Improving the potency of *N*-aryl-2,5-dimethylpyrroles against multidrug-resistant and intracellular mycobacteria.

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Supporting Information

EXPERIMENTAL PART

Homology Model building

The crystal structures of *Mycobacterium smegmatis* MmpL3 in complex with Rimonabant, AU1235 and ICA38 (PDB 6AJI, 6AJH and 6AJJ)[1] were downloaded from the Protein Data Bank and used to develop a homology model for *Mycobacterium tuberculosis* (Mtb) MmpL3.

A 3D model of *Mycobacterium tuberculosis* MmpL3 was constructed using Modeller 9.21.5.[2] The amino acid sequence of MmpL3 was obtained from GenBank and downloaded in a FAST ALL format (FastA). A pairwise alignment of the Mtb MmpL3 sequence and the *M. smegmatis* MmpL3 template was performed using SwissModel.[3] This revealed high sequence identities of 73.2, 74.54 and 74.14% for 6AJH, 6AJI and 6AJJ respectively. The online Basic Local Alignment Search Tool (BLAST) was utilised to search the Protein Databank and did not yield a template with a higher sequence identity.[4] A multiple sequence alignment procedure was utilised with 6AJH, 6AJI and 6AJJ, the crystal structures of MmpL3, as inputs for Modeller to produce 50 protein models.[2]

Comparison of homology models and crystal structures

The quality of the homology models was assessed using stereochemical parameters including Ramachandran plot, G-score and Z-score using ProSA-web and PDBSum.[5] Root-mean-squared deviation (RMSD) of the predicted protein model was calculated in comparison with the template protein structure, 6AJH used as the template.

Binding Site Refinement

The side-chains positions of the ligand binding site residues in model_28 were refined by docking BM212, AU128 and SQ109 using the Induced Fit Docking (IFD) module of Schrodinger.[6] The docking site box was set at 28 x 28 x 28 angstroms centred around Tyr640. Tyr640 is an important residue involved in the electrochemical proton gradient and in ligand interactions at the centre of the active site. A maximum of 20 poses per ligand were created and the 10 best poses were selected. The van der Waals (vdW) radii and partial atomic charges of the ligand and receptor were set at 0.5. The ligands were then redocked into the new receptor conformations using the standard vdW radii and charge scaling (1.0 receptor, 0.8 ligand).

Homology modelling

Figure S1 displays the top homology models superimposed together. These models cover a wide range of the potential conformations available to the protein. Not surprisingly, the loops display a wider degree of variation compared with the transmembrane region. The significant conformational space that is covered by the MmpL3 models is beneficial for exploring ligand binding while maintaining a similar fold to the template protein is beneficial in its application for docking studies.

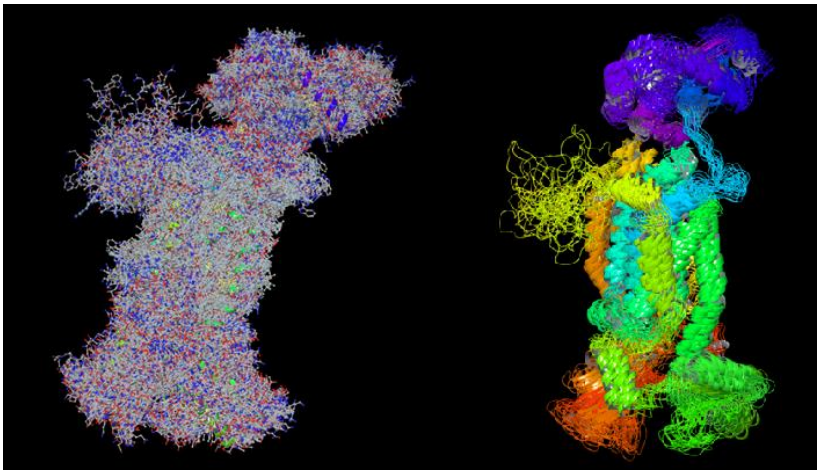


Figure S1. Superimposition of the top MmpL3 *Mycobacterium tuberculosis* models

Ramachandran plot score, G-score, Z-score and RMSD were calculated using the PDBsum tool, the Maestro software package and ProSA-web for the models obtained with the results shown in Table S1.[1] The top models had residues in the most favoured region, additionally allowed, generally allowed and disallowed regions in the range of 83.3-94.3%, 5.5-12.5%, 0-3.4% and 0.3-1.8% respectively. The model with the best stereo-chemical quality, model_17, was assessed using a Ramachandran plot. The top model was made using advanced multiple modelling which showed residues at 93.3%, 6.2%, 0.3% and 0.4% for the favoured regions, allowed regions, generally allowed regions and disallowed regions respectively. The models were generally geometrically reasonable.

Table S1. Ramachandran, G-score, Z-score and RMSD of selected MmpL3 homology models.

			Ramachandran plot score				
Model	**Z-Score**	**G-Score**	**Number of residues in favoured regions (%)**	**Additionally allowed regions (%)**	**Generously allowed regions (%)**	**Disallowed regions (%)**	**RMSD**
6AJH	-13.56	0.27	94.1	5.7	0.1	0	
Model_1	-8.03	-0.03	92.1	6.8	0.9	0.3	1.01
Model_02	07.33	-0.08	93.3	5.5	0.9	0.4	1.184
Model_05	-4.27	-0.39	83.3	12.5	2.9	1.4	1.768
Model_08	-4.55	-0.05	83	11.8	3.4	1.8	2.129
Model_09	-6.99	-0.16	91.7	7.2	0.8	0.4	0.926
Model_10	-6.91	-0.15	91.4	7.2	0.9	0.5	2.204
Model_11	-7.78	-0.05	92.7	6.4	0.6	0.3	1.020
Model_17	-7.68	-0.04	93.2	6.2	0.3	0.4	1.182

Docking

Hydrogen atoms, bond orders, and setting of formal charges at pH 7 of the homology model were performed using the Protein Preparation Wizard of the Maestro software package.[7] The water molecules in the protein structures were removed. The protein underwent energy minimisation using the OPLS_2005 (Optimised Potential for Liquid Simulations) force field. The active site of the Mtb MmpL3 protein was defined based on the crystallised ligands using

the receptor grid generation panel. The interactions of ligands with the protein residue in the active site were visualised using ligand interaction diagram in Schrodinger suite version 11.4.[8]

Docking was performed using the Glide software as implemented in Schrodinger's Maestro using the standard precision (SP) protocol. The IFD protocol was utilized for binding site refinement.

Biological Assays - Materials and methods

Time-kill assay

In 125 ml Erlenmeyer flasks, the volume of 50 ml of a *Mycobacterium tuberculosis* culture with approximately 10^6 CFU/ml was added. The compounds were added in the flasks at the concentration 0.32µg/mL for **5d**; 0.07and 0.35µg/mL to MOX (the antibiotic used as control) and a flask with only the bacterial inoculum (without addition of drug) was the growth control. The flasks were incubated at 37 ° C in shaker and aliquots were regularly recovered at 1st, 2nd, 3rd, 6th, 9th and 12th day. A serial decimal dilution was performed and aliquots of 100 µL of each dilution were plated on 7H11 solid medium plates supplemented with OADC. These plates were incubated per 3-4 weeks 37ºC, with 5% CO_2, to count the colonies. The results are expressed in Log_{10} CFU/mL (mean and standard error of three experiments done independently - y-axis) as a function of time exposure in days (x-axis).[9]

Intramacrophagic activity

Murine macrophages (lineage J774A.1 ATCC TIB-67) was cultured in RPMI medium with 10% fetal bovine serum (FBS) (37°C and 5% CO_2) until cell confluency. The cell concentration was adjusted to $5x10^4$ cells/well in a 24 well plate. After 24h for macrophages adhesion, the mycobacterial suspension (It was previously cultured in 7H9 medium supplemented with 10% OADC, diluted in RPMI medium at approximately 10^6 CFU/ml) was added for phagocytosis. After 2 h, the extracellular mycobacteria were removed by three successive washes with phosphate-buffer saline (PBS)**.** The treatment was added at concentrations non-toxic to the macrophage in 72h (previously determined) and it remained in contact with the infected cells for 72h. After this time, the supernatant was discarded, the cells washed with PBS to remove treatment residue and the macrophages had their membranes ruptured (Triton 0,01%). The intramacrophagic content was diluted and seeded onto solid media plates (7H11 with 10% OADC). The colonies were counted and the action of the compound was exhibited by the percentage inhibition of mycobacterial growth in relation to a control that did not receive treatment.[10]

Resazurin assay

The antibacterial activity was assessed on 96 well plates with 7HGC media (Middlebrook 7H9 broth) supplemented with OADC (oleic acid, albumin, dextrose, catalase; BD BACTEC). *M.tuberculosis* subspecies tuberculosis (Zopf) Lehmann and Neumann $H_{37}RV$ (ATCC 25618) was serially diluted with starting concentration of 4µg/ml of compound from working stock of

20µg/ml. Growth of bacterial population in the presence of MDR strains for 7days at 37°C. At day 7, 20µl of resazurin dye sodium salt [0.01% (w/v) mix (Sigma-Aldrich) was added to all wells with further incubation of 48h at 37°C. Observation of colour change from blue (no to pink indicated (bacterial growth) to determine MICC. Whilst blue resazurin indicated no growth.

Eukaryotic cytotoxicity assay

The cytotoxicity assay was performed in a 96-well plate based on REMA assay. In the first row, 200 µL of RPMI-1640 media containing 10% Fetal Bovine Serum (FBS) was added and in the consequent rows, 100 µL of the media was added. 4 µL of molecules were added in the first well and two-fold serial dilution was made by transferring 100 mL was repeated until the second last row as the last row was kept as a control in which no compound was added (media only). In each well, 100µL murine macrophage cell line, which has been passaged and normalised to a concentration of $5x10^5$ cells/mL, was added. The plate was incubated at 37°C, 5% CO_2 for 48h. Following a 48h incubation, the plate was washed with 1X PBS and fresh RPMI-1640 containing 10% FBS was added to each well. 30 µL of 0.01% freshly prepared resazurin solution was added to the media and this plate was then incubated overnight at 37°C, 5% CO_2. The following day, the colour change was observed. The experiment was performed three times to consider biological replicates and each time two technical replicates were taken.[11]

Toxicity testing in Galleria mellonella larvae

Larvae of the sixth development stage of *Galleria mellonella* were purchased from Livefoods Pet Shop (London) and stored at 15 °C in a sealed, ventilated container prior to use. Groups of 10 larvae (± 0.25 g) were injected with either 10 µL of 1% acetic acid or 0.3 mg/mL of **5d** solubilised in 1% acetic acid into the hindmost proleg using a Hamilton microsyringe. The larvae were then incubated at 37 °C in Petri dishes for a period of 5 days and mortality was scored every 24 hours. A larva was considered dead when melanisation occurred and/or when it failed to respond to physical stimuli; also taking into account inconsistencies that may arise since non-standardized larvae were used.[12]

Table S2: Survival counts of *Galleria mellonella* larvae post injection with pyrrole derivative **5d**

Entry	Time (h)	1% Acetic acid	5d	
		Number of larvae	Number of larvae	
1	0	10	10	10
2	24	10	10	10
3	48	10	10	10
4	72	10	10	10
5	96	8	10	9

6	120	8	10	9

The mortality of two larvae in the blank assay with 1% acetic acid is attributable to natural causes and not to the assay condition.

Intracellular high throughput spot-culture growth inhibition (HT-SPOTi) assay

The intracellular survival of *Mycobacterium aurum* was assessed using murine macrophages (RAW 264.7) HT-SPOTi model.[11] The macrophage cells (2×10^5 cells/well) were infected with *M. aurum* to achieve a multiplicity of infection (MOI) of 10:1 and were incubated at 37 °C, 5% CO_2 for 2 hr in RPMI-1640 (Fisher Scientific, UK) containing 10% Fetal Bovine Serum (FBS) (Fisher Scientific, UK) in 24-well plates. After the infection stage, the culture was washed with RPMI-1640 twice and incubated for 48 hr with different concentrations of the compounds (2x, 1x, 0.5x, 0.25x and 0.12x MIC) in RPMI-1640 media containing 10% FBS. Following the incubation, the cells were washed twice with RPMI-1640 and lysed in 500 µL of sterile distilled water at room temperature (RT) for 10 min. The lysed cells were centrifuged 16,000 g for 10 min at RT. They were resuspended into 50 µL of sterile distilled water, and then 5 µL was spotted onto the 24-well plates containing Middlebrook 7H10 agar containing 10% (v/v) of OADC supplement. The plates were incubated at 37°C for 3 days to determine intracellular survival.

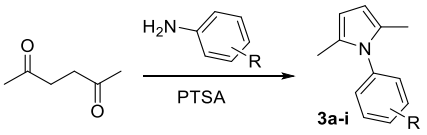
Chemistry - Materials and methods

^{1}H NMR and ^{13}C NMR spectra were recorded with an AVANCE 400 spectrometer Bruker, Germany at room temperature (rt) operating at the frequencies indicated. Chemical shifts (δ) are in ppm, referenced to tetramethylsilane. Coupling constants (J) are reported in Hertz and rounded to 0.5 Hz. Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or some combination of them. Infrared spectra were obtained using a Durascope diamond ATR system. Mass spectra (HRMS) were recorded at the EPSRC National Mass Spectrometry Service Centre on a Thermo Scientific LTQ Orbitrap XL Mass Spectrometer using low-resolution ESI or high-resolution nanoESI techniques. The purity of the compounds was assessed by reverse-phase liquid chromatography coupled with a mass spectrometer (Agilent series 1100 LC/MSD) with a UV detector at k = 254 nm and an electrospray ionization source (ESI). HPLC analyses were performed at 0.4 mL/min flow rate and using a binary solvent system of 95:5 methyl alcohol/water. All the solvents were of HPLC grade. Mass spectra were acquired in positive mode scanning over the mass range of 50–1500. The following ion source parameters were used: drying gas flow, 9 mL/min; nebulize pressure, 40 psig; and drying gas temperature, 350 ºC. All target compounds possessed a purity of ≥ 95% as verified by HPLC analyses. TLC was performed using commercially available pre-coated plates and visualized with UV light at 254 nm; $KMnO_4$ was used to reveal the products. Flash column chromatography was carried out using Fluorochem Davisil 40-63 u 60 Å. All reactions were conducted under a nitrogen atmosphere in oven-dried glassware unless stated otherwise. THF was distilled under nitrogen from sodium using a benzophenone indicator. Dichloromethane was purchased from Aldrich. Acetonitrile was further dried over 4 Å oven-

activated molecular sieves for 1 h prior to use. Petrol refers to the fraction of light petroleum ether boiling between 40 and 65 °C. All other solvents and commercially available reagents were used as received.

Synthesis of pyrroles 3a-i[13]

Pyrroles **3a-i** were synthesised as previously described in the literature.



1-(4-chlorophenyl)-2,5-dimethyl-1H-pyrrole (3a).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.46 (d, 2H, *J* = 8Hz), 7.20 (d, 2H, *J* = 8Hz), 5.92 (s, 2H), 2.05 (2 s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 133.4, 129.3, 128.8, 105.9, 77.0, 76.7, 13.0 ppm.

1-(3,5-dichlorophenyl)-2,5-dimethyl-1H-pyrrole (3b).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.40 (m, 1H), 7.14 (m, 2H), 5.88 (s, 2H), 2.05 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 140.9, 135.1, 128.4, 127.9, 127.0, 12.9 ppm.

1-(2,5-dimethylphenyl)-2,5-dimethyl-1H-pyrrole (3c).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.20-7.18 (m, 1H), 7.14-7.12 (m, 1H), 6.98 (m, 1H), 5.91 (s, 2H), 2.36 (s, 3H), 1.89 (s, 3H), 1.98 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.9, 136.6,133.7, 130.4, 129.3, 129.0, 128.2, 105.0, 20.8, 16.6, 12.6 ppm.

2,5-dimethyl-1-(2-(trifluoromethyl)phenyl)-1H-pyrrole (3d).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.07-7.16 (m, 4H), 5.96 (s, 2H), 2.00 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 135.1, 130.0, 129.9, 128.8, 116.2, 116.0, 106.0, 13.0 ppm.

1-(4-fluorophenyl)-2,5-dimethyl-1H-pyrrole (3e).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.30-7.26 (m, 2H), 7.13-7.11 (d, 2H, *J* = 8Hz), 5.90 (s, 2H), 2.05 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 169.4, 136.5, 128.9, 127.9, 126.9, 105.3, 13.0 ppm.

1-(4-isopropylphenyl)-2,5-dimethyl-1H-pyrrole (3f).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.30-7.26 (m, 2H), 7.12 (d, 2H, *J* = 8Hz), 5.89 (s, 1H), 2.97 (m, 1H), 2.03 (s, 6H), 1.31 (s, 3H), 1.29 (s, 1.29) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 148.6, 136.5, 128.9, 127.9, 126.9, 105.3, 33.7, 23.9, 13.0 ppm.

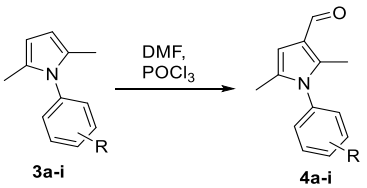
1-(2-fluorophenyl)-2,5-dimethyl-1H-pyrrole (3g).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.42 (s,1H), 7.28 (m, 3H), 5.96 (s, 2H) 2.04 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 158.4, 130.6, 129.7, 129.1, 126.6, 124.4, 116.6, 106.0, 12.4 ppm.

1-(4-cyanophenyl)-2,5-dimethyl-1H-pyrrole (3h).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.81 (d, 2H, *J* = 8Hz), 7.38 (d, 2H, *J* = 8Hz), 5.98 (s, 2H), 2.08 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 143.1, 133.2, 128.8, 128.5, 118.3, 111.3, 107.1, 12.4 ppm.

1-(4-methoxyphenyl)-2,5-dimethyl-1H-pyrrole (3i).
^{1}H-NMR (400 MHz $CDCl_3$) δ 7.01 (d, 2H, *J* = 8Hz), 6.85 (d, 2H, *J* = 8Hz), 5.78 (s, 2H), 3.73 (s, 3H), 1.90 (s, 6H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 158.9, 131.9, 129.1, 129.0, 114.4, 105.2, 55.3, 12.9 ppm.

Synthesis of aldehydes 4a-i

Aldehydes **4a-i** were synthesised as previously described in the literature.[13]



1-(4-chlorophenyl)-2,5-dimethyl-1H-pyrrole-3-carbaldehyde (4a)
^{1}H-NMR (400 MHz $CDCl_3$) δ 10.09 (s, 1H), 7.59 (d, 2H, J = 8Hz), 7.22 (d, 2H, J = 8Hz), 6.44 (s, 1H), 2.30 (s, 3H), 2.01 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 185.3, 162.6, 138.7, 135.5, 130.9, 129.6, 129.3, 122.1, 106.1, 12.7, 11.2 ppm.

1-(3,5-dichlorophenyl)-2,5-dimethyl-1H-pyrrole-3-carbaldehyde (4b).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.86 (s, 1H), 7.52 (s, 1H), 7.17 (s, 2H), 6.39 (s, 1H), 2.32 (s, 3H), 2.03 (s, 3H) ppm.
^{13}C-NMR (100 MHz $CDCl_3$) δ 185.3, 138.8, 138.3, 135.9, 130.6, 129.4, 126.8, 122.3, 106.5, 12.6, 11.2 ppm.

1-(2,5-dimethylphenyl)-2,5-dimethyl-1H-pyrrole-3-carbaldehyde (4c).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.98 (s, 1H), 7.35 (m, 2H), 6.98 (s, 1H), 6.47 (s, 1H), 2.42 (s, 3H), 2.24 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H) ppm.
^{13}C-NMR (100 MHz $CDCl_3$) δ 184.9, 138.3, 136.9, 135.7, 132.8, 130.9, 130.3, 130.1, 128.5, 121.7, 105.5, 20.7, 16.5, 12.2, 10.8.

1-(2-(trifluoromethyl)phenyl)-2,5-dimethyl-1H-pyrrole-3-carbaldehyde (4d).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.75 (s, 1H), 7.57-7.80 (m, 3H), 7.20 (d, 1H, *J* = 8Hz), 6.29 (s, 1H), 2.10 (s, 3H), 1.81 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 185.2, 139.9, 134.9, 133.4, 131.8, 131.0, 130.0, 128.9, 128.6, 127.6, 127.5, 122.1, 105.7, 12.1, 10.8 ppm.

1-(4-fluorophenyl)-2,5-dimethyl-1H-pyrrole-3-carbaldehyde (4e).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.88 (s, 1H), 7.51 (d, 2H, *J* = 8Hz), 7.16 (d, 2H, *J* = 8Hz), 6.38 (s, 1H), 2.28 (s, 3H), 1.99 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 187.3, 129.8, 120.4, 117.2, 116.8, 116.6, 105.9, 12.7, 11.2 ppm.

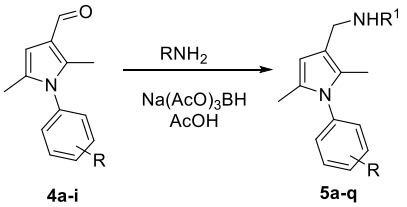
1-(4-isopropylphenyl)-2,5 dimethyl-1H-pyrrole-3-carbaldehyde (4f).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.88 (s, 1H), 7.37 (d, 2H, *J* = 8Hz), 7.10 (d, 2H, *J* = 8Hz), 6.55 (s, 1H), 2.97-3.04 (m, 1H), 2.26 (s, 3H), 2.00 (s, 3H), 1.35 (d, 6H, *J* = 4Hz) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 185.1, 149.6, 139.0, 134.4, 131.1, 127.6, 127.5, 121.7, 105.5, 33.8, 23.9, 12.7, 11.2 ppm.

1-(2-fluorophenyl)-2,5-dimethyl-1H-pyrrole-3-carboxaldehyde (4g).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.77 (s, 1H), 7.80-7.59 (m, 3H), 7.21 (m, 1H), 6.29 (s, 1H), 2.10 (s, 3H), 1.81 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 185.2, 139.9, 134.9, 133.4, 131.8, 131.0, 130.0, 128.9, 128.6, 127.6, 127.5, 122.5, 105.7, 12.1, 10.8 ppm.

1-(4-cyanophenyl)-2,5-dimethyl-1H-pyrrole-3-carboxaldehyde (4h).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.76 (s, 1H), 7.80 (d, 2H, *J* = 8Hz), 7.33 (d, 2H, *J* = 8Hz), 6.31 (s, 1H), 2.23 (s, 3H), 1.94 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 190.2, 145.6, 143.8, 139.0, 135.6, 134.4, 127.1, 123.3, 116.9, 110.9, 17.6, 15.9 ppm.

1-(4-methoxyphenyl)-2,5-dimethyl-1H-pyrrole-3-carboxaldehyde (4i).
^{1}H-NMR (400 MHz $CDCl_3$) δ 9.77 (s, 1H), 7.04 (d, 2H, *J* = 8Hz), 6.39 (d, 2H, *J* = 8Hz), 6.29 (s, 1H), 3.80 (s, 3H), 2.18 (s, 3H), 1.90 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 185.2, 159.6, 139.3, 131.3, 129.4, 128.9, 121.7, 114.7, 105.4, 55.5, 12.6, 11.1 ppm.

Synthesis of antitubercular pyrroles 5a-q



4a-i 5a-q

The appropriate aldehyde **4** (1 mmol) was dissolved in 5 mL of THF in a round bottom flask. Then AcOH (1 mmol) and the appropriate amine (1 mmol) were added to the stirring solution at room temperature. The mixture was allowed to stir at room temperature for 20 minutes before $NaB(AcO)_3H$ (3 mmol) was added. The mixture was allowed to stir at room temperature for 18h. Then, after completion, the reaction was quenched with NaOH 1M solution (25 mL). The mixture was then allowed to stir for 30 minutes. The reaction mixture was then diluted with EtOAc (10 mL), washed two times with EtOAc (10 mL) and once with brine (20 mL). The organic extracts were collected and then dried over $MgSO_4$, filtered and concentrated under

reduced pressure. The residue was then purified by flash chromatography (hexane-AcOEt, 6:4 v/v) affording the desired compounds **5**.

1-[1-(4-chlorophenyl)-2,5-dimethyl-pyrrol-3-yl]-*N*-[(2-propylcyclohexyl)methyl]methanamine (5a).
^{1}H NMR (400MHz $CDCl_3$) δ 7.35 (d, 2H, *J* = 8Hz), 7.05 (d, 2H, *J* = 8Hz), 5.86 (s, 1H), 3.51 (m, 2H), 2.61 (m, 1H), 2.17 (m, 2H), 1.92 (s, 3H), 1.90 (s, 3H), 1.55-1.38 (m, 4H), 1.18-1.02 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.5, 133.4, 129.5, 129.3, 128.0, 107.2, 61.0, 53.4, 45.1, 40.5, 35.7, 35.0, 28.8, 27.0, 12.8, 10.7 ppm. HRMS (ESI): calcd for $C_{20}H_{26}ClN_2$ (M + H^+) 329.1779, found 329.1779.

***N*-[[1-(4-chlorophenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]-1-phenyl-ethanamine (5b).**
1H NMR (400MHz $CDCl_3$) δ 7.33 (m, 5H), 7.19 (s, 2H), 7.03 (m, 2H), 5.87 (s, 1H), 3.80 (q, 1H, *J* = 4Hz), 3.37 (s, 2H), 1.93 (s, 3H), 1.79 (s, 3H), 1.31 (d, 3H, J = 4Hz) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.5, 133.4, 129.5, 129.3, 128.4, 128.0, 126.9, 126.8, 125.7, 107.0, 57.8, 43.3, 24.4, 12.8, 10.6 ppm. HRMS (EI): calcd for $C_{21}H_{22}ClN_2^+$ (M - H)$^+$ 337.1466, found 337.1466.

***N*-[[1-(4-chlorophenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]-N'-phenyl-ethane-1,2-diamine (5c).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.37 (d, 2H, *J* = 8Hz), 7.05 (m, 4H), 6.59 (m, 3H), 5.86 (s, 1H), 3.56 (s, 2H), 3.17 (t, 2H, *J* = 4Hz), 2.89 (t, 2H, *J* = 4Hz), 1.93 (s, 3H), 1.89 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 148.4, 137.4, 133.5, 129.5, 129.3, 129.2, 128.1, 125.9, 117.2, 112.9, 107.0, 47.8, 45.1, 43.1, 12.8, 10.7 ppm. HRMS (ESI): calcd for $C_{21}H_{25}ClN_3$ (M + H^+) 354.1732, found 354.1732.

1-[1-(4-chlorophenyl)-2,5-dimethyl-pyrrol-3-yl]-N-(cyclohexylmethyl)methanamine (5d).
^{1}H NMR (400MHz $CDCl_3$) δ 7.36 (d, 2H, *J* = 8Hz), 7.07 (d, 2H, *J* = 8Hz), 5.85 (s, 1H), 3.58 (s, 2H), 2.45 (d, 2H, *J* = 4Hz), 1.92 (s, 3H), 1.90 (s, 3H), 1.72-1.58 (m, 5H), 1.20-1.10 (m, 4H), 0.8 (m, 2H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.3, 133.5, 129.5, 129.3, 128.1, 126.1, 107.2, 55.4, 45.2, 37.8, 31.4, 26.6, 26.0, 12.8, 10.7 ppm. HRMS (ESI): calcd for $C_{20}H_{28}ClN_2$ (M + H^+) 331.1936, found 331.1937.

***N*-[[1-(3,5-dichlorophenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]cyclohexanamine (5e).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.34 (t, 1H, *J* = 4Hz), 7.05 (d, 2H, *J* = 2Hz), 5.86 (s, 1H), 3.55 (s, 2H), 2.46 (m, 1H), 1.95 (s, 3H), 1.93 (s, 3H), 1.86 (m, 2H), 1.70-1.66 (m, 4H), 1.23-1.06 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 140.9, 135.1, 127.9, 127.0, 125.4, 107.7, 57.4, 56.4, 42.3, 33.2, 26.1, 25.0, 12.8, 10.7 ppm. HRMS (ESI): calcd for $C_{19}H_{25}Cl_2N_2$ (M + H^+) 351.1389, found 351.1390.

***N*-[[1-(2,5-dimethylphenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]cyclohexanamine (5f).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.12-7.10 (m, 1H), 7.06 (m, 1H), 6.88 (m, 1H), 5.84 (s, 1H), 3.57 (s, 2H), 2.46 (m, 1H), 2.27 (s, 3H), 1.87 (m, 2H), 1.82 (s, 3H), 1.79 (s, 3H), 1.79 (s, 3H), 1.69-1.57 (m, 4H), 1.22-1.06 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 138.0, 136.3, 133.7,

130.3, 129.4, 129.0, 127.4, 125.0, 117.1, 105.9, 56.3, 42.7, 33.3, 33.2, 31.6, 26.2, 25.1, 22.6, 16.6, 14.1, 12.4, 10.3 ppm. HRMS (ESI): calcd for $C_{21}H_{31}N_2$ (M + H^+) 311.2482, found 311.2483.

***N*-[[2,5-dimethyl-1-[2-trifluoromethyl)phenyl]pyrrol-3-yl]methyl]cyclohexanamine (5g).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.48-7.76 (m, 3H), 7.20 (m, 1H), 5.86 (s, 1H), 3.59 (s, 2H), 2.44 (m, 1H), 1.82 (s, 3H), 1.78 (3H), 1.86-1.54 (m, 6H), 1.18-1.10 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.6, 132.8, 131.8, 129.2, 128.8, 127.2, 127.1, 126.9, 124.2, 106.6, 55.8, 45.2, 33.1, 32.8, 26.1, 25.1, 25.0, 12.3, 10.2 ppm. HRMS (ESI): calcd for $C_{20}H_{26}F_3N_2$ (M + H^+) 351.2043, found 351.2045.

***N*-[[1-(4-fluorophenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]cyclohexanamine (5h).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.08 (m, 4H), 5.86 (s, 1H), 3.59 (s, 2H), 2.50 (s, 1H), 1.91 (s, 3H), 1.89 (s, 3H), 1.92-1.87 (m, 2H), 1.70-1.54 (m, 4H), 1.19-1.12 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 163.0, 160.6, 134.9, 129.9, 129.8, 128.3, 126.1, 116.1, 115.8, 106.9, 56.0, 41.9, 32.5, 25.9, 25.0, 12.7, 10.6 ppm. HRMS (ESI): calcd for $C_{19}H_{26}FN_2$ (M + H^+) 301.2075, found 301.2072.

***N*-[[1-(4-isopropylphenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]cyclohexanamine (5i).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.22-7.20 (d, 2H, *J* = 8Hz), 7.01 (d, 2H, *J* = 8Hz), 5.86 (s, 1H), 3.62 (s, 2H), 2.89 (m, 1H), 2.56 (m, 1H), 1.93 (s, 3H), 1.91 (m, 3H), 1.90 (m, 2H), 1.68-1.57 (m, 4H), 1.23 (d, 6H, *J* = 4Hz), 1.17 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 148.2, 136.4, 128.3, 128.0, 126.9, 126.2, 106.4, 55.9, 41.9, 33.7, 32.5, 26.0, 25.0, 23.9, 12.8, 10.7 ppm. HRMS (ESI): calcd for $C_{22}H_{33}N_2$ (M + H^+) 325.2638, found 325.2640.

***N*-[[1-(2-fluorophenyl)-2,5-dimethyl-pyrrol-3-yl-]methyl]cyclohexanamine (5j).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.34-7.30 (m, 1H), 7.19-7.13 (m, 3H), 5.95 (s, 1H), 3.61 (s, 2H), 2.52 (m, 1H), 1.91 (s, 3H), 1.90 (s, 3H), 1.90 (m, 2H), 1.70-1.54 (m, 4H), 1.16 (m, 4H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 157.2 ,130.7, 129.8, 129.7, 128.5, 126.5, 126.2, 124.5, 124.4, 116.7, 116.5, 107.1, 56.2, 42.2, 32.9, 32.8, 26.0, 25.1, 25.0, 12.3, 10.2 ppm. HRMS (ESI): calcd for $C_{19}H_{26}FN_2$ (M + H^+) 301.2075, found 301.2073.

***N*-[[1-(4-cyanophenyl)-2,5-dimethyl-pyrrol-3-yl-]methyl]cyclohexanamine (5k).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.70 (d, 2H, *J* = 8Hz), 7.24 (d, 2H, *J* = 8Hz), 5.94 (s, 1H), 3.61 (s, 2H), 2.54 (m, 1H), 1.94 (s, 3H), 1.93 (s, 3H), 1.89 (m, 2H), 1.70-1.55 (m, 3H), 1.16 (m, 5H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 143.0, 133.1, 129.0, 127.9, 125.6, 118.2, 111.4, 108.3, 56.0, 41.8, 32.6, 26.0, 25.6, 25.0, 12.9, 10.8 ppm. HRMS (ESI): calcd for $C_{20}H_{26}N_3$ (M + H^+) 308.2121, found 308.2119.

***N*-[[1-(4-methoxyphenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]cyclohexanamine (5l).**
^{1}H NMR (400MHz $CDCl_3$) δ 7.03 (d, 2H, *J* = 8Hz), 6.87 (d, 2H, *J* = 8Hz), 5.88 (s, 1H), 3.78 (s, 3H), 3.68 (s, 2H), 2.58 (m, 1H), 1.93 (m, 2H), 1.90 (s, 3H), 1.89 (s, 3H), 1.69-1.54 (m, 3H), 1.20 (m, 5H) ppm.^{13}C-NMR (100 MHz $CDCl_3$) δ 158.8, 131.6, 129.3, 128.5, 126.5, 114.1,

106.5, 55.8, 53.4, 41.7, 32.2, 25.9, 25.6, 25.0, 12.7, 10.6 ppm. HRMS (ESI): calcd for $C_{20}H_{27}N_2O^+$ $(M - H)^+$ 311.2118, found 311.2120.

***N*-[[1-(2,5-dimethylphenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]-1-phenyl-methanamine (5m).**

^{1}H NMR (400MHz $CDCl_3$) δ 7.34-7.27 (m, 5H), 7.10-7.04 (m, 3H), 5.92 (s, 1H), 3.83 (s, 2H), 3.66 (s, 2H), 2.27 (s, 3H), 1.81 (s, 3H), 1.80 (s, 3H), 1.72 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.6, 136.8, 136.4, 133.6, 130.4, 129.3, 129.2, 128.9, 128.6, 128.0, 127.6, 126.5, 106.3, 50.8, 43.2, 20.8, 16.6, 12.4, 10.3 ppm. HRMS (ESI): calcd for $C_{22}H_{27}N_2$ (M + H+) 319.2169, found 319.2169.

***N*-[[1-(3,5-dichlorophenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]-1-phenyl-methanamine (5n)**.

^{1}H NMR (400MHz $CDCl_3$) δ 7.37-7.29 (m, 6H), 7.01 (s, 2H), 5.98 (s, 1H), 3.88 (s, 2H), 3.68 (s, 2H), 1.91 (s, 3H), 1.90 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 140.5, 135.3, 134.2, 129.3, 128.8, 128.7, 128.5, 128.3, 127.9, 127.7, 127.0, 108.1, 50.0, 42.9, 12.7, 10.6 ppm. HRMS (ESI): calcd for $C_{20}H_{21}Cl_2N_2$ (M + H+) 359.1076, found 359.1074.

***N*-[[1-(4-isopropylphenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]-1-phenyl-methanamine (5o).**

^{1}H NMR (400MHz $CDCl_3$) δ 7.40-7.25 (m, 7H), 7.08 (m, 2H), 5.95 (s, 1H), 3.89 (s, 2H), 3.67 (s, 2H), 2.97 (m, 1H), 2.00 (s, 3H), 1.93 (s, 3H), 1.29 (d, 6H, J = 8Hz) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 148.3, 128.5, 128.5, 128.4, 128.0, 127.2, 127.0, 126.5, 106.5, 52.1, 50.1, 33.7, 23.9, 12.8, 10.7 ppm. HRMS (ESI): calcd for $C_{23}H_{29}N_2$ $(M + H^+)$ 333.2325, found 333.2324.

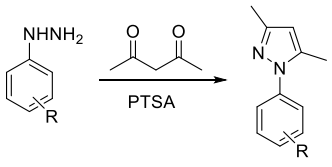
***N*-[[1-(4-fluorophenyl)-2,5-dimethyl-pyrrol-3-yl]methyl]-1-phenyl-methanamine (5p).**

^{1}H NMR (400MHz $CDCl_3$) δ 7.36-7.27 (m, 5H), 7.07 (m, 4H), 5.92 (s, 1H), 3.85 (s, 2H), 3.61 (s, 2H), 1.91 (s, 3H), 1.91 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 130.1, 129.9, 128.4, 128.2, 117.9, 116.2, 115.9, 106.8, 53.6, 45.2, 12.9, 10.8 ppm. HRMS (ESI): calcd for $C_{20}H_{21}FN_2$ $(M + H^+)$ 308.1689, found 308.1685.

***N*-[[1-(2,5-dimethyl)-2-trifluoromethyl-phenyl-pyrrol-3-yl]methyl]-1-phenyl-methanamine (5q).**

^{1}H NMR (400MHz $CDCl_3$) δ 7.75-7.73 (m, 1H), 7.59 (m, 1H), 7.53 (m, 1H), 7.35-7.25 (m, 5H), 7.19 (m, 1H), 5.93 (s, 1H), 3.78 (dd, 2H, J = 12, 8Hz), 3.63 (dd, 2H, J = 12, 8Hz), 1.82 (s, 3H), 1.71 (s, 3H) ppm. ^{13}C-NMR (100 MHz $CDCl_3$) δ 137.1, 136.7, 132.9, 131.7, 129.7, 129.0, 128.9, 128.6, 128.1, 127.6, 127.2, 113.7, 106.8, 50.4, 42.9, 12.3, 10.2 ppm. HRMS (ESI): calcd for $C_{21}H_{22}F_3N_2$ $(M + H^+)$ 359.1730, found 359.1727.

Synthesis of the pyrazoles 6a-b



6a-b

Acetylacetone (10 mmol) was dissolved in ethanol (15 mL). The appropriate phenylhydrazine (10 mmol) was added to the reaction mixture followed by the addition of *p*-toluene-sulfonyl acid (0.5 mmol). The resulting mixture was stirred at 150 ºC for 2 hours. The reaction was quenched with a saturated solution of $NaHCO_3$ and the product was extracted three times with EtOAc (25 mL). The organic extracts were collected and then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was than purified by flash column chromatography (hexane-AcOEt, 7:3 v/v) to afford the pyrazoles **6a-b**.

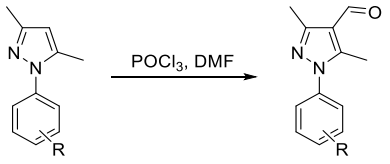
3,5-Dimethyl-1-phenyl-1H-pyrazole (6a).

^{1}H NMR (400 MHz $CDCl_3$) δ 7.45-7.50 (m, 2H), 7.35-7.40 (m, 2H), 7.27 (m, 1H), 6.03 (s, 1H), 2.33 (s, 3H), 1.9 (s, 3H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 149.5, 139.9, 139.4, 128.9, 127.3, 124.7, 106.9, 13.5, 12.4 ppm.

1-(4-Chlorophenyl)-3,5-dimethyl-1H-pyrazole (6b).

^{1}H NMR (400 MHz $CDCl_3$) δ 7.25 (d, 2H, *J* = 8Hz), 7.20 (d, 2H, *J* = 8Hz), 5.88 (s, 1H), 2.17 (s, 3H), 2.14 (s, 3H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 148.2, 138.3, 137.4, 131.6, 128.1, 124.6, 106.4, 12.4, 11.3 ppm.

Synthesis of the pyrazole aldehydes S7a-b.



6a-b **S7a-b**

A solution of $POCl_3$ (4 mmol) was added dropwise to a round-bottom flask containing ice-cooled DMF (5 mL) under N_2 atmosphere. The mixture was allowed to warm to room temperature in 15 min. The pyrazole **6a-b** dissolved in DMF (2 mL) were then added and the reaction mixture was then stirred at 100 ºC for 3 hours. After completion, the reaction was quenched with 10% w/v NaOH solution (10 mL) while the flask was placed in the ice bath. The mixture was stirred in such conditions for 30 minutes. The organic layers were separated, dried over sodium sulfate and concentrated in vacuo. The residue was purified by flash column chromatography (hexane-AcOEt, 4:1 v/v) affording the desired compounds **S7a-b**.

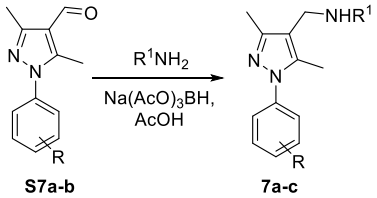
3,5-Dimethyl-1-phenyl-1H-pyrazole-4-carbaldehyde (S7a).

^{1}H NMR (400 MHz $CDCl_3$) δ 9.90 (s, 1H), 7.70 (m, 1H), 7.43- 7.37 (m, 2H), 7.35- 7.30 (m, 2H), 2.85 (s, 3H), 2.77 (s, 3H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 185.1, 151.5, 144.8, 138.1, 129.3, 128.7, 125.3, 118.8, 12.5, 11.2 ppm.

1-(4-Chlorophenyl)-3,5-dimethyl-1H-pyrazole-4-carbaldehyde (S7b).

^{1}H NMR (400 MHz $CDCl_3$) δ 9.70 (s, 1H), 7.27- 7.24 (m, 2H), 7.23-7.20 (m, 2H), 2.17 (s, 3H), 2.14 (s, 3H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 185.0, 151.9, 144.8, 136.7, 134.5, 129.4, 126.5, 119.1 12.5, 11.3 ppm.

Synthesis of the pyrazoles 7a-c.



S7a-b **7a-c**

In a round-bottom flask containing THF (5 mL) under N_2 atmosphere, the appropriate carbaldehyde (1 mmol), amine (1 mmol) and glacial acetic acid (1 mmol) were added dropwise. After 40 min of stirring, sodium $Na(AcO)_3BH$ (3 mmol) was added, and the mixture was left stirring overnight. The reaction was quenched with 1 M NaOH (10 mL) and diluted with AcOEt (10 mL). The mixture was extracted three times with AcOEt (10 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography (hexane-AcOEt, 1:4 v/v) to afford the desired compounds **7a-c**.

***N*-((3,5-Dimethyl-1-phenyl-1H-pyrazol-4-yl)methyl)cyclohexanamine (7a).**

^{1}H NMR (400 MHz $CDCl_3$) δ 7.38-7.35 (m, 2H), 7.34-7.30 (m, 2H), 7.25 (m, 1H), 4.20 (br s, 1H), 3.60 (s, 2H), 2.53-2.45 (m, 1H), 2.25 (s, 3H), 2.22 (s, 3H), 1.90-1.85 (m, 4H), 1.70-1.65 (m, 4H), 1.55 (m, 2H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 148.2, 139.9, 137.6, 129.0, 127.3, 124.9, 56.5, 39.7, 32.9, 26.0, 25.0, 11.9, 11.0 ppm. HRMS (ESI): calcd for $C_{18}H_{26}N_3$ ($M + H^+$) 284.2121, found 284.2119.

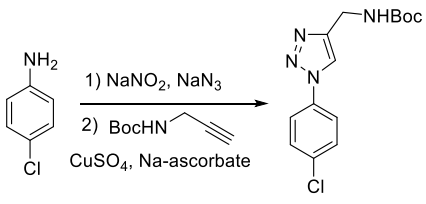
***N*-((1-(4-chlorophenyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)cyclohexanamine (7b).**

^{1}H NMR (400 MHz $CDCl_3$) δ 7.33- 7.28 (m, 2H), 7.26-7.23 (m, 2H), 3.53 (s, 2H), 2.73 (s, 1H), 2.22 (s, 3H), 2.19 (s, 3H), 1.94 (m, 2H), 1.87-1.81 (m, 4H), 1.69- 1.63 (m, 4H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 148.5, 138.5, 137.3, 132.7, 129.2, 125.8, 56.5, 39.9, 33.3, 26.2, 25.0, 11.7, 11.0 ppm. HRMS (ESI): calcd for $C_{18}H_{25}ClN_3$ ($M + H^+$) 318.1732, found 318.1731.

***N*-benzyl-1-(1-(4-chlorophenyl)-3,5-dimethyl-1H-pyrazol-4-yl)methanamine (7c).**

^{1}H NMR (400 MHz $CDCl_3$) δ 7.35-7.29 (m, 4H), 7.25-7.2 (m, 4H), 7.15 (m, 1H), 4.5 (br s, 1H), 3.75 (s, 2H), 3.55 (s, 2H), 2.19 (s, 3H), 2.14 (s, 3H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 148.8, 139.9, 138.5, 137.7, 132.8, 129.3, 128.5, 128.3, 127.2, 125.8, 116.6, 53.3, 42.1, 11.9, 11.0 ppm. HRMS (ESI): calcd for $C_{19}H_{21}ClN_3$ ($M + H^+$) 326.1419, found 326.1414.

Synthesis of the ^{t}butyl-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)carbamate (8).

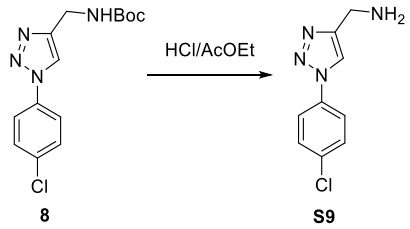


8

p-Chloroaniline (2 mmol) was dissolved in HCl 6 M (10 mL) and cooled at 0 ^{o}C. $NaNO_2$ (5.63 mmol) was added and the resulting mixture was stirred at 0 ^{o}C for 10 minutes. Then, NaN_3 (5.63 mmol) was carefully added and the reaction mixture was stirred at room temperature for 2 hours. The mixture was then diluted with water and extracted three times with AcOEt (4 mL). The combined organic layers were dried on $MgSO_4$, filtered and evaporated to give the crude *p*-chlorophenylazide. The *N*-Boc-propargylamine (3.10 mmol) and the freshly prepared *p*-chlorophenylazide were suspended in a 1:1 mixture of water and ^{t}BuOH (7 mL each). To this solution sodium ascorbate (0.563 mmol) and copper(II) sulfate (0.0563 mmol) were added and the reaction was left stirring overnight at room temperature. The suspension was filtered using a Buckner funnel. A precipitate was obtained, collected and dried and resulted to be the desired triazole **8** with a 22% yield and used in the next step without any further purification.

^{1}H NMR (400 MHz $CDCl_3$) δ 7.90 (s, 1H), 7.65-7.6 (d, 2H, J = 8Hz), 7.45-7.4 (d, 2H, J = 8Hz), 7.2 (s, 1H), 4.4 (s, 2H), 1.38 (s, 9H) ppm. LRMS (ESI+): m/z = 309 $[M + H]^+$.

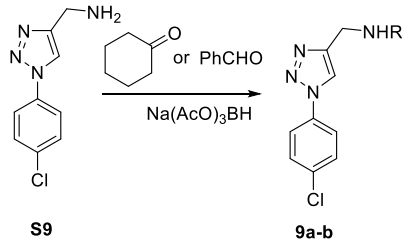
Synthesis of the (1-(4-chlorophenyl)-1H-1,2,3-triazol-4yl)methanaminium chloride (S9).[14]



8 **S9**

The triazole **8** (1.22 mmol) was placed in a round-bottom flask and added with a freshly prepared solution of HCl/AcOEt (5 mL). The mixture was stirred overnight and then the solvent was removed under reduced pressure. The residue was washed several times with small portions of cold Et_2O affording the desired compound **S9** which was used in the next step without any further purification.

Synthesis of triazoles (9a-b).



S9 **9a-b**

In a round-bottom flask containing THF (5 mL) under N_2 atmosphere, triazole **S9** (1 mmol), cyclohexanone or benzaldehyde (1 mmol) and glacial acetic acid (1 mmol) were added. After 40 min of stirring, $Na(AcO)_3BH$ (3 mmol) was added and the mixture was left stirring overnight. The reaction was then quenched with 1M NaOH and diluted with AcOEt (10 mL each). The mixture was extracted three times with AcOEt (10 mL). The organic layers were

then combined, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (MeOH-AcOEt, 1:9 v/v) to afford the desired compounds **9a-b**.

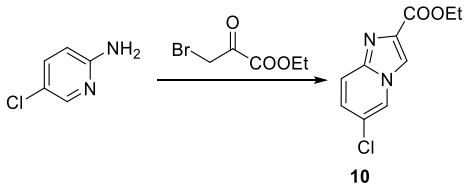
***N*-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)cyclohexanamine (9a).**

^{1}H NMR (400 MHz $CDCl_3$) δ 7.92 (s, 1H), 7.67 (d, *J* = 8.7Hz, 2H), 7.48 (d, *J* = 8.7Hz, 2H), 4.0 (br s, 1H), 3.7 (s, 2H), 2.5 (m, 1H), 1.9-1.5 (m, 10H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 147.8, 135.7, 134.4, 129.9, 121.6, 119.9, 68.0, 56.4, 41.7, 33.3, 26.0, 25.6, 25.0 ppm. HRMS (ESI): calcd for $C_{15}H_{20}ClN_4$ ($M + H^+$) 291.1371, found 291.1371.

***N*-benzyl-1-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methanamine (9b).**

^{1}H NMR (400 MHz $CDCl_3$) δ 7.82 (s, 1H), 7.63-7.60 (d, 2H), 7.59-7.57 (d, 2H), 7.45-7.43 (m, 2H), 7.42-7.40 (m, 2H), 7.20 (s, 1H), 5.23 (br s, 1H), 4.06 (s, 2H), 4.04 (s, 2H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 140.1, 135.5, 134.5, 133.2, 129.9, 128.5, 128.4, 127.3, 122.4, 120.0, 65.8, 47.2 ppm. HRMS (ESI): calcd for $C_{16}H_{16}ClN_4$ ($M + H^+$) 299.1058, found 299.1056.

Synthesis of the ethyl imidazo[1.2-a]pyridine-2-carboxylate 10.

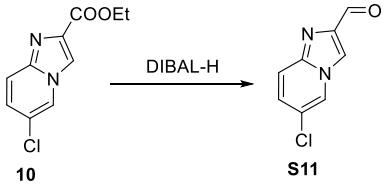


10

A solution of 2-amino-5-chloropyridine (1 mmol) in ethanol (20 mL) was added with ethyl bromopyruvate (1.2 mmol) and the mixture was stirred under reflux for 1 hour. After this time, the reaction was concentrated under vacuum to remove the solvent. The residue was partitioned between dichloromethane and a saturated aqueous solution of Na_2CO_3. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane-AcOEt, 1:1 v/v) to afford the desired compound **10**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.11 (s, 1H), 7.53 (d, *J* = 9.6Hz, 1H), 7.12 (d, *J* = 9.4Hz, 1H), 4.37 (q, *J* = 6.9Hz, 2H), 1.34 (t, *J* = 7.0Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.8, 143.5, 137.6, 127.8, 124.0, 122.2, 119.3, 117.1, 61.2, 14.4.

Synthesis of the imidazo[1,2-a]pyridine-2-carbaldehydes S11.



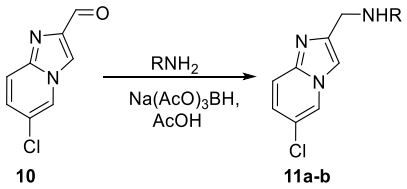
10 **S11**

To a solution of **10** (1 mmol) in CH_2Cl_2 (5 mL), DIBAL-H (1.0 M in methylene chloride, 2 mmol) was added dropwise at -78 ºC. The reaction mixture was stirred at -78 ºC for 1.5 h. The reaction mixture was then quenched with cold methanol and stirred for additional 10 min. The

resulting mixture was poured into a biphasic mixture of saturated $NaHCO_3$ (8 mL) and CH_2Cl_2 (10 mL) and allowed to warm to room temperature with occasional stirring. The organic layer was then separated, while the aqueous layer was extracted three additional times with CH_2Cl_2 (5 mL). The combined filtrate and extracts were washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the aldehyde **S11** which was used in the next step without any further purification.

^{1}H NMR (400 MHz $CDCl_3$) δ 10.07 (s, 1H), 8.18 (m, 1H), 8.07 (s, 1H), 7.58 (d, *J* = 9.7Hz, 1H), 7.19 (m, 1H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 187.7, 144.3, 130.1, 128.4, 124.4, 122.9, 119.7, 115.5 ppm

Synthesis of the imidazo[1,2-a]pyridine 11a-b.



10 **11a-b**

In a round-bottom flask containing THF (5 mL) under N_2 atmosphere the aldehyde **S11** (1 mmol), the appropriate amine (1 mmol) and glacial acetic acid (1 mmol) were added. After 40 min of stirring, $Na(AcO)_3BH$ (3 mmol) was added to the mixture and was left stirring overnight. The reaction was then quenched with 1M NaOH and diluted with AcOEt (10 mL each). The mixture was extracted three times with AcOEt (10 mL). The organic layers were then combined, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (AcOEt, 100%) to afford the desired compounds **11a-b**.

***N*-((6-chloroimidazo[1,2-a]pyridin-2-yl)methyl)cyclohexanamine (11a).**

^{1}H NMR (400 MHz $CDCl_3$) δ 8.8 (s, 1H), 7.8 (m, 1H), 7.7 (m, 1H), 7.5 (m, 1H), 4.2 (s, 2H), 2.5 (m, 1H), 1.7 (m, 2H), 1.6- 1.2 (m, 8H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 147.2, 143.6, 125.7, 123.4, 120.2, 117.5, 110.2, 56.5, 44.5, 33.3, 26.1, 25.0 ppm. HRMS (ESI): calcd for $C_{14}H_{19}ClN_3$ ($M + H^+$) 264.1262, found 264.1258.

***N*-benzyl-1-(6-chloroimidazo[1,2-a]pyridin-2-yl)methanamine (11b).**

^{1}H NMR (400 MHz $CDCl_3$) δ 8.15 (s, 1H), 7.77 (m, 1H), 7.74 (m, 1H), 7.45 (m, 1H), 7.37 (m, 2H), 7.34 (m, 2H), 7.29 (m, 1H), 3.76 (s, 2H), 3.74 (s, 2H) ppm. ^{13}C NMR (100 MHz $CDCl_3$) 146.7, 143.6, 128.4, 128.3, 127.1, 125.8, 123.4, 120.3, 117.5, 110.4, 53.4, 46.8 ppm. HRMS (ESI): calcd for $C_{15}H_{15}ClN_3$ ($M + H^+$) 272.0949, found 272.0944.

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