved physicochemical properties. Moreover, in order to gain some understanding on how these pyrroles interact with the MmpL3 transporter, we run a docking analysis using methods previously developed [15].

2. Results and discussion

2.1 Design

The main objectives of the **BM635** optimization program consisted in enhancing drug-like properties, such as lipophilicity and solubility, as well as finding compounds with the right potency/safety balance. For doing so, we followed different approaches like breaking planarity, reducing aromaticity (by introducing either benzyl ring or alkyl substituents) and introducing polarity (pyridines, polar substituents). Therefore, the new analogues were prepared by modifying three main motifs: the N1 phenyl ring (N1Ph), the C5 phenyl ring (C5Ph), and the C3 morpholine moiety (C3M) (Fig. 1). Both N1Ph and C5Ph modifications (compounds **1-42**) consisted in: *i)* removal of the phenyl ring; *ii)* replacement with a benzyl ring; *iii)* replacement with heterocycles; *iv)* replacement of either the fluorine or the isopropyl substituents; *v)* introduction of polar substituents; and *vi)* replacement with alkyl or cycloalkyl substituents (Figs 2 and 3). C3M modifications (compounds **43-45**) included substitution of the morpholine moiety with a 3-substituted piperidine one (Fig. 3).

2.2 Chemistry

Compounds **1-35** and **38-45** were prepared following a straightforward synthetic pathway previously optimized in our group and reported in Scheme 1 [10]. Briefly, 1,4-diketones **48a-k** were obtained by treating the suitable aldehydes **46a-k** with methyl vinyl ketone **47** in a sealed glass tube in the microwave reactor. Microwave assisted cyclization of **48a-k** in the presence of the appropriate amine gave the expected pyrroles **49a-n'** in good yields. Finally, by treating pyrroles **49a-n'** with formaldehyde and the appropriate amine, following Mannich reaction conditions, target compounds **1-35** and **38-45** were obtained. Compounds **36** and **37** were obtained, in turn, by deprotecting **35** with TFA and subsequent reductive methylation of **36** by means of formaldehyde and sodium triacetoxy borohydride ($Na(OAc)_3BH$), respectively (Scheme 2).

2.3 Antimycobacterial activities and cytotoxicities of compounds 1-45

For each compound, we determined the minimum concentration required to inhibit (MIC) *Mtb* growth in culture as well as, for some of them, the activity (expressed as IC_{50}) against intracellular *Mtb* growth in macrophages (THP-1 cells) (Table 1). Moreover, we evaluated compound cytotoxicities by measuring the concentration of compound resulting in 50% inhibition (TOX_{50}) of HepG2 cell line growth, and occasionally the potential for drug-induced human hepatotoxicity using an *in vitro* high-content cell-based assay (Cell Health or CH assay) [16] (Table 1).

Several compounds proved to be active against *Mtb* both in culture and in macrophages with MICs ranging from 0.08 to 0.6 µM (compounds **7**, **9**, **13**, **17-21**, and **29,** Table 1) and IC_{50}s ranging from 0.08 to 0.63 µM (compounds **9**, **13**, **17-21**, and **29,** Table 1). Some of the active compounds showed statistically relevant toxicity against HepG2 cells (Table 1) even though compounds **7**, **13**, **17**, **18**, **20**, **21**, and **29** had good selectivity indexes (Tox_{50}/MIC) ranging from 100 to 333. Moreover, compounds **21** and **29** exhibited initial clean profiles regarding hepatotoxicity (Cell health > 200 µM, Table 1).

Table 1. *In vitro* characterization of compounds **1-45**, **BM635** and isoniazid.

Compound	MIC *Mtb* (µM)	Tox_{50} (µM) HepG2	Selectivity index (Tox_{50}/MIC)	Intracell IC_{50} *Mtb* (µM)	Cell health[a]
1	>5	>100	nc[b]	>10	nd[c]
2	>5	50	nc[b]	>10	nd[c]
3	>125	>100	nc[b]	>10	>200/>200/>200
4	>5	80	nc[b]	>10	nd[c]
5	1.25	80	40	3.3	nd[c]
6	5	>100	nc[b]	>10	>200/>200/>200
7	0.6	>100	167	nd[c]	nd[c]
8	>5	10		nd[c]	nd[c]
9	0.6	20	33	0.6	50/63/80
10	1.25	16	13	2.5	nd[c]
11	>40	>100	nc[b]	nd[c]	nd[c]

12	40	>100	nc[b]	nd[c]	nd[c]
13	0.08	20	250	0.16	nd[c]
14	40	>100	nc[b]	nd[c]	nd[c]
15	12.5	1.6	nc[b]	>10	nd[c]
16	1.25	16	13	1.25	40/40/50
17	0.15	20	133	0.16	25/32/32
18	0.2	20	100	0.08	nd[c]
19	0.3	20	67	0.2	40/40/50
20	0.08	20	250	0.08	25/25/32
21	0.3	>100	>267	0.63	>200/>200/>200
22	1.3	50.1	39	1.32	nd[c]
23	1.3	nd[c]	nc[b]	1.25	32/40/50
24	2.5	40	nc[b]	2.5	50/100/126
25	>40	>100	nc[b]	nd[c]	nd[c]
26	>40	>100	nc[b]	nd[c]	nd[c]
27	2.5	80	nc[b]	2.5	80/80/100
28	2.5	50.1	nc[b]	nd[c]	nd[c]
29	0.15	50	333	0.3	>200/>200/>200
30	>5	>100	nc[b]	5.28	nd[c]
31	5	32	nc[b]	5	40/50/63
32	>40	20	nc[b]	nd[c]	nd[c]
33	>5	20	nc[b]	>10	nd[c]
34	>5	nd[c]	nc[b]	>10	nd[c]
35	30	16	nc[b]	nd[c]	nd[c]
36	>80	16	nc[b]	nd[c]	nd[c]
37	>40	25	nc[b]	nd[c]	nd[c]
38	2.5	nd[c]	nc[b]	nd[c]	nd[c]
39	2.5	nd[c]	nc[b]	nd[c]	nd[c]
40	2.5	20	nc[b]	nd[c]	nd[c]
41	1.25	>100	>167	nd[c]	nd[c]
42	1.25	20	67	nd[c]	nd[c]
43	2.5	10	nc[b]	nd[c]	nd[c]

44	7.5	20	nc[b]	nd[c]	nd[c]
45	2	12.6	nc[b]	nd[c]	nd[c]
BM635	0.12	40	333.34	0.1	>40/>40/ >40
Isoniazid	1.8	-	-	-	-

[a]Nuclear morphology/membrane permeability/mitochondrial potential. nc[b], not calculated. nd[c], not determined.

2.4 SAR analysis

C5Ph modifications: modifications of this position were not well tolerated leading to inactive compounds (**1**-**6** and **8**, Table 1) with the exception of compound **7** which showed a remarkable activity (MIC of 0.6 μM). N1Ph modifications were more tolerated even if limited to: *i)* the replacement of the 4-F-phenyl ring with either a 4-F-pyridin-2-yl or 4-F-benzyl ring (compounds **9** and **13**, respectively, Table 1) and *ii)* the replacement with alkyl, cycloalkyl or hetero cycloalkyl substituents (compounds **17**-**21** and **29**, Table 1). C3M modifications, however, just led to a drastic loss of activity (compounds **43**-**45**, Table1).

2.5 In vitro safety and DMPK profile

To identify the best hits for progression to *in vivo* studies, compounds with MICs <1 μM were evaluated for their *in vitro* safety and DMPK profile. In detail, we determined the hydrophobicity using the chromatography technique to generate Chrom log $D_{pH7.4}$ values, the artificial membrane permeability, the percentage of binding to human serum albumin (HSA), the kinetic solubility *via* chemiluminescent nitrogen detection (CLND), the solubility in the biorelevant medium to simulate the fasted states *in vivo* (FaSSIF), as well as human Ether-a-go-go-related Gene (hERG) binding (Table 2).

The replacement of the isopropyl substituent with a trifluoromethyl one at the *para* position of the C5 phenyl ring as well as the introduction of alkyl or cycloalkyl substituents at N1 gave 0.42-0.89 units reduction of hydrophobicity respect to **BM635** (compounds **7**, **17**, and **19**, Table 2), while the introduction of either a pyridine or a tetrahydropyrane ring led to a 1.25 unit reduction (compounds **9** and **29**, Table 2). The reduction of hydrophobicity led also to a decrease in HSA binding, with compounds **9, 17, 19,** and **29** showing the best values (Table 2). All the tested compounds proved to be highly permeable in the artificial membrane permeability assay with values in the 10^{-5} range (Table 2). Compounds **9**, **13**, **17**-**20** and **29**

proved to be more soluble than **BM635** with CLND values ranging from 11 to 276 µM. Compounds **17, 19** and **29** showed grater values in the FaSSIF medium (249, 511 and 43 µg/ml, respectively). Finally, compounds **7** and **21** proved a clean profile in the hERG activity.

Table 2. Drug-like properties and safety profile of compounds **7, 9, 13, 17-21, 29** and **BM635**.

Compound	Chrom log $D_{pH7.4}$	Membrane permeability cm/sec	%HSA binding	CLND solubility (µM)	FaSSIF solubility (µg/ml)	hERG IC_{50} (µM)
7	7.67	1.5×10^{-5}	98.33	<1	nd[a]	>50
9	6.85	6.4×10^{-5}	94.13	276	nd[a]	8
13	8.2	3.0×10^{-5}	98.47	11	nd[a]	5
17	7.68	7.2×10^{-5}	94.11	199	249	16
18	8.46	4.3×10^{-5}	97.12	47	nd[a]	16
19	7.21	nd[a]	96.49	181	511	8
20	8.13	5.5×10^{-5}	98.12	71	nd[a]	8
21	9.13	2.7×10^{-5}	98.1	<1	nd[a]	50
29	6.86	7.3×10^{-5}	92.85	241	43	10
BM635	8.1	2.4×10^{-5}	98.37	<1	5	10

nd[a], not determined.

2.6 In vivo profiling of best hits

The most promising derivatives, compounds **17** and **29** were chosen to determine their *in vivo* bioavailability as well as their therapeutic efficacy.

Pharmacokinetic profiles of **17** and **29** were evaluated in female C57 mice following intravenous and oral administration. After intravenous administration, **17** showed a moderate mean volume of distribution in blood (Vss), which exceeded near 3-fold the total body water in mouse, while **29** showed a greater volume of distribution (near 6-fold the total body water in mouse). While **29** showed a very high *in vivo* clearance of 125 ml/min/kg, suggesting that it would be rapidly removed from the body, **17** presented a low value of mean clearance, compared to the hepatic blood flow in mouse, and a moderate mean half-life of 1.7 hours

(Table 3). After oral administration, **17** showed a mean C_{max} value of 177 ng/ml while **29** a practically three-fold higher C_{max} value of 548 ng/mL (Table 4). Compound **29** showed a moderate mean bioavailability value of 23 % and compound **17** a very low mean bioavailability of 1.2% (Table 4).

Table 3. Pharmacokinetic parameters of **17** and **29** after intravenous administration to C57 mice.

Compound	Dose (mg/kg)	Vss (L/kg)	AUC_{inf} (ng·h/ml)	$DNAUC_{inf}$ (ng·h/ml per mg/kg)	Clearance (ml/min/kg)	$t_{1/2}$ (h)
17	4	1.8	4024	1006	16.9	1.7
29	1	3.5	138	138	125	0.4

Table 4. Pharmacokinetic parameters of **17** and **29** after oral administration to C57 mice.

Compound	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	Tlast (h)	%F
17	50	0.7	177	7.3	1.2
29	50	0.9	548	8	23

Compounds **17** and **29** were progressed to the *in vivo* acute murine model of *Mtb* infection. Compounds **17** and **29** were orally administered to C57 mice once a day for eight days starting on day 1 after infection. Although **17** showed a very poor bioavailability (Table 4), it induced a statistically significant difference in lung bacterial counts compared to untreated mice (Table 5). Compound **29**, on the other hand, did not inhibit the growth of bacterial burden in the lungs of infected mice (Table 5).

Table 5. Therapeutic efficacy of **17**, **29** and moxifloxacin. Differences in the lung microorganism burden (log10 CFUs/lungs) with respect to untreated controls (Day 9 after infection).

Compound	Dose (mg/kg)	Administration	Difference in logCFU/lungs	P^2
17	50	Once a day	1.5	$p<0.05$
29	50	Once a day	0.1	$p<0.05$
Moxifloxacin	30	Once a day	3.1	$p<0.05$

2.7 Molecular docking of compounds into MmpL3

In order to gain insight regarding the interaction between these derivatives and MmpL3, we run a docking analysis using methods previously reported [15]. The docking analyses started with the parent compound **BM212** [4,11] to verify whether the binding site as optimized in the previous study was also able to properly accommodate the larger pyrrole derivatives. Figure 4 (A) shows the main interactions stabilizing the putative complex between MmpL3 and **BM212** as simulated in its protonated form due to the high basicity of the piperazine ring. Indeed, Figure 4 (A) reveals the key ionic contact between the protonated piperazine nitrogen atom and Asp640, a contact reinforced by the charge transfer interaction with Tyr252. Even though the relevance of Asp640 has recently been confirmed by a mutational study showing that the D640C mutant failed to rescue an *Msmg mmpL3* knockout mutant [15], the concrete role of this ion-pair might be limited by the closeness of Arg259 which interacts with Asp640 and which elicits an extended charge transfer interaction with the pyrrole ring and, to minor extent, with the two phenyl rings. The non-protonated piperazine nitrogen atom is involved in H-bonds with Ser288 and Ser325, while the carbon skeleton of the piperazine ring is engaged in hydrophobic contacts with surrounding apolar side chains (e.g. Ile248 and Val643). Besides the already mentioned charge transfer interaction, the N1 phenyl ring elicits electronic interactions with Phe644 and the chlorine atom stabilizes a halogen bond with Thr280. Similarly, the chlorine atom of the phenyl ring at C5 is engaged in a halogen bond with Thr277, while the aromatic ring contacts only alkyl side chains such as Ile256, Val285, Leu329 and Pro330.

The described complex affords a rather convincing validation of the capacity of the previously optimized binding site to properly accommodate also the pyrrole derivatives and, therefore, the MmpL3 homology model was exploited in the docking simulations involving

the herein reported novel derivatives. When considering these novel compounds in their neutral state, two possible binding modes can be detected. The first binding mode, as here exemplified by the computed complex for **23** (Fig. 4 (B)), is very similar to that already seen for **BM212** with the morpholine ring which replaces the piperazine as seen in **BM212**. In detail, the pyrrole stabilizes charge transfer interaction with Arg259, while the morpholine ring elicits H-bonds with Tyr252, Ser288 and Ser321. The contacts stabilized by the phenyl ring at C5 are largely dominated by the apolar contacts that the para-isopropyl group can afford with the surrounding hydrophobic residues such as Ile256, Val278, Leu329, Pro330 and Leu333. Finally, the N-linked methoxy butyl chain stabilizes H-bonds with Thr280 reinforced by hydrophobic contacts with Ala281 and Ile256.

In the second observed binding mode, as exemplified by the putative complex for **13** (Fig. 4 (C)), the isopropyl phenyl moiety replaces the morpholine or the piperazine ring as seen in the previous complexes and markedly reinforces the charge transfer interactions which the bound molecule can stabilize with Arg259. Moreover, **13** elicits extended π-π stacking contacts, which involve the phenyl moiety with Tyr252 as well as the benzyl group with Phe644, while the *para*-isopropyl group contacts Ile248 and Val643. Finally, the morpholine ring elicits H-bonds with Thr277 reinforced by apolar contacts with Leu329, Pro330 and Leu333. When analyzing the best poses assumed by all simulated ligands, one may observe that all ligands can assume both binding modes even though with different relevance in terms of both docking scores and relative abundance. As a rule, the relevance of the second binding mode depends on the ligand's capacity to stabilize extended charge transfer and π-π stacking contacts with the Arg259-Asp640 interacting dyad and the surrounding aromatic residues. Interestingly, such a second mode appears to be the prevailing one for several potent compounds. For example, the binding mode 2 is the preferred one for 13 out of the 42 simulated analogues when considering the PLP score function, and the compounds preferring binding mode 2 show at most a MIC value of 2.5 μM.

Notably, the complexes computed when considering the inhibitors in their protonated state reveal the same binding modes already seen in the neutral forms even though with different frequency. For example, Fig. 4 (D) shows the first binding mode as assumed by **21** in its protonated state and reveals a pattern of interactions already seen for neutral ligands further reinforced by the clear ion pair between the protonated morpholine ring and Asp640. Nevertheless, the capacity to yield such a salt bridge does not vastly alter the relative abundance of the two possible binding modes. The number of compounds preferring the binding mode 2 increases compared to what was observed for neutral inhibitors, since 18

protonated forms out of 42 show a prevailing binding mode 2, thus confirming that the ion-pair stabilized by the protonated morpholine ring has a very limited role. In addition, several potent inhibitors preferentially assume the second binding mode, and indeed the compounds with a prevailing binding mode 2 show a MIC value lower than 5 µM. A clear example of potent inhibitor preferring the second binding mode is offered by **13** in which the protonated morpholine ring stabilizes a reinforced H-bond with Ser325 (complex not shown).

Regardless of the ionized forms and different binding modes, the obtained docking results allow for some general considerations. Firstly, the hydrophobic contacts appear to play a dominant role: this result is in line with what was observed in the previous study[LIT] and is confirmed by the following correlative study in which the best relationships are afforded by docking scores parameterizing for apolar contacts (see Supporting Information, Table S1), while scores encoding for ionic interactions do not yield interesting results. This finding can be justified by considering that the explored binding site is lined by ion-pairs (*i.e.*, Arg259-Asp640 and Arg648-Glu263) so, that an ionized ligand cannot stabilize clear ionic interactions without being also repelled. This can explain the limited effect of protonated morpholine rings and can rationalize why a second ionizable group in the moiety linked to the nitrogen atom of the pyrrolidine ring has always a detrimental role (as seen for **11** or **36**). Even though the aromatic rings of the ligand can engage in charge transfer interactions, the *N*-linked aromatic ring can be conveniently replaced by an aliphatic (cyclic or acyclic) moiety (as seen for **29**), a result which is explainable considering the richness of alkyl side chains flanking the binding site, while only one aromatic residue (Phe644) can contact this ligand portion. The presence of some H-bonding residues can explain the positive role of the ligand's halogen atoms, which can be involved in halogen bonding while contributing to the ligand apolarity.

Correlative studies (see Supporting Information) confirmed the relevance of the second binding mode and suggested that both ionized and neutral forms can be involved in ligand recognition with a relative weight which is related to their nucleophilicity. Overall, the encouraging developed equations represent a mutual validation of both the MmpL3 homology model and docking results.

3. Conclusion

1,5-Diphenyl pyrroles were previously identified as a class of anti-mycobacterial compounds endowed with *in vivo* efficacy within the range of commonly employed tuberculosis drugs. Herein we presented our medicinal chemistry efforts aiming at improving

the physicochemical properties and drug-likeness of this series while retaining their anti-mycobacterial activity. We have designed, synthesized, and biologically evaluated a series of 45 **BM635** analogues. Some of the new analogues showed improved physicochemical properties and drug-likeness respect to the hit compound with good artificial membrane permeability, high water solubility, no interaction with hERG, as well as excellent anti-mycobacterial activity.

The best compound in the series was derivative **17**, which showed both good intracellular and extracellular anti-mycobacterial activities together with a good drug-like profile. Additionally, compound **17** induced a statistically significant difference in lung bacterial counts compared to untreated controls.

Finally, a putative homology model for the MmpL3 transporter has been used for docking studies providing valuable further insights into the SAR of the compound series discussed.

4. Experimental section

4.1 Synthesis

Reagents and solvents were obtained from commercial sources (Fluka, Sigma-Aldrich, Alfa Aesar). All reactions were carried out under normal atmosphere with magnetic stirring. Microwave-assisted reactions were performed using a focused microwave reactor (Discover, CEM Corporation, Matthews, NC, USA). Analytical thin layer chromatography (TLC) was performed on Merck silica gel (60F254) pre-coated plates (0.25 mm). The compounds were visualized under UV light (254 nm) and/or stained with a relevant reagent. Flash column chromatography was performed on silica gel with pore size 60 Å, 230−400 mesh particle size, and 40−63 μm particle size, with the indicated solvents. The yields refer to purified products, and were not optimized. All solid compounds were obtained as amorphous solids, and melting points were not measured. ^{1}H NMR spectra were recorded on a Bruker Avance III NMR spectrometer and a Bruker DPX Avance 400 MHz spectrometer equipped with a QNP probe and are reported in ppm using tetramethylsilane an internal standard. ^{13}C NMR spectra were recorded on a Bruker Avance III NMR spectrometer at 295 K and are reported in ppm using solvent as an internal standard (DMSO-d_6 at 39.5 ppm; $CDCl_3$ at 77.0 ppm). Mass spectra data and high resolution mass measurements were performed on a VG-Analytical Autospec Q mass spectrometer. Analytical purity was ≥95% unless stated otherwise. The purities of the final compounds were checked using a Waters ZQ2000 coupled with LC Waters 2795 and Waters 2996 PDA detector. All mass spectra were performed using electrospray ionization.

4.1.1 General procedure for the preparation of pentane-1,4-diones 48a-k.

Compound **48a** was commercially available. Pentanediones **48b-k** were prepared as following. In a sealed glass tube equipped with a stirring bar, aldehydes **46b-k** (0.09 mol), triethylamine (19.5 mL, 0.14mol), methyl vinyl ketone **47** (0.09 mol), and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (3.53 g, 0.014 mol) were mixed together. The flask was heated in the cavity of the microwave reactor for 15 min (150W, internal temperature 70 °C, and internal pressure 60 psi). At the end, the obtained crude residue was stirred with 10 ml of 2N HCl for 30 min. After extraction with ethyl acetate, the organic layers were washed with aqueous sodium bicarbonate and brine. The organic fractions were dried over Na_2SO_4, filtered, and concentrated to give a crude orange liquid. Chromatography on aluminum oxide (activity II–III, according to Brockmann) (cyclohexane/ethyl acetate, 3:1 v/v) gave the desired **48b-k.**

4.1.2 General procedure for the preparation of pyrroles 49a-n'.

The proper 1,4-pentanedione **48** (2.28 mmol) and the suitable amine (2.28 mmol) were dissolved in ethanol (2 ml) in a sealed glass tube equipped with a stirring bar in the presence of *p*-toluenesulfonic acid (30 mg, 0.17 mmol). The tube was heated in the cavity of the microwave reactor for 30 min (150W, internal temperature 160 °C, and internal pressure 150 psi). At the end, the reaction mixture was cooled down and concentrated. The crude material was purified by chromatography on aluminum oxide (activity II–III, according to Brockmann) with cyclohexane to give the expected pyrroles **49a-n'** as solids in satisfactory yields.

4.1.3 General procedure for the preparation of compounds 1-35 and **38-45**.

To a stirred solution of the appropriate pyrrole **49** (5.6 mmol) in acetonitrile (20 ml), a mixture of the appropriate amine (0.57 g, 5.6 mmol), formaldehyde (0.18 g, 5.6 mmol) (40% in water), and 5 ml of glacial acetic acid was added drop-wise in 5 min. Following addition, the mixture was stirred at room temperature for 1 h and then treated with a solution of sodium hydroxide (20%, w/v) and extracted with ethyl acetate. The organic extracts were combined, washed with brine, and dried over Na_2SO_4. The obtained residue after solvent evaporation was purified by column chromatography, using silica gel and petroleum ether/ethyl acetate (3:1 v/v) to give **1-35** and **38-45** as solids in satisfactory yields.

4.1.3.1 4-((1-(4-Fluorophenyl)-2,5-dimethyl-1*H*-pyrrol-3-yl)methyl)morpholine (1). Yellow oil (yield 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.15 (m, 4H), 5.91 (s, 1H), 3.73 (t, 4H, *J*= 4.8 Hz), 3.36 (s, 2H), 2.48 (m, 4H), 1.99 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (400

MHz, $CDCl_3$): δ ppm= 159.8, 134.8, 129.6, 126.8, 122.5, 121.9, 116.8, 111.5, 67.1, 56.2, 55.3, 12.9, 10.2. MS-ESI: m/z 289 ($M + H^+$).

4.1.3.2 4-((5-Cyclohexyl-1-(4-fluorophenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (2). White cristals (yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.15 (m, 4H), 5.90 (s, 1H), 3.74 (t, 4H, *J*= 4.7 Hz), 3.38 (s, 2H), 2.48 (m, 4H), 2.19 (tt, 1H, *J*= 11.6, 3.3 Hz), 1.91 (s, 3H), 1.74-1.58 (m, 6H), 1.29 (m, 2H), 1.09 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 159.6, 134.5, 132.1, 125.7, 121.5, 120.9, 115.8, 110.8, 66.5, 55.9, 54.6, 34.3, 33.2, 25.8, 24.9, 10.2. MS-ESI: m/z 357 ($M + H^+$).

4.1.3.3 4-((5-(5-Chloropyridin-2-yl)-1-(4-fluorophenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (3). White solid (yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 8.26 (d, 1H, *J*= 2.8 Hz), 7.37 (dd, 1H, *J*= 8.6, 2.8 Hz), 7.13 (m, 4H), 7.07 (d, 1H, *J*= 8. 6 Hz), 6.72 (s, 1H), 3.73 (t, 4H, *J*= 4.2 Hz), 3.43 (s, 2H), 2.51 (m, 4H), 2.05 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 159.8, 153.9, 151.2, 146.5, 135.9, 132.7, 129.8, 125.9, 124.2, 121.9, 121.6, 116.2, 110.8, 66.8, 56.6, 55.8, 10.4. MS-ESI: m/z 386 ($M + H^+$).

4.1.3.4 4-((5-(6-Chloropyridin-3-yl)-1-(4-fluorophenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (4). White powder (yield 41%). ^{1}HNMR (400 MHz, $CDCl_3$): δ ppm= 8.10 (d, 1H, *J*= 2.5 Hz), 7.19 (dd, 1H, *J*= 8.3, 2.5 Hz), 7.11 (m, 5H), 6.44 (s, 1H), 3.75 (t, 4H, *J*= 4.3 Hz), 3.43 (s, 2H), 2.52 (m, 4H), 2.08 (s, 3H). ^{13}CNMR (400 MHz, $CDCl_3$): δ ppm= 159.5, 153.6, 150.2, 146.3, 143.5, 132.0, 129.4, 125.4, 121.8, 121.2, 120.9, 115.8, 110.3, 66.3, 56.2, 55.1, 10.0. MS-ESI: m/z 387 ($M + H^+$).

4.1.3.5 *N*-(4-(1-(4-fluorophenyl)-5-methyl-4-(morpholinomethyl)-1*H*-pyrrol-2-yl)phenyl)acetamide (5). White solid (yield 22%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.29 (d, 2H, *J*= 8.4 Hz), 7.10 (m, 4H), 6.98 (d, 2H, *J*= 8.4 Hz), 6.33 (s, 1H), 3.75 (s broad, 4H), 3.44 (s, 2H), 2.54 (s broad, 4H), 2.14 (s, 3H), 2.07 (s, 3H). ^{13}CNMR (400 MHz, $CDCl_3$): δ ppm= 168.9, 159.8, 146.7, 138.2, 135.8, 134.7, 128.2, 126.1, 122.0, 121.5, 116.3, 113.3, 66.9, 56.4, 55.8, 23.8, 10.6. MS-ESI: m/z 408 ($M + H^+$).

4.1.3.6 4-((5-(4-Chloro-2-methylphenyl)-1-(4-fluorophenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (6). White solid (yield 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.08 (d, 1H, *J*= 2.3 Hz), 6.98-6.92 (m, 6H), 6.91 (d, 1H, *J*= 8.6 Hz), 6.15 (s, 1H), 3.75 (m, 4H), 3.46 (s, 2H), 2.54 (m, 4H), 2.11 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 159.5, 146.3, 137.6, 134.5, 133.8, 130.1, 128.3, 127.4, 126.1, 125.4, 121.6, 121.0, 115.9, 112.9, 66.6, 56.0, 55.4, 17.8, 9.8. MS-ESI: m/z 399 ($M + H^+$).

4.1.3.7 4-((1-(4-Fluorophenyl)-2-methyl-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrrol-3-yl)methyl)morpholine (7). White solid (yield 67%). ^{1}HNMR (400 MHz, $CDCl_3$): δ ppm=

7.39 (d, 2H, *J*= 7.5 Hz), 7.11 (m, 6H), 6.47 (s, 1H), 3.75 (m, 4H), 3.44 (s, 2H), 2.53 (m, 4H), 2.09 (s, 3H). ^{13}CNMR (400 MHz, $CDCl_3$): δ ppm= 159.9, 146.8, 143.2, 134.8, 131.3, 126.8, 126.1, 124.0, 121.9, 121.4, 116.6, 113.5, 66.8, 56.5, 55.7, 10.5. MS-ESI: m/z 419 ($M + H^+$).

4.1.3.8 4-((5-Benzyl-1-(4-fluorophenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (8).Yellow oil (yield 46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.25-7.08 (m, 7H), 6.89 (m, 2H), 5.73 (s, 1H), 3.64 (s, 2H), 3.53 (m, 4H), 3.22 (s, 2H), 2.33 (m, 4H), 1.86 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 159.5, 146.4, 138.0, 134.2, 129.0, 128.4, 126.8, 125.3, 121.9, 121.0, 116.0, 113.0, 66.4, 56.0, 55.3, 32.1, 10.1. MS-ESI: m/z 365 ($M + H^+$).

4.1.3.9 4-((1-(5-Fluoropyridin-2-yl)-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (9) White solid (yield 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 8.44 (d, 1H, *J*= 3.1 Hz), 7.34 (dd, 1H, *J*= 8.7, 3.1 Hz), 7.01 (m, 2H, *J*= 8.7, 3.1 Hz), 6.92 (m, 3H), 6.32 (s, 1H), 3.73 (t, 4H, *J*= 4.0 Hz), 3.43 (s, 2H), 2.82 (sept, 1H, *J*= 7.1 Hz), 2.51 (s broad, 4H), 2.16 (s, 3H), 1.20 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 158.3, 154.9, 148.6, 137.2, 135.1, 133.8, 128.5, 126.0, 125.3, 121.6, 120.2, 115.8, 111.3, 66.9, 57.4, 56.2, 33.7, 23.5, 10.2. MS-ESI: m/z 394 ($M + H^+$).

4.1.3.10 4-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)-*N*,*N*-dimethylaniline (10).White solid (yield 48%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 6.98 (m, 6H), 6.69 (m, 2H), 6.18 (s, 1H), 3.55 (t, 4H, *J*= 4.2 Hz), 3.29 (s, 2H), 2.91 (s, 6H), 2.76 (sept, 1H, *J*= 7.1 Hz), 2.37 (m, 4H), 1.93 (s, 3H), 1.11 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.4, 147.2, 140.2, 136.8, 134.3, 128.3, 125.8, 125.2, 121.5, 117.2, 113.3, 112.1, 66.6, 56.3, 55.8, 41.5, 33.1, 23.1, 10.1. MS-ESI: m/z 418 ($M + H^+$).

4.1.3.11 4-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)benzoic acid (11). White solid (yield 32%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.98 (d, 2H, *J*= 8.3 Hz), 7.11 (d, 2H, *J*= 8.3 Hz), 6.95 (m, 4H), 6.26 (s, 1H), 3.95 (m, 4H), 3.88 (s, 2H), 3.09 (m, 4H), 2.77 (sept, 1H, *J*= 6.8 Hz), 2.02 (s, 3H), 1.17 (d, 6H, *J*= 6.8 Hz). ^{13}CNMR (400 MHz, $CDCl_3$): δ ppm= 169.6, 156.2, 148.2, 137.1, 134.2, 130.9, 129.6, 128.1, 127.2, 125.8, 125.0, 121.7, 113.5, 66.8, 56.5, 55.9, 33.6, 23.4, 10.5. MS-ESI: m/z 419 ($M + H^+$).

4.1.3.12 3-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)benzoic acid (12). White solid (yield 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 12.50 (s broad, 1H), 8.05 (t, 1H, 2.5 Hz), 8.00 (dt, 1H, *J*= 7.8 Hz, 2.5 Hz), 7.39 (t, 1H, *J*= 7.8 Hz), 7.22 (dt, 1H, *J*= 7.8 Hz, 2.5 Hz), 6.91 (d, 2H, *J*= 8.3 Hz), 6.82 (d, 2H, *J*= 8.3 Hz), 6.47(s, 1H), 4.36 (m, 2H), 4.16 (s broad, 2H), 4.02 (m, 2H), 3.58 (m, 2H), 3.00 (s broad, 2H), 2.78 (sept, 1H, *J*= 7.1 Hz), 2.19 (s, 3H), 1.16 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, DMSO-d_6):

δ ppm= 166.7, 148.3, 141.7, 136.9, 134.0, 130.5, 129.0, 127.9, 126.8, 126.5, 125.4, 125.0, 121.2, 113.1, 107.2, 66.5, 56.1, 55.3, 33.0, 23.1, 10.2. MS-ESI: m/z 419 ($M + H^+$).

4.1.3.13 2-Methyl-3-[(morpholino)-methyl]-5-[4-(*i*-propyl)phenyl]-1-(4-fluorobenzyl)-1*H*-pyrrole (13). White solid (yield 36%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.18-7.10 (m, 6H), 6.84 (m, 2H), 6.06 (s, 1H), 5.11 (s, 2H), 3.54 (t, 4H, *J*= 4.2 Hz), 3.29 (s, 2H), 2.84 (sept, 1H, *J*= 7.1 Hz), 2.34 (m, 4H), 1.99 (s, 3H), 1.16 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 160.2, 148.6, 136.9, 134.5, 133.2, 128.9, 128.3, 126.2, 125.7, 121.5, 115.6, 113.6, 66.8, 56.7, 55.9, 47.1, 33.5, 23.4, 10.4. MS-ESI: m/z 407 ($M + H^+$).

4.1.3.14 4-((5-(4-Isopropylphenyl)-2-methyl-1-(pyrimidin-2-yl)-1*H*-pyrrol-3-yl)methyl)morpholine (14). Yellow syrup (yield 13%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 8.70 (d, 2H, *J*= 4.9 Hz), 7.19 (t, 1H, *J*= 4.9 Hz), 6.92 (d, 2H, *J*= 8.2 Hz), 6.6.90 (d, 2H, *J*= 8.2 Hz), 6.33 (s,1H), 3.71 (t, 4H, *J*= 4.2 Hz), 3.42 (s, 2H), 2.81 (sept, 1H, *J*= 6.9 Hz), 2.50 (m, 4H), 2.27 (s, 3H), 1.19 (d, 6H, *J*= 6.9 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 169.7, 155.8, 148.2, 136.6, 133.5, 128.0, 125.2, 124.7, 118.5, 116.9, 110.6, 66.4, 55.8, 54.9, 32.9, 23.0, 10.0. MS-ESI: m/z 376 ($M + H^+$).

4.1.3.15 4-((5-(4-Isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine formate (15). White solid (yield 15%) ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 8.44 (s, 1H), 8.34 (s broad, 1H), 7.36 (d, 2H, *J*= 8.3), 7.22 (d, 2H, *J*= 8.3 Hz), 6.35 (s, 1H), 3.91 (t, 4H, *J*= 4.7 Hz), 3.87 (s, 2H), 2.97 (m, 4H), 2.90 (sept, 1H, *J*= 7.1 Hz), 2.32 (s, 3H), 1.26 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.4, 133.6, 130.8, 128.3, 127.8, 125.7, 118.5, 108.6, 66.7, 55.8, 55.9, 33.9, 23.6, 11.5. MS-ESI: m/z 345 ($M + H^+$).

4.1.3.16 4-((5-(4-Isopropylphenyl)-1,2-dimethyl-1*H*-pyrrol-3-yl)methyl)morpholine (16). Yellowish solid (yield 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.29 (m, 2H), 7.24 (m, 2H), 6.10 (s, 1H), 3.73 (t, 4H, *J*= 4.3 Hz), 3.51 (s, 3H), 3.41 (s, 2H), 2.93 (sept, 1H, *J*= 6.8 Hz), 2.50 (m, 4H), 2.25 (s, 3H), 1.28 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.2, 143.1, 130.0, 128.0, 127.3, 125.2, 119.1, 110.6, 66.7, 55.6, 55.0, 33.0, 25.6, 23.3, 10.2. MS-ESI: m/z 313 ($M + H^+$).

4.1.3.17 4-((1-Isopropyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (17). Yellow oil (yield 2%) ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.25 (d, 2H, *J*= 8.3 Hz), 7.17 (d, 2H, *J*= 8.3 Hz), 5.80 (s, 1H), 4.42 (sept, 1H, *J*= 7.1 Hz), 3.52 (t, 4H, *J*= 4.2 Hz), 3.21 (s, 2H), 2.89 (sept, 1H, *J*= 7.1 Hz), 2.32 (s broad, 4H), 2.29 (s, 3H), 1.35 (d, 6H, *J*= 7.1 Hz), 1.22 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.4, 143.1, 130.4, 127.9, 127.3, 125.2, 119.1, 111.0, 66.7, 55.7, 55.1, 50.9, 33.0, 23.3, 10.8. MS-ESI: m/z 341 ($M + H^+$).

4.1.3.18 4-((1-Isobutyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (18). Yellow oil (yield 15%). ^{1}H NMR (400 MHz, DMSO- d_6): δ ppm= 7.23 (m, 4H), 5.89 (s, 1H), 3.75 (d, 2H, *J*= 7.8 Hz), 3.52 (t, 4H, *J*= 4.3 Hz), 3.25 (s, 2H), 2.88 (sept, 1H, *J*= 7.1 Hz), 2.31 (m, 4H), 2.18 (s, 3H), 1.57 (sept, 1H, *J*= 7.8 Hz), 1.20 (d, 6H, *J*= 7.1 Hz), 0.54 (d, 6H, *J*= 7.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.6, 143.5, 130.7, 128.7, 127.6, 125.7, 119.6, 111.6, 66.9, 57.9, 56.8, 55.8, 33.4, 30.1, 19.7, 23.3, 10.6. MS-ESI: m/z 355 ($M + H^+$).

4.1.3.19 4-((1-Cyclopropyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (19). Yellow oil (yield 22%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.36 (d, 2H, *J*= 8.1 Hz), 7.20 (d, 2H, *J*= 8.1 Hz), 5.91 (s, 1H), 3.51 (t, 4H, *J*= 4.5 Hz), 3.35 (m, 1H), 3.20 (s, 2H), 2.88 (sept, 1H, *J*= 6.8 Hz), 2.31 (m, 4H), 2.25 (s, 3H), 1.21 (d, 6H, *J*= 6.8 Hz), 0.86 (m, 2H), 0.40 ppm (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.5, 143.3, 130.6, 128.7, 127.8, 125.9, 119.4, 111.6, 66.8, 56.8, 56.0, 33.4, 30.5, 23.4, 10.9, 4.9. MS-ESI: m/z 339 ($M + H^+$).

4.1.3.20 4-((1-Cyclobutyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (20). Yellow oil (yield 24%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.23 (d, 2H, *J*= 8.1 Hz), 7.17 (d, 2H, *J*= 8.1 Hz), 5.80 (s, 1H), 4.76 (quint, 1H, *J*= 8.9 Hz), 3.52 (t, 4H, *J*= 4.2 Hz), 3.21 (s, 2H), 2.89 (sept, 1H, *J*= 6.8 Hz), 2.33 (m, 9H), 2.22 (m, 2H), 1.62 (m, 2H), 1.21 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.4, 142.9, 130.1, 128.1, 127.2, 125.2, 119.2, 110.9, 66.7, 56.1, 55.4, 54.3, 33.0, 31.4, 23.3, 15.9, 11.0. MS-ESI: m/z 353 ($M + H^+$).

4.1.3.21 4-((1-Cyclohexyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (21). Uncolorless oil (yield 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.22 (s, 4H), 5.99 (s, 1H), 4.07 (tt, 1H, *J*= 12.2, 4.0 Hz), 3.71 (t, 4H, *J*= 4.0 Hz), 3.37 (s, 2H), 2.94 (sept, 1H, *J*= 7.1 Hz), 2.47 (m, 4H), 2.38 (s, 3H), 1.99-1.79 (m, 6H), 1.64 (m, 2H), 1.29 (d, 6H, *J*= 7.1 Hz), 1.20 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.5, 143.2, 130.5, 128.3, 127.6, 125.7, 119.4, 111.3, 66.9, 63.8, 56.2, 55.7, 35.2, 33.4, 25.8, 25.0, 3.5, 10.9. MS-ESI: m/z 381 ($M + H^+$).

4.1.3.22 4-((5-(4-Isopropylphenyl)-1-(2-methoxyethyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (22). Yellow oil (yield 26%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.27 (m, 4H), 5.90 (s, 1H), 4.00 (t, 2H, *J*= 6.2 Hz), 3.53 (m, 4H), 3.36 (t, 2H, *J*= 6.2 Hz), 3.24 (s, 2H), 3.09 (s, 3H), 2.89 (sept, 1H, *J*= 6.8 Hz), 2.32 (m, 4H), 2.20 (s, 3H), 1.21 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.2, 142.9, 130.0, 127.7,

126.8, 125.0, 119.1, 110.8, 75.4, 66.7, 58.6, 55.9, 55.5, 47.8, 33.0, 23.3, 10.6. MS-ESI: m/z 357 ($M + H^+$).

4.1.3.23 4-((5-(4-Isopropylphenyl)-1-(3-methoxypropyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (23). Yellow oil (yield 32%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.24 (m, 4H), 5.90 (s, 1H), 3.92 (t, 2H, *J*= 7.3 Hz), 3.52 (t, 4H, *J*= 4.5 Hz), 3.31 (s, 2H), 3.09 (t, 2H, *J*= 5.8 Hz), 3.04 (s, 3H), 2.89 (sept, 1H, *J*= 6.8 Hz), 2.32 (m, 4H), 2.18 (s, 3H), 1.62 (quint, 2H, *J*= 5.8 Hz), 1.27 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.6, 143.3, 130.4, 128.1, 127.5, 125.8, 119.6, 111.3, 70.2, 66.8, 59.5, 56.7, 55.8, 45.8, 33.3, 31.7, 23.4, 10.7. MS-ESI: m/z 371 ($M + H^+$).

4.1.3.24 1-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)-2-methylpropan-2-ol (24). Orange oil (yield 15%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.21 (m, 4H), 5.87 (s, 1H), 4.34 (s, 1H), 3.96 (s broad, 2H), 3.53 (m, 4H), 3.26 (s, 2H), 2.88 (sept, 1H, *J*= 7.1 Hz), 2.32 (m, 4H), 2.25 (s, 3H), 1.20 (d, 6H, *J*= 7.1 Hz), 0.68 (s, 6H). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.7, 143.2, 130.4, 128.5, 127.6, 125.9, 119.7, 111.3, 71.4, 66.7, 56.2, 55.8, 33.4, 27.8, 23.5, 10.7. MS-ESI: m/z 371 ($M + H^+$).

4.1.3.25 3-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)propanoic acid (25). White solid (yield 27%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 12.40 (s broad, 1H), 7.28 (m, 4H), 6.17 (s, 1H), 4.13 (m, 4H), 3.96 (d, 2H, *J*= 12.2 Hz), 3.68 (t, 2H, *J*= 12.2 Hz), 3.05 (m, 2H), 2.93 (sept, 1H, *J*= 6.8 Hz), 2.32 (s, 3H), 1.21 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 171.8, 148.2, 143.0, 130.0, 128.1, 127.3, 125.4, 119.2, 111.0, 66.7, 56.0, 55.4, 45.3, 35.1, 33.2, 23.1, 10.6. MS-ESI: m/z 371 ($M + H^+$).

4.1.3.26 4-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1H-pyrrol-1-yl)butanoic acid (26). White solid (yield 17%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 11.91 (s, 1H), 7.30 (m, 4H), 6.17 (s, 1H), 4.41 (t, 2H, *J*= 6.5 Hz), 4.03 (m, 6H), 3.66 (m, 2H), 2.96 (m, 3H), 2.41 (s, 3H), 2.11 (m, 2H), 1.88 (m, 2H), 1.30 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 178.7, 148.5, 143.6, 130.4, 128.4, 127.7, 125.5, 119.4, 111.1, 66.7, 56.8, 55.9, 48.2, 35.2, 33.5, 25.9, 23.3, 10.7. MS-ESI: m/z 385 ($M + H^+$).

4.1.3.27 4-((5-(4-Isopropylphenyl)-2-methyl-1-(oxetan-3-yl)-1*H*-pyrrol-3-yl)methyl)morpholine (27). Yellow oil (yield 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.22 (d, 2H, *J*= 8.2 Hz), 7.14 (d, 2H, *J*= 8.2 Hz), 6.05 (s, 1H), 5.53 (quint,1H, *J*= 7.5 Hz), 4.82 (t, 2H, *J*= 7.5 Hz), 4.76 (t, 2H, *J*= 7.5 Hz), 3.73 (m, 4H), 3.40 (s broad, 2H), 2.98 (sept, 1H, *J*= 6.8 Hz), 2.51 (m, 4H), 2.38 (s, 3H), 1.27 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.0, 143.1, 130.1, 127.8, 126.9, 125.2, 118.7, 110.6, 79.3, 66.6, 57.1, 55.8, 55.0, 33.0, 23.3, 10.9. MS-ESI: m/z 355 ($M + H^+$).

4.1.3.28 4-((5-(4-Isopropylphenyl)-2-methyl-1-(tetrahydro-2*H*-pyran-4-yl)-1H-pyrrol-3-yl)methyl)morpholine (28). White solid (yield 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.23 (m, 4H), 6.01 (s, 1H), 4.31 (tt, 1H, *J*= 12.0, 4.4 Hz), 4.03 (dd, 2H, *J*= 12.0, 4.4 Hz), 3.73 (s broad, 4H), 3.49 (t, 1H, *J*= 12.0 Hz), 3.34 (m, 4H), 2.96 (sept, 1H, *J*= 7.7 Hz), 2.49 (s broad, 3H), 2.41 (s, 3H), 2.32 (td, 2H, *J*= 12.5, 4.4 Hz), 1.76 (dd, 2H, *J*= 12.5, 2.0 Hz), 1.29 (d, 6H, *J*= 7.7 Hz), 1.21 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 147.5, 142.9, 129.7, 127.5, 126.8, 124.9, 119.0, 110.5, 66.2, 64.9, 55.6, 55.0, 53.8, 32.8, 31.7, 23.0, 10.7. MS-ESI: m/z 383 ($M + H^+$).

4.1.3.29 4-((5-(4-Isopropylphenyl)-2-methyl-1-(tetrahydro-2*H*-pyran-3-yl)-1*H*-pyrrol-3-yl)methyl)morpholine (29). White solid (yield 15%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.27 (d, 2H, *J*= 8.1 Hz), 7.19 (d, 2H, *J*= 8.1 Hz), 5.85 (s, 1H), 4.12 (m, 1H), 3.76-3.69 (m, 3H), 3.42 (m, 1H), 3.52 (t, 4H, *J*= 4.0 Hz), 3.25 (m, 1H), 3.21 (s, 2H), 2.91 (sept, 1H, *J*= 6.8 Hz), 2.31 (s, 6H), 2.15 (qd, 1H, *J*= 13.0, 4.0 HZ), 1.92 (d, 1H, *J*= 13.0 Hz), 1.68 (d, 1H, *J*= 13.0 Hz), 1.48 (m, 1H), 1.22 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.6, 143.3, 130.5, 128.3, 127.7, 125.8, 119.5, 111.5, 75.1, 69.8, 66.7, 58.2, 56.3, 55.6, 33.0, 23.3, 20.5, 10.2. MS-ESI: m/z 383 ($M + H^+$).

4.1.3.30 4-((5-(4-Isopropylphenyl)-2-methyl-1-((tetrahydro-2*H*-pyran-4-yl)methyl)-1*H*-pyrrol-3-yl)methyl)morpholine (30). White solid (yield 28%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.24 (s, 4H), 5.88 (s, 1H), 3.84 (d, 2H, *J*= 7.3 Hz), 3.62 (d, 2H, *J*= 10.9 Hz), 3.52 (m, 4H), 3.24 (s, 2H), 3.01 (t, 2H, *J*= 10.9 Hz), 2.91 (sept, 1H, *J*= 6.8 Hz), 2.31 (s broad, 4H), 2.20 (s, 3H), 1.56 (m, 1H), 1.21 (d, 6H, *J*= 6.8 Hz), 1.09 (d, 2H, *J*= 12.0 Hz), 0.86 (qd, 2H, *J*= 12.0, 4.0 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.2, 143.0, 130.0, 128.1, 127.5, 125.3, 119.2, 111.0, 69.3, 66.7, 56.3, 55.7, 52.1, 33.1, 30.8, 29.1, 23.3, 10.6. MS-ESI: m/z 397 ($M + H^+$).

4.1.3.31 4-((5-(4-Isopropylphenyl)-2-methyl-1-((tetrahydrofuran-2-yl)methyl)-1*H*-pyrrol-3-yl)methyl)morpholine (31). Yellowish oil (yield 25%). ^{1}H NMR (400 MHz, DMSO-d_6): δ= 7.28 (d, 2H, *J*= 8.6 Hz), 7.23 (d, 2H, *J*= 8.6 Hz), 5.89 (s, 1H), 3.93 (d, 2H, *J*= 5.8 Hz), 3.80 (quint, 1H, *J*= 5.8 Hz), 3.52 (m, 6H), 3.24 (s, 2H), 2.88 (sept, 1H, *J*= 6.8 Hz), 2.32 (s broad, 4H), 2.21 (s, 3H), 1.62 (m, 3H), 1.26 (m, 1H), 1.20 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.8, 143.6, 130.8, 128.7, 127.9, 125.8, 119.5, 111.8, 81.7, 68.5, 66.7, 56.6, 55.9, 53.1, 28.4, 33.5, 24.9, 23.8, 10.7. MS-ESI: m/z 383 ($M + H^+$).

4.1.3.32 3-(5-(4-Isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)cyclohexan-1-ol (32). Yellow syrup (yield 9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.23 (m, 4H), 6.16 (s, 1H), 4.62 (m, 1H), 4.23 (m, 1H), 3.70 (s broad, 4H), 3.48 (s, 2H), 2.80-

2.93 (m, 6H), 2.42 (s, 3H), 2.15 (m, 1H), 1.99 (m, 1H), 1.91 (m, 2H), 1.75 (m, 3H), 1.43 (m, 1H), 1.29 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm=148.5, 143.0, 130.1, 127.8, 126.9, 124.8, 118.6, 110.8, 69.0, 66.0, 59.2, 55.6, 55.0, 39.8, 35.6, 34.0, 32.9, 23.0, 16.5, 10.8. MS-ESI: m/z 397 ($M + H^+$).

4.1.3.33 4-((5-(4-Isopropylphenyl)-2-methyl-1-(1-methylpiperidin-4-yl)-1*H*-pyrrol-3-yl)methyl)morpholine (33). Yellow oil (yield 29%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.25 (d, 2H, *J*= 8.1 Hz), 7.17 (d, 2H, *J*= 8.1 Hz), 5.82 (s, 1H), 3.95 (tt, 1H, *J*= 12.0, 4.0 Hz), 3.52 (t, 4H, *J*= 4.0 Hz), 3.34 (s broad, 1H), 2.90 (sept, 1H, *J*= 6.8 Hz), 2.78 (d, 2H, *J*= 12.0 Hz), 2.30 (m, 7H), 2.11 (m, 5H), 1.76 (t, 2H, *J*= 11.8 Hz), 1.69 (d, 2H, *J*= 11.8 Hz), 1.22 (m, 7H). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 148.7, 143.6, 130.9, 128.5, 127.6, 125.8, 119.7, 111.6, 66.9, 56.6, 56.2, 55.8, 53.2, 47.5, 33.8, 30.1, 23.5, 11.0. MS-ESI: m/z 396 ($M + H^+$).

4.1.3.34 *Tert*-butyl-4-(5-(4-isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)piperidine-1-carboxylate (34). Colourless oil (yield 40%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.24 (d, 2H, *J*= 8.1 Hz), 7.18 (d, 2H, *J*= 8.1 Hz), 5.82 (s, 1H), 4.16 (tt, 1H, *J*= 12.0, 4.0 Hz), 3.99 (m, 2H), 3.52 (m, 4H), 3.21 (s broad, 2H), 2.89 (sept, 1H, *J*= 7.1 Hz), 2.66 (m, 2H), 2.32 (m, 4H), 2.25 (s, 3H), 1.85 (m, 2H), 1.72 (m, 2H), 1.36 (m, 9H), 1.21 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 160.4,149.0, 144.2, 130.8, 128.8, 127.5, 126.1, 119.9, 111.8, 80.3, 67.2, 56.7, 56.3, 55.9, 45.4, 33.5, 30.8, 27.3, 24.1, 11.2. MS-ESI: m/z 482 ($M + H^+$).

4.1.3.35 *Tert*-butyl-(*S*)-3-(5-(4-isopropylphenyl)-2-methyl-3-(morpholinomethyl)-1*H*-pyrrol-1-yl)piperidine-1-carboxylate (35). Yellow oil (yield 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.23 (m, 4H), 6.02 (s, 1H), 4.11 (m, 3H), 3.71 (t, 4H, *J*= 4.2 Hz), 3.36 (s, 2H), 3.18 (m, 1H), 2.93 (sept, 1H, *J*= 7.0 Hz), 2.56 (t, 1H, *J*= 12.0 Hz), 2.47 (s broad, 4H), 2.37 (s, 3H), 2.07-1.93 (m, 3H), 1.71 (d, 1H, *J*= 12.0 Hz), 1.40 (s, 9H), 1.27 (d, 6H, *J*= 7.0 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 154.8, 148.8, 143.7, 130.5, 128.6, 127.9, 125.4, 119.8, 110.9, 80.1, 66.7, 58.5, 56.4, 55.5, 52.8, 49.3, 33.4, 31.5, 29.6, 23.5, 22.0, 10.9. MS-ESI: m/z 482 ($M + H^+$).

4.1.3.36 4-((5-(4-isopropylphenyl)-2-methyl-1-(piperidin-3-yl)-1H-pyrrol-3-yl)methyl)morpholine (36) Yellow solid (yield 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.22 (m, 4H), 5.99 (s, 1H), 4.18 (m, 1H), 3.73 (m, 4H), 3.41 (s, 2H), 3.10 (m, 2H), 2.96 (m 3H), 2.52 (s broad, 4H), 2.39 (s, 3H), 2.09-20.2 (m, 2H), 1.68 (m broad, 1H), 1.47 (m, 2H), 1.28 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.5, 143.12, 132.6, 128.7,

127.8, 126.6, 118.5, 111.7, 66.9, 61.2, 55.9, 54.8, 49.8, 48.7, 33.8, 30.12, 23.5, 22.8, 10.8. MS-ESI: m/z 382 ($M + H^+$).

4.1.3.37 4-((5-(4-isopropylphenyl)-2-methyl-1-(1-methylpiperidin-3-yl)-1H-pyrrol-3-yl)methyl)morpholine (37) White solid (yield 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.20 (m, 4H), 5.99 (s, 1H), 4.35 (m, 1H), 3.71 (m, 4H), 3.36 (s, 2H), 2.88 (m, 2H), 2.79 (m 1H), 2.47 (s broad, 4H), 2.39 (s, 3H), 2.26 (s, 3H), 1.91-1.62 (m, 6H), 1.28 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.5, 142.12, 132.6, 128.7, 126.8, 125.6, 118.5, 111.7, 65.9, 61.2, 55.9, 54.8, 49.8, 48.7, 33.8, 30.12, 22.96, 23.5, 22.8, 10.8. MS-ESI: m/z 396 ($M + H^+$).

4.1.3.38 4-((5-(4-Chlorophenyl)-1-isopropyl-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (38) Yellow solid (yield 12%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.34 (m, 2H), 7.24 (m, 2H), 6.00 (s, 1H), 4.47 (sept, 1H, *J*= 7.1 Hz), 3.73 (m, 4H), 3.38 (s broad, 2H), 2.49 (m, 4H), 2.37 (s, 3H), 1.43 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 143.0, 133.6, 129.8, 127.8, 127.5, 126.6, 118.5, 110.7, 66.0, 55.8, 54.9, 49.7, 22.8, 10.8. MS-ESI: m/z 333 ($M + H^+$).

4.1.3.39 4-((1-Isopropyl-5-(4-methoxyphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)morpholine (39) White solid (yield 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm= 7.17 (d, 2H, *J*= 8.6 Hz), 6.94 (d, 2H, *J*= 8.6 Hz), 5.75 (s, 1H), 4.36 (sept, 1H, *J*= 7.1 Hz), 3.76 (s, 3H), 3.52 (t, 4H, *J*= 4.0 Hz), 3.20 (s, 2H), 2.31 (s broad, 4H), 2.28 (s, 3H), 1.32 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm= 161.2, 144.5, 128.7, 127.8, 126.0, 120.1, 115.3, 112.0, 67.1, 56.8, 56.0, 55.3, 51.6, 23.5, 10.9. MS-ESI: m/z 329 ($M + H^+$).

4.1.3.40 4-((1-Isopropyl-2-methyl-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrrol-3-yl)methyl)morpholine (40) Colourless oil (yield 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.61 (d, 2H, *J*= 7.5 Hz), 7.41 (d, 2H, *J*= 7.5 Hz), 6.08 (s, 1H), 4.50 (sept, 1H, *J*= 6.8 Hz), 3.72 (s broad, 4H), 3.36 (s, 2H), 2.47 (s broad, 4H), 2.38 (s, 3H), 1.45 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 142.6, 135.9, 129.2, 126.6, 125.4, 124.8, 123.2, 119.3, 111.5, 65.9, 55.8, 54.7, 49.6, 22.8, 10.7. MS-ESI: m/z 367 ($M + H^+$).

4.1.3.41 4-((1-Cyclohexyl-2-methyl-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrrol-3-yl)methyl)morpholine (41) Colourless oil (yield 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.60 (d, 2H, *J*= 8.1 Hz), 7.39 (d, 2H, *J*= 8.1 Hz), 6.08 (s, 1H), 4.02 (tt, 1H, *J*= 12.0 Hz, 3.8 Hz), 3.72 (t, 4H, *J*= 3.8 Hz), 3.37 (s broad, 2H), 2.47 (m, 4H), 2.40 (s, 3H), 1.98-1.82 (m, 6H), 1.68 (d, 1H, *J*= 11.4 Hz) 1.29-1.17 (m, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm=

138.2, 131.6, 127.9, 127.0, 126.3, 124.1, 120.5, 112.4, 66.9, 64.6, 56.6, 55.8, 35.2, 26.1, 25.3, 11.0. MS-ESI: m/z 407 ($M + H^+$).

4.1.3.42 4-((1-Cyclobutyl-2-methyl-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrrol-3-yl)methyl)morpholine (42) Colourless oil (yield 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.60 (d, 2H, *J*= 8.5 Hz), 7.40 (d, 2H, *J*= 8.5 Hz), 6.06 (s, 1H), 4.79 (quint, 1H, *J*= 8.7 Hz), 3.71 (t, 4H, *J*= 4.0 Hz), 3.37 (s, 2H), 2.48 (m, 4H), 2.41 (s, 3H), 2.38-2.28 (m, 4H), 1.73 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 145.0, 137.4, 132.3, 128.2, 126.8, 125.7, 124.3, 120.6, 112.5, 66.9, 56.8, 55.4, 54.0, 31.8, 16.3, 10.9. MS-ESI: m/z 379 ($M + H^+$).

4.1.3.43 1-((1-(4-Fluorophenyl)-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)piperidin-3-ol (43) Colourless oil (yield 15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.14 (m, 2H), 7.05 (m, 2H), 6.99 (d, 2H, *J*= 7.1 Hz), 6.95 (d, 2H, *J*= 7.1 Hz), 6.28 (s, 1H), 3.85 (s broad, 1H), 3.45 (s, 2H), 2.80 (sept, 1H, *J*= 6.8 Hz), 2.62 (m, 2H), 2.49 (s broad, 1H), 2.28 (m, 1H), 2.05 (s, 3H), 1.83-1.57 (m, 5H), 1.18 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 158.7, 147.2, 145.9, 136.3, 133.1, 127.8, 124.6, 124.2, 121.4, 120.7, 115.4, 112.8, 66.4, 63.8, 55.9, 54.8, 33.0, 29.3, 20.2, 10.1. MS-ESI: m/z 407 ($M + H^+$).

4.1.3.44 1-((1-Isopropyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)piperidin-3-ol (44). Colourless oil (yield 15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.23 (s, 4H), 5.95 (s, 1H), 4.54 (sept, 1H, *J*= 6.8 Hz), 3.91 (s broad, 1H), 3.52 (s, 2H), 2.94 (sept, 1H, *J*= 6.8 Hz), 2.73 (m, 2H), 2.43 (s, 3H), 2.37 (s, 3H), 1.93 (s broad, 1H) 1.60 (s broad, 3H), 1.43 (d, 6H, *J*= 6.8 Hz), 1.28 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 147.9, 142.5, 129.3, 127.4, 126.8, 124.7, 118.5, 110.6, 65.7, 63.6, 56.3, 55.8, 49.4, 32.5, 31.9, 23.3, 23.0, 19.9, 10.7. MS-ESI: m/z 355 ($M + H^+$).

4.1.3.45 1-((1-Isopropyl-5-(4-isopropylphenyl)-2-methyl-1*H*-pyrrol-3-yl)methyl)-3-methoxypiperidine (45). Colourless oil (yield 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.23 (m, 4H), 5.98 (s, 1H), 4.54 (sept, 1H, *J*= 7.1 Hz), 3.43 (s, 2H), 3.35 (s, 3H), 3.32 (m, 1H), 3.05 (m, 1H), 2.93 (sept, 1H, *J*= 7.1 Hz), 2.74 (m, 1H), 2.36 (s, 3H), 1.98-1.74 (m, 3H), 1.71 (m, 2H), 1.50 (m, 1H), 1.44 (dd, 6H, *J*= 7.1, 1.8 Hz Hz), 1.28 (d, 6H, *J*= 7.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.1, 142.9, 129.8, 128.0, 127.2, 125.3, 119.0, 110.9, 76.4, 61.2, 57.2, 56.3, 55.8, 50.1, 33.0, 28.9, 23.0, 22.8, 21.3, 10.7. MS-ESI: m/z 369 ($M + H^+$).

4.1.4 Preparation of compound 36.

To a stirred solution of **35** in dichloromethane (2.5 ml), TFA (2.5 ml) was added and the reaction mixture was stirred at room temperature for 1.5 h. After solvent evaporation, sat. $NaHCO_3$ was added (2.5 ml), the mixture was extracted with dichloromethane, washed with

brine and dried under $MgSO_4$. After solvent evaporation, **36** was obtained in satisfactory yield without further purification.

4.1.4.1 *(S)*-4-((5-(4-isopropylphenyl)-2-methyl-1-(piperidin-3-yl)-1H-pyrrol-3-yl)methyl)morpholine (36). Yellow syrup (yield 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.22 (m, 4H), 5.99 (s, 1H), 4.18 (tt, 1H, *J*= 12.0, 4.0 Hz), 3.73 (t, 4H, *J*= 4.0 Hz), 3.41 (s broad, 2H), 2.97-3.17 (m, 5H), 2.52 (s broad, 4H), 2.39 (s, 3H), 2.06 (m, 2H), 1.70 (s broad, 1H), 1.47 (m, 2H), 1.28 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 147.8, 142.5, 129.6, 127.7, 126.8, 124.9, 118.1, 108.6, 66.0, 60.1, 55.4, 54.0, 50.4, 46.8, 32.8, 3.7, 23.3, 20.9, 13.2. MS-ESI: m/z 382 ($M + H^+$).

4.1.5 Preparation of compound 37.

To a stirred solution of **36** (0.038 mmol) in dichloroethane (5ml), formaldehyde (0.042 mmol) and a drop of acetic acid, sodium (triacetoxy)borohydride was added in one portion at 0° C. The mixture was stirred at room temperature for 15 hours. The reaction was quenched with NaOH 2N (10 ml), extracted with ethyl acetate, washed with brine and dried over $MgSO_4$. After solvent evaporation, **37** was obtained in satisfactory yield without further purification.

4.1.5.1 (*S*)-4-((5-(4-Isopropylphenyl)-2-methyl-1-(1-methylpiperidin-3-yl)-1*H*-pyrrol-3-yl)methyl)morpholine (37). Yellow syrup (yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm= 7.20 (m, 4H), 5.99 (s, 1H), 4.35 (m, 1H), 3.71 (m, 4H), 3.36 (s, 2H), 2.91 (m, 3H), 2.88 (m, 1H), 2.47 (s broad, 4H), 2.38 (s, 3H), 2.26 (s, 3H), 1.91-1.73 (m, 5H), 1.29 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm= 148.0, 142.7, 129.8, 127.6, 126.9, 125.0, 118.9, 110.7, 66.7, 63.2, 6.4, 57.2, 55.8, 55.0, 48.3, 33.2, 30.8, 23.3, 20.9, 10.7. MS-ESI: m/z 396 ($M + H^+$).

4.1.6 Declaration of Purity. All assayed final compounds (**1-45**) were more than 95% pure by UPLC-MS analysis (see Supporting Information).

4.2 Biological assays

4.2.1 Materials and methods

The human biological samples were sourced ethically and their research use was according to the terms of the informed consent. 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. This research was approved by the Comité Etico de Experimentacion Animal de GSK R&D, protocolos number: PROEX 63/14 and PROEX 71/14

714 **4.2.2 MIC determination**

715 The measurement of the minimum inhibitory concentration (MIC) against *M. tuberculosis*
716 strains for each tested compound was performed in 96-well flat-bottom, polystyrene
717 microtiter plates in a final volume of 100 ml. Ten two-fold drug dilutions in neat DMSO
718 starting at 50 mM were performed. Drug solutions were added to Middlebrook 7H9 medium
719 (Difco) and isoniazid (Sigma Aldrich) was used as a positive control with two-fold dilutions
720 of isoniazid starting at 160 mg/ml. The inoculum was standardized to approximately $16e^7$
721 cfu/ml and diluted 1 in 100 in Middlebrook 7H9 broth (Difco). This inoculum (100 ml) was
722 added to the entire plate but G-12 and H-12 wells were used as blank controls. All plates
723 were placed in a sealed box to prevent drying out of the peripheral wells and incubated at 37
724 °C without shaking for six days. A Resazurin solution was prepared by dissolving one tablet
725 of resazurin (Resazurin Tablets for Milk Testing; Ref 330884Y' VWR International Ltd) in
726 30 ml of sterile PBS (phosphate buffered saline). Of this solution, 25 ml were added to each
727 well. Fluorescence was measured (Spectramax M5 Molecular Devices, Excitation 530 nm,
728 Emission 590 nm) after 48 hours to determine the MIC value.

729 **4.2.3 In vivo assay.**

730 Specific pathogen-free, 8-10 week-old female C57BL/6 mice were purchased from Harlan
731 Laboratories and were allowed to acclimate for one week. In brief, mice were intratracheally
732 infected with 100.000 CFU/mouse (*M. tuberculosis* H37Rv strain). Products were
733 administered for 4 consecutive days starting on day five after infection. Lungs were harvested
734 on day nine (24 hours after the last administration). All lung lobes were aseptically removed,
735 homogenized and frozen. Homogenates were plated in 10% OADC-7H11 medium for 14
736 days at 37 ºC.

737 **4.2.4 Docking.**

738 The proposed docking simulations are based on the homology model for the MmpL3 channel
739 previously generated [15]. Briefly, the homology model was developed by an iterative
740 approach which analyzed different suitable templates to finally select the model based on the
741 Multidrug Exporter MEXB (PDB ID 2V50) as the template. The model was then refined and
742 validated by combining molecular docking simulations as reported elsewhere [15]. The
743 considered compounds were simulated in both neutral and ionized forms. Indeed, the weak
744 basicity of the morpholine ring should favor the neutral form at physiological pH although
745 the protonated state cannot be completely excluded depending on the micro-environment of
746 the MmpL3 binding site. In detail, the conformational profile of the simulated compounds
747 was explored by MonteCarlo simulations, which generated 1000 conformers by randomly

rotating the rotors. The so obtained lowest energy geometry was then optimized by PM7 semi-empirical calculations and underwent docking simulations by using PLANTS [17]. In detail, docking simulations were focused on a 10 Å radius sphere around the barycenter of the two interacting residues Agr259-Asp640. For each docking run, 10 poses were generated and scored by using the ChemPLP function with speed equal to 1. All computed complexes were then minimized by keeping fixed all atoms outside a 10 Å radius sphere around a bound inhibitor and finally used to recalculate the scoring functions by using the ReScore+ tool as implemented in the VEGA suite of programs [18]. The so minimized and rescored complexes were then visually inspected in order to extract the best complexes for the two possible binding modes as above detailed. All mentioned minimizations were performed using the conjugate gradient algorithm until the r.m.s. gradient was smaller than 0.01 kcal mol^{-1} $Å^{-1}$. All calculations were carried out by Namd2.10 with the force-field CHARMm v22 and the Gasteiger's atomic charges.

Supplementary data

Additional protocols, synthesis, and experimental properties of intermediate compounds.

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Fig. 1. Chemical structure of **BM635** and design of compounds **1**-**45**.

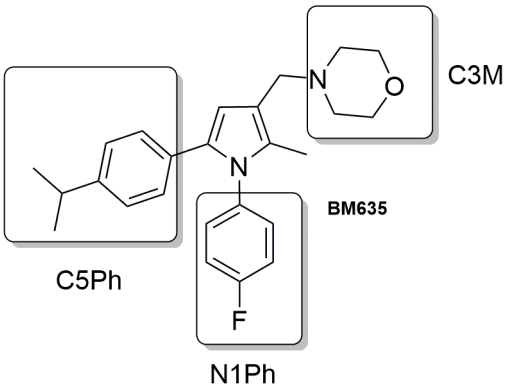
Fig. 2. Chemical structures of compounds **1**-**37**.

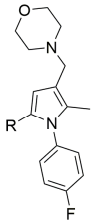
Fig. 3. Chemical structures of compounds **38**-**45**.

Fig. 4. (A) Key interactions stabilizing the computed complexes between the MmpL3 homology model (based on PDB ID 2V50 as reported in ref. 15) and **BM212** in its protonated state as a reference ligand; (B) **23** in its neutral form assuming the proposed binding mode 1; (C) **13** in its neutral form assuming the proposed binding mode 2; (D) **21** in its protonated state assuming the proposed binding mode 1. Notice that usually the alkyl side-chains are not shown for clarity.

Scheme 1. Synthetic pathway for compounds **1-35, 38**-**45**.

Scheme 2. Synthetic pathway for compounds **36** and **37**.





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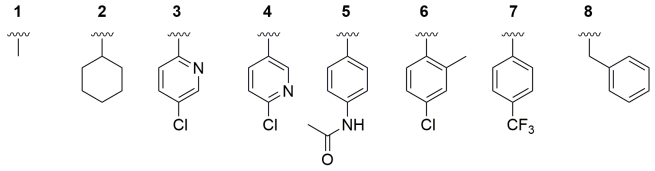

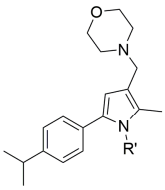

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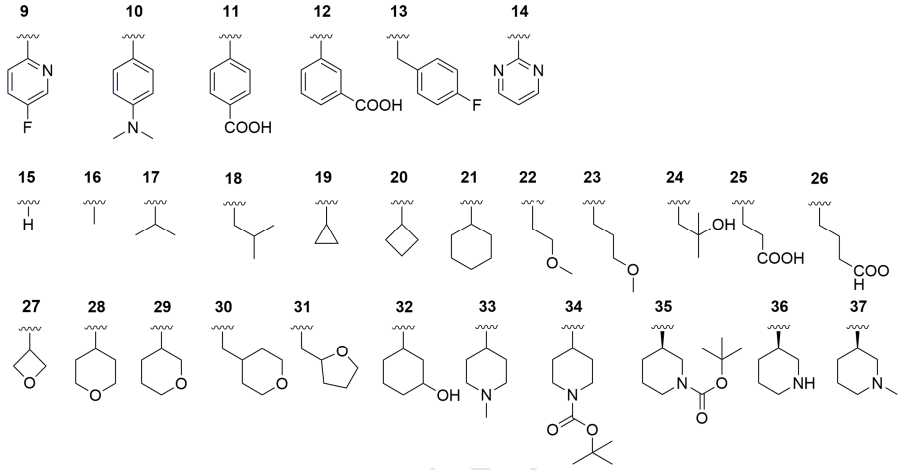
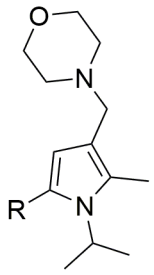
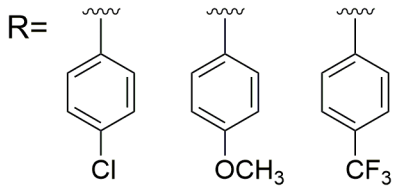
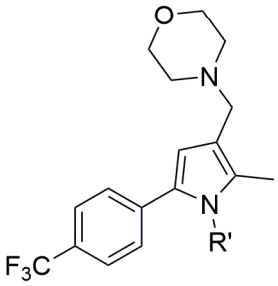




R=

38 **39** **40**





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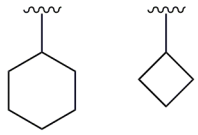
41 **42**



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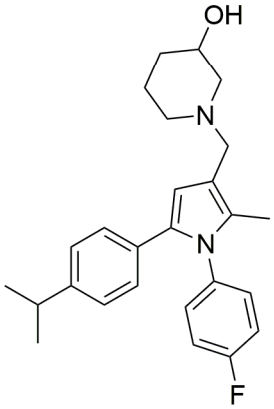

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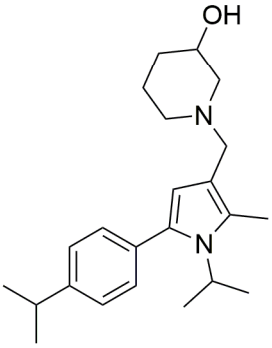

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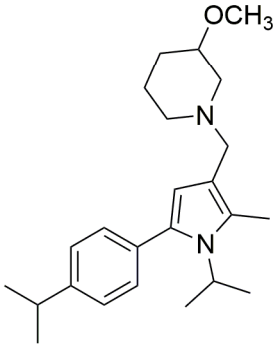
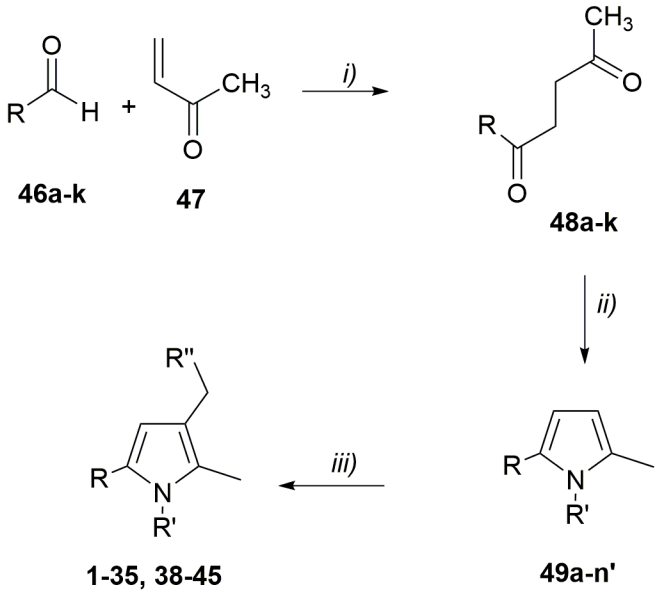
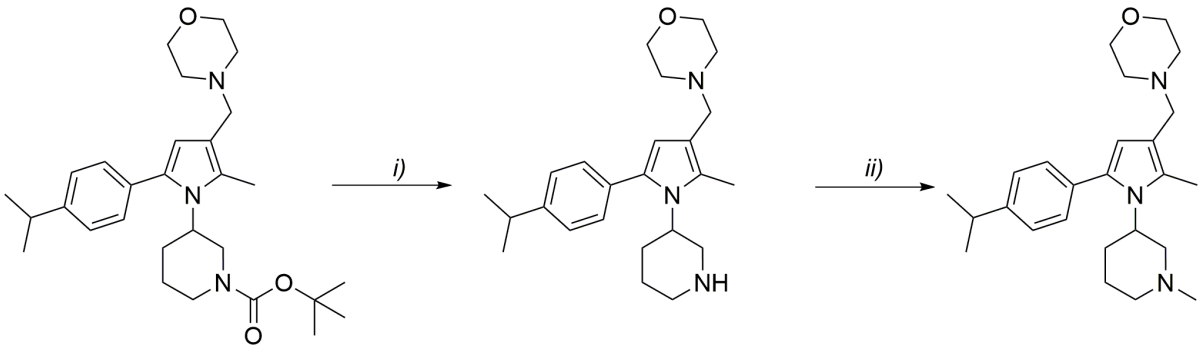




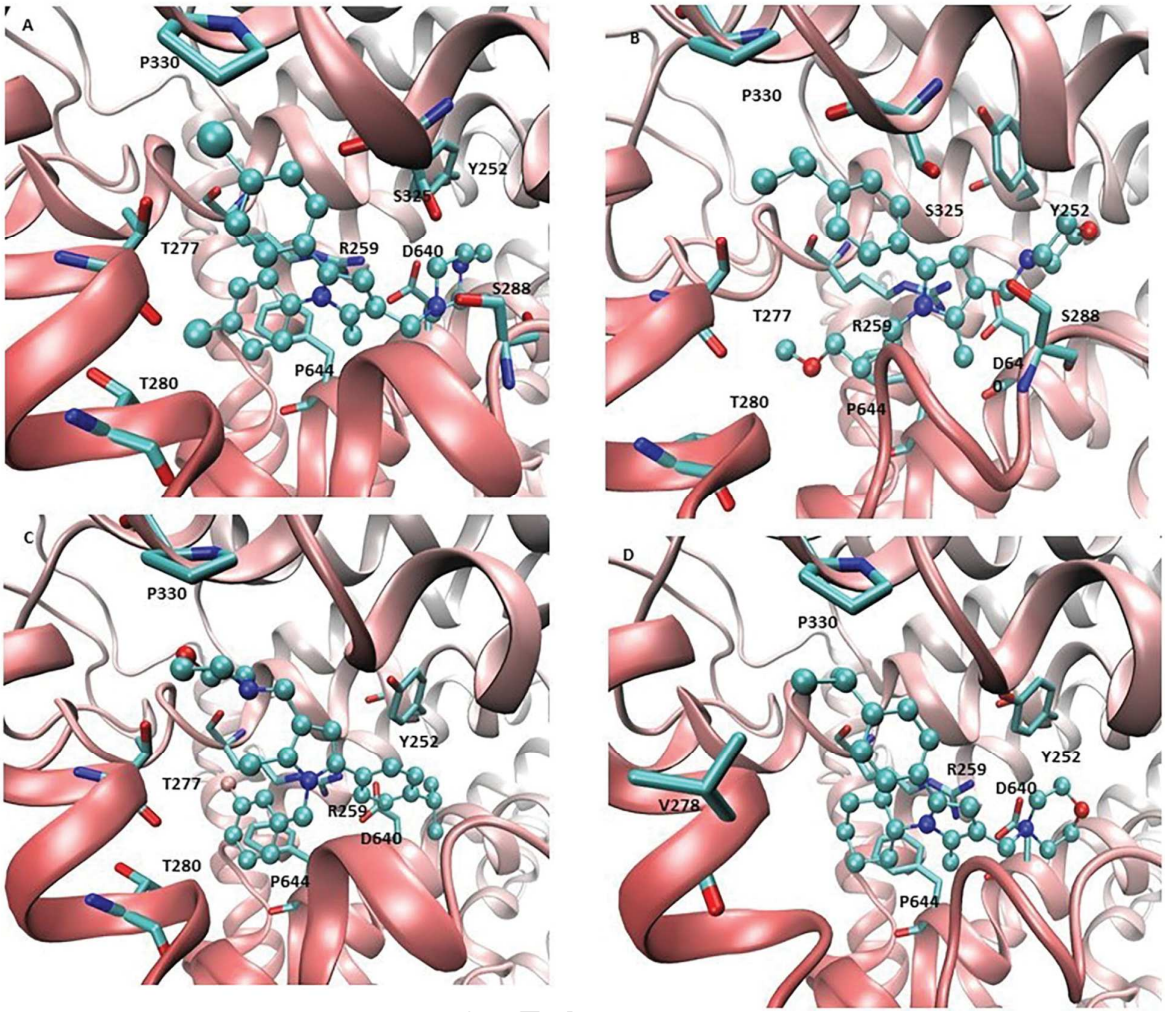
46a-k **47** **48a-k** **49a-n'** **1-35, 38-45**

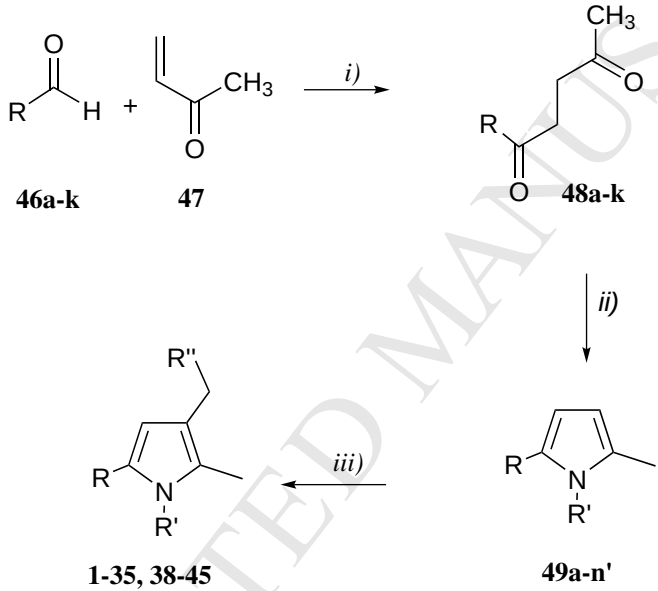
Reagents and conditions: i) 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, TEA, microwave, 15 min; ii) amine, p-toluensulfonic acid, EtOH, microwave, 30 min; iii) amine, CH3CN, HCHO, CH3COOH, room temperature, 1 h.



35 **36** **37**

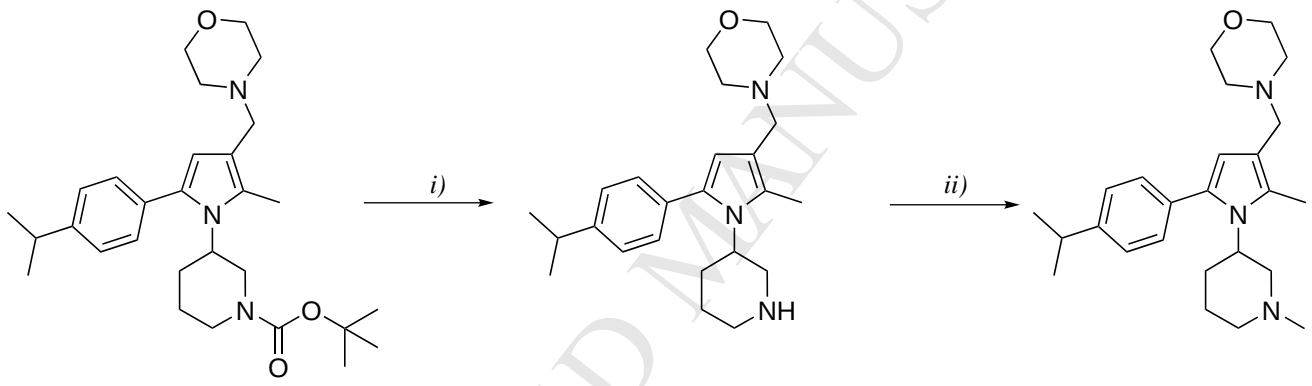
Reagents and conditions: i) TFA, DCM, room temperature, 1,5 h; ii) HCHO, CH3COOH, Na(OAc)3BH, DCE, room temperature, 15 h.





46a-k **47** **48a-k** **49a-n'** **1-35, 38-45**

Reagents and conditions: *i)* 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, TEA, microwave, 15 min; *ii)* amine, *p*-toluensulfonic acid, EtOH, microwave, 30 min; *iii)* amine, CH_3CN, HCHO, CH_3COOH, room temperature, 1 h.



35 **36** **37**

Reagents and conditions: i) TFA, DCM, room temperature, 1,5 h; *ii)* HCHO, CH_3COOH, $Na(OAc)_3BH$, DCE, room temperature, 15 h.

Highlights

- The discovery and development of new medicines is a major keystone for tuberculosis treatment and control.
- BM635 optimization program is proposed herein.
- Improved analog showed significant reduction of lung bacterial counts in infected mice.