Structural rigidification of *N*-aryl-pyrroles into indoles active against intracellular and drug-resistant mycobacteria.

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Methods

A. Molecular docking

The homology model of MmpL3 was built as previously described.[1]

Ligand Preparation with LigPrep

The indole ligands were imported as SMILES (simplified molecular-input line-entry system) strings to the Schrodinger Suite. For each ligand, a single low energy 3D conformer was generated using the LigPrep module, resulting in a database of ligands store in mol2. The OPLS3 (Optimised Potential for Liquid Simulations) force field and charges were used in the ligand preparation. Additionally, all possible protonation and ionisation states were generated at pH of 7.0 ±2. Up to 32 stereoisomers per ligand were generated for the ligands with unassigned stereoisomers. Additionally, the ligands were de-salted and tautomerized. The conformations with the lowest energy state were selected for each ligand.

Docking

The Protein Preparation Wizard of the Schrodinger Software package was used to prepare the Mtb MmpL3 protein. The process involved the addition of missing hydrogen atoms, followed by the adjustment of bond orders for amino acid residues in the protein and the setting of formal charges at pH 7. The protein underwent energy minimisation using the OPLS3 force field with a proximity of heavy atoms set to an RMSD of 0.3Å.

The OPLS3 force field was used in the preparation of the receptor grid. The active site of the Mtb MmpL3 protein was defined based on the location of the crystallised ligands from *M. smegmatis* MmpL3 protein. Following from this, the ligand database was docked into the generated receptor grid using the Glide software as implemented in Schrodinger's Maestro using the standard precision (SP) protocol. The partial atomic charge was set at a cutoff of 0.15 for the nonpolar ligand atoms and a 0.8 scaling factor was applied for van der Waals radii. The interactions of ligands with the protein residue in the active site were visualised using ligand interaction diagram.

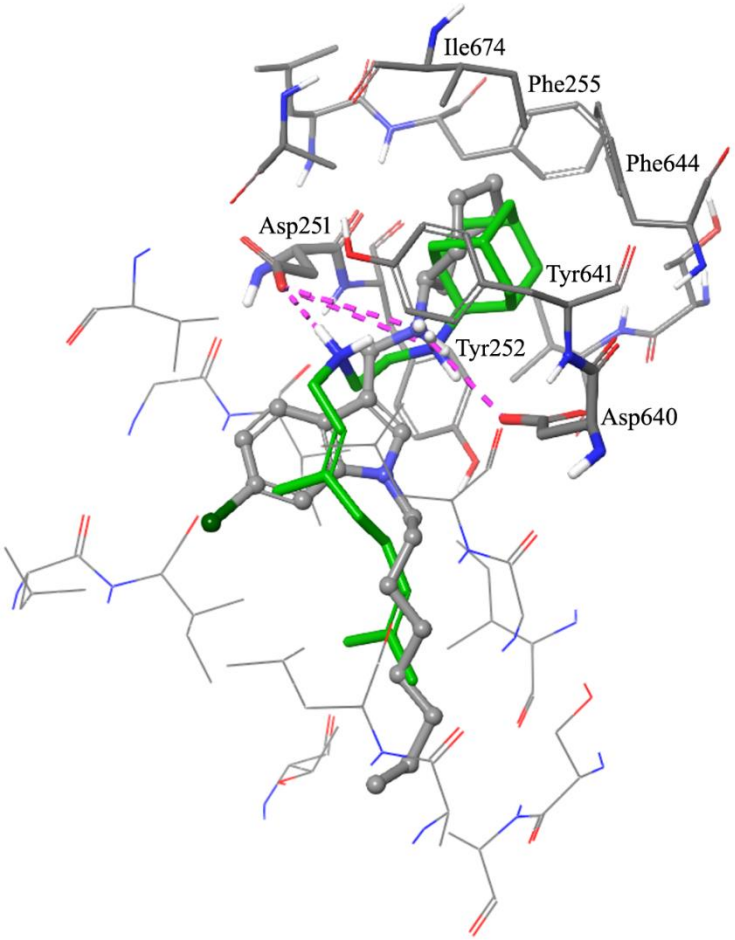


Figure S1. Comparison of the top scoring docking pose of **7j** and SQ109 into the Mtb MmpL3 homology model. Amino acids of the hydrophobic cage surrounding the heptylamino side chain are represented by thin tubes and some of them are also labeled, Asp251 and Asp640 responsible for two salt bridges with the basic amino groups of both inhibitors are represented by thick tubes, while the remaining amino acids that constitute the ligand binding site are in wire representation. The ligand **7j** is in ball&stick representation, while SQ109 is represented by thick tubes with atom type notation and green carbon atoms.

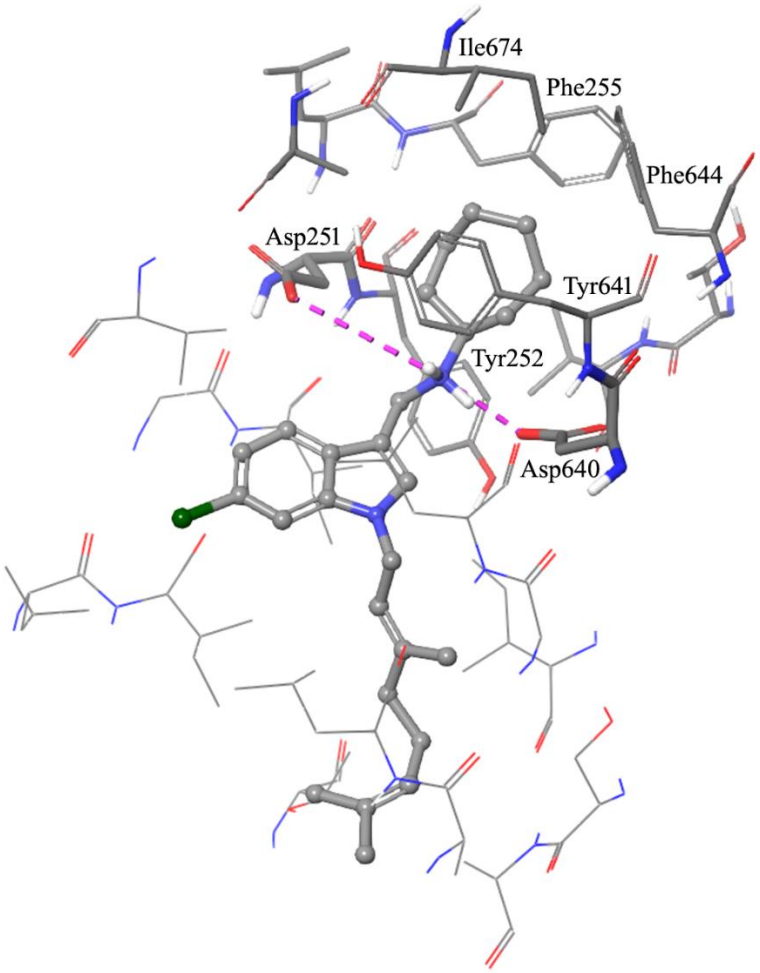


Figure S2. Top scoring docking pose of **7m** into the Mtb MmpL3 homology model. Amino acids of the hydrophobic cage surrounding the heptylamino side chain are represented by thin tubes and some of them are also labeled, Asp251 and Asp640 responsible for two salt bridges with the basic amino group are represented by thick tubes, while the remaining amino acids that constitute the ligand binding site are in wire representation. The ligand is in ball&stick representation.

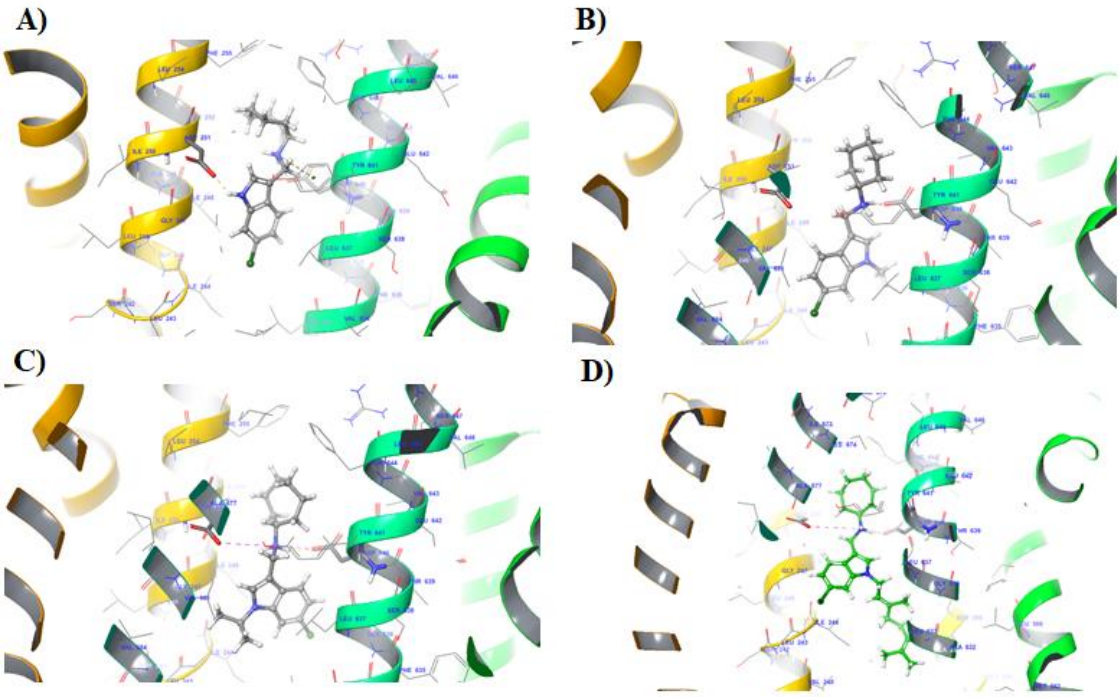


Figure S3. Docking poses of *N*-alkyl indoles into the MmpL3 Mtb homology model: A) indole **5f**, B) indole **7a**, C) indole **7h**, D) indole **7m**

Comparison of the homology model used in this work and the recently disclosed cryo-EM structure of Mycobacterium tuberculosis

Recently, a three-dimensional structure of MmpL3 from *Mycobacterium tuberculosis* from cryo-EM experiments has been deposited in the Protein Data Bank (entry 7nvh, 3 Å resolution). A comparison between this structure and the homology model resulted in an rmsd = 1.490 Å (calculated on the backbone atoms of the overall structures). A similar comparison between the homology model and the structure of MmpL3 (from *Mycobacterium smegmatis*) in the complex with SQ109 (entry 6ajg) resulted in a rmsd = 1.277 Å. Importantly, taking the amino acids that constitute the binding site of SQ109 in 6ajg (defined by the amino acids within 5 Å from the ligand structure) as the reference structure, their superposition with those that constitute the binding site of the pyrrole compound in the homology model resulted in a rmsd = 0.043 Å. In a similar way, a superposition of the same amino acids of the homology model and the corresponding residues of 7nvh resulted in a rmsd of 0.038 Å. The negligible structural differences found between these structures and, in particular, between the

homology model and the cryo-EM structure of *Mycobacterium tuberculosis* strongly suggest that the modeled MmpL3 represents a reliable structure for molecular modelling simulations.

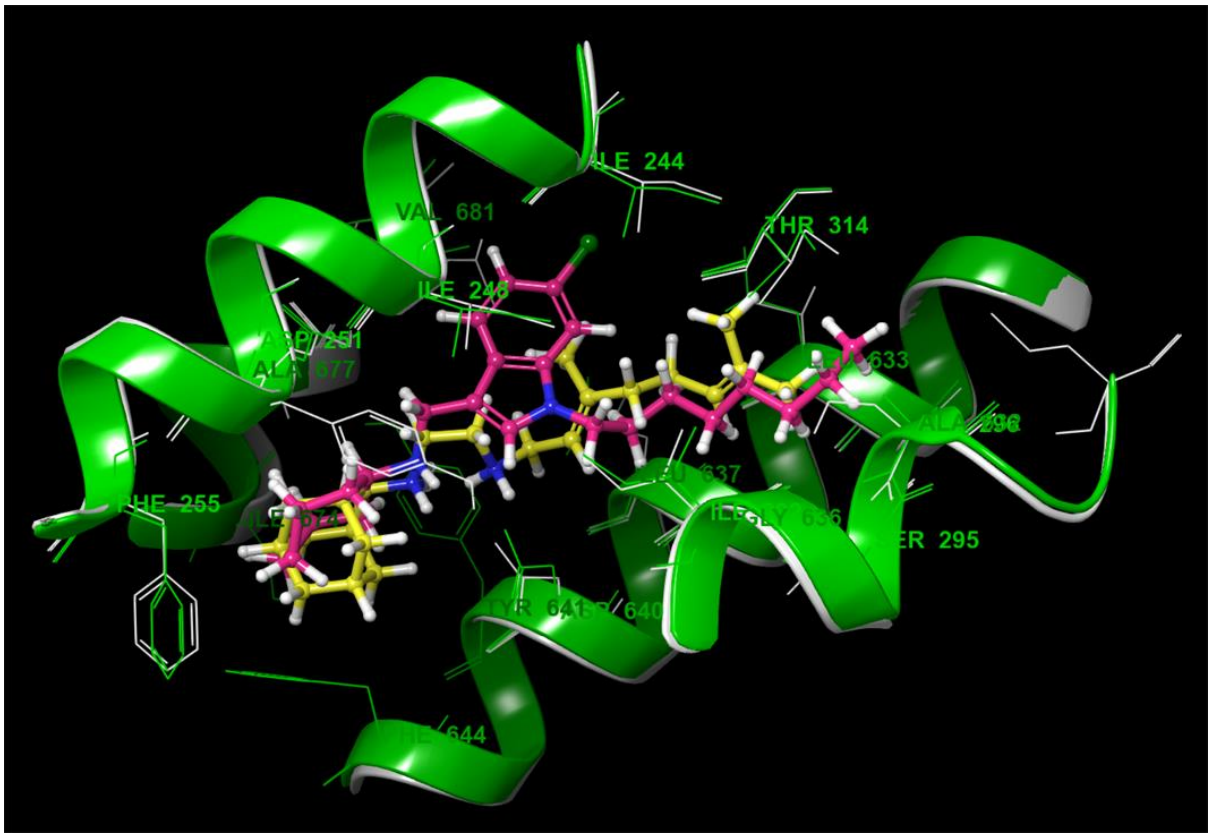
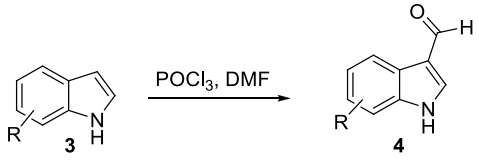


Figure S4. Graphical representation of the superposition between SQ109 (blue, ball&stick notation) in the complex with MmpL3 from *Mycobacterium smegmatis* (entry 6ajg of the Protein Data Bank) and **7j** (pink, ball&stick notation) in the complex with the modeled MmpL3. Both ligands share the same binding pocket within the protein. Amino acids are numbered according to the sequence of MmpL3 from *Mycobacterium tuberculosis* (Uniprot code P9WJV5).

B. Chemical synthesis

General. All reagents and solvents were purchased from commercial suppliers and utilized without further purification. Chemical reactions were carried out under a nitrogen atmosphere in over-dried glassware unless stated otherwise. The reactions were monitored by TLC using commercially available pre-coated plates and visualized with UV light at 254 nm; KMnO4 was used to reveal the products. Flash column chromatography was carried out using Sigma Aldrich silica gel (particle size 40-63 µm, pore size 60 Å). ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Ascend 400 spectrometer, at room temperature (rt) operating at the frequencies indicated. Chemical shifts are expressed in parts-per-million (ppm) relative to the internal solvent peak or tetramethylsilane (TMS). Coupling constants (*J*) are reported in Hertz (Hz). Spin multiplicities are denoted by the following abbreviations and combinations thereof: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). High resolution mass spectral (HRMS) data were obtained at King's College London Chemistry Department on a Waters Xevo G2-XS QTOF, LC on a Waters Acquity UPLC with reverse phase chromatography. All target compounds possessed a purity of ≥ 95% as verified by HPLC analyses (See page S16).

*General procedure for Vilsmeier-Haack formylation – synthesis of aldehydes **4**.*[2]



To a stirring solution of indole **3** (1.0 mmol) in DMF phosphorus oxychloride (1.2 mmol) was added dropwise at 0 °C. The mixture was allowed to warm to room temperature thereafter heated to 40 °C for 1 h. After cooling down, crushed ice was added along with a solution of 2 M NaOH and the mixture was heated under reflux for 16 h. The mixture was then allowed to cool to room temperature; the precipitate that formed was collected by vacuum filtration, washed with water and dried under high vacuum to obtain the desired indole carbaldehyde **4**.

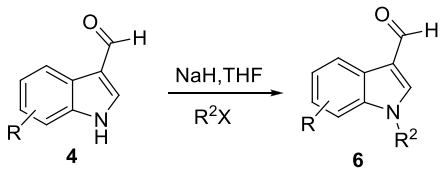
6-Chloro-1H-indole-3-carbaldehyde (4a). Yield: 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 8.34 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 7.25 (dd, *J* = 8.4, 1.7 Hz, 1H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ 185.5, 139.8, 138.0, 128.4, 123.3, 122.9, 122.5, 118.4, 112.7 ppm.

5-Chloro-1H-indole-3-carbaldehyde (4b). Yield: 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (br. s., 1H), 9.98 (s, 1H), 8.42 (s, 1H), 8.12 (s, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 1H) ppm; ^{13}C NMR (101 MHz, DMSO-d_6) δ 185.1, 139.5, 135.4, 126.7, 125.2, 123.5, 119.8, 117.5, 114.1 ppm.

5-Methyl-1H-indole-3-carbaldehyde (4c). Yield: 64%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H), 9.23 (br. s., 1H), 8.13 (s, 1H), 7.82 (d, *J* = 1.0 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.15 (d, *J* = 9.8 Hz, 1H), 2.48 (s, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.4, 135.9, 135.1, 132.8, 125.9, 124.7, 121.6, 119.2, 111.3, 21.5 ppm.

1H-Indole-3-carbaldehyde (4d).[3] (commercially available)

*General procedure for N-alkylation of formylated indoles **4** - synthesis of aldehydes **6.***



To a cooled solution of indole-3-carbaldehyde **4** (1.0 mmol) in THF NaH (60% dispersion in mineral oil, 2.0 mmol) was added portionwise under an inert atmosphere of nitrogen and the mixture was allowed to stir for 30 min. Thereafter, the appropriate alkylating agent was added dropwise and the mixture was allowed to stir for 14 h. After complete consumption of the starting material as monitored by TLC, the reaction was quenched with water and extracted with EtOAc. The organic extracts were combined, washed with brine, dried over $MgSO_4$ and thereafter concentrated under reduced pressure. The resulting residue was purified by silica gel flash column chromatography (EtOAc/Hexane, 1:9 v/v) to give the desired product **6**.

6-Chloro-1-methyl-1H-indole-3-carbaldehyde (6a). Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 7.65 (s, 1H), 7.35 (d, *J* = 1.4 Hz, 1H), 7.28 (dd, *J* = 8.4, 1.7 Hz, 1H), 3.83 (s, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.3, 139.6, 138.3, 129.8, 123.7, 123.6, 123.1, 118.1, 110.2, 33.9 ppm.

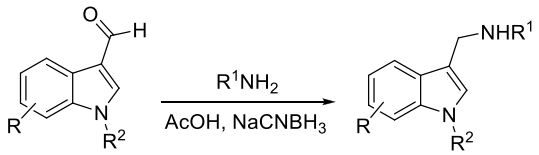
6-Chloro-1-isopropyl-1H-indole-3-carbaldehyde (6b). Yield: 49%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 8.14 (m, 1H), 7.75 (d, *J* = 3.6 Hz, 1H), 7.33 (d, *J* = 0.6 Hz, 1H), 7.19

(m, 1H), 4.56 (m, 1H), 1.51 (m, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.3, 137.3, 135.0, 129.8, 124.0, 123.5, 123.1, 118.2, 110.4, 48.4, 22.6 ppm.

6-Chloro-1-octyl-1H-indole-3-carbaldehyde (6c). Yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 8.12 (d, *J* = 8.5 Hz, 1H), 7.59 (s, 1H), 7.26 (d, *J* = 1.4 Hz, 1H), 7.17 (dd, *J* = 8.5, 1.7 Hz, 1H), 4.01 (t, *J* = 7.2 Hz, 2H), 1.79 (m, 2H), 1.21 (m, 10H), 0.79 (t, *J* = 6.9 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.2, 138.6, 137.7, 129.9, 123.9, 123.4, 123.1, 118.0, 110.2, 47.4, 31.7, 29.7, 29.1, 29.0, 26.8, 22.6, 14.0 ppm.

(E)-6-Chloro-1-(3,7-dimethylocta-2,6-dienyl)-1H-indole-3-carbaldehyde (6d). Yield: 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 3.5 Hz, 1H), 7.24 (d, *J* = 1.6 Hz, 1H), 7.17 (m, 1H), 5.30 (td, *J* = 7.0, 1.0 Hz, 1H), 4.97 (m, 1H), 4.58 (t, *J* = 6.2 Hz, 2H), 2.04 (m, 4H), 1.74 (m, 3H), 1.58 (s, 3H), 1.51 (s, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.3, 143.0, 138.4, 137.7, 132.3, 129.7, 124.0, 123.5, 123.4, 122.9, 117.9, 117.2, 110.4, 44.8, 39.4, 26.1, 25.7, 17.7, 16.6 ppm.

*General procedure for reductive amination – synthesis of compounds **5** and **7**.*



4 *or* **6** **5** *or* **7**

To a solution of carbaldehyde (1.0 mmol) **4 or 6** in THF AcOH (1.0 mmol) was added and the solution was allowed to stir at room temperature for 15 min. The appropriate amine (1.0 mmol) was added and the mixture was allowed to stir at room temperature for 48 h. $NaCNBH_3$ was then added and the mixture was allowed to stir for a further 18 h. The reaction was quenched with 1M NaOH and extracted with EtOAc. The organic extracts were combined, washed with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting crude material was purified by silica gel flash column chromatography (EtOAc/Hexane, 8:2 v/v) to afford the desired product **5** or **7**.

N-((6-Chloro-1H-indol-3-yl)methyl)cyclohexanamine (5a). Yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (br. s., 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.28 (m, 1H), 7.06 (m, 2H), 3.97 (s, 2H), 2.97 (br. s., 1H), 2.62 (m, 1H), 1.97 (m, 2H), 1.77 (m, 2H), 1.64 (m, 1H), 1.23 (m,

5H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.6, 128.1, 125.5, 123.9, 120.3, 119.3, 113.3, 111.4, 56.7, 41.2, 32.8, 25.9, 25.0 ppm; HRMS (ESI): *m/z* calcd for $C_{15}H_{20}ClN_2$ $[M + H]^+$ 263.1315, found 263.1305.

N-((5-Chloro-1H-indol-3-yl)methyl)cyclohexanamine (5b). Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (br. s., 1H), 6.98 (m, 1H), 6.88 (d, *J* = 1.0 Hz, 1H), 6.79 (br. s., 1H), 6.74 (s, 1H), 3.65 (s, 2H), 2.29 (br. s., 1H), 1.52 (m, 7H), 0.84 (m, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.7, 128.1, 125.1, 124.2, 122.2, 118.1, 114.2, 112.4, 56.8, 41.5, 33.3, 26.1, 25.1 ppm; HRMS (ESI): *m/z* calcd for $C_{15}H_{20}ClN_2$ $[M + H]^+$ 263.1315, found 263.1306.

N-((5-Methyl-1H-indol-3-yl)methyl)cyclohexanamine (5c). Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (br. s., 1H), 7.42 (s, 1H), 7.25 (s, 1H), 7.13 (d, *J* = 1.0 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 4.01 (s, 2H), 2.62 (br. s., 1H), 2.47 (s, 3H), 1.75 (br. s., 2H), 1.22 (m, 8H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.6, 128.8, 127.2, 123.7, 123.0, 118.2, 113.9, 110.9, 56.4, 41.6, 33.2, 26.1, 25.1, 21.6 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{23}N_2$ $[M + H]^+$ 243.1861, found 243.1859.

1-(6-Chloro-1H-indol-3-yl)-N-(cyclohexylmethyl)methanamine (5d). Yield: 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (br. s., 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 1.3 Hz, 1H), 7.08 (m, 2H), 3.93 (s, 2H), 2.54 (d, *J* = 6.7 Hz, 2H), 2.13 (br. s., 1H), 1.73 (m, 4H), 1.49 (m, 1H), 1.21 (m, 4H), 0.91 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.7, 128.0, 125.7, 123.3, 120.2, 119.7, 114.9, 111.2, 56.3, 44.8, 37.8, 31.5, 26.6, 26.0 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{22}ClN_2$ $[M + H]^+$ 277.1471, found 277.1461.

N-((1H-Indol-3-yl)methyl)-1-cyclohexylmethanamine (5e). Yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (br. s., 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.42 (m, 1H), 7.36 (m, 1H), 7.27 (s, 1H), 4.23 (s, 2H), 3.14 (br. s., 1H), 2.83 (d, *J* = 6.8 Hz, 2H), 1.92 (m, 6H), 1.45 (br. s., 5H) ppm; ^{13}C NMR (101 MHz, Acetone-d_6) δ 128.4, 124.8, 122.2, 122.0, 119.6, 119.2, 112.1, 111.9, 51.0, 45.4, 38.4, 32.2, 27.4, 26.8 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{23}N_2$ $[M + H]^+$ 243.1861, found 243.1859.

N-((6-Chloro-1H-indol-3-yl)methyl)cycloheptanamine (5f). Yield: 46%. ^{1}H NMR (400 MHz, MeOD) δ 7.61 (d, *J* = 8.5 Hz, 1H), 7.40 (m, 2H), 7.08 (dd, *J* = 8.5, 1.8 Hz, 1H), 4.22 (s, 2H), 3.09 (m, 1H), 2.07 (m, 2H), 1.77 (m, 2H), 1.54 (m, 8H) ppm; ^{13}C NMR (101 MHz, MeOD) δ 137.0, 127.7, 126.6, 125.3, 119.9, 118.8, 111.1, 107.2, 58.4, 39.7, 31.5, 29.4, 27.4, 23.9 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{22}ClN_2$ $[M + H]^+$ 277.1466, found 277.1468.

N-((5-Methyl-1H-indol-3-yl)methyl)cycloheptanamine (5g). Yield: 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (br. s., 1H), 7.42 (s, 1H), 7.25 (s, 1H), 7.21 (d, *J* = 1.9 Hz, 1H), 7.03 (dd, *J* = 8.3, 1.4 Hz, 1H), 4.01 (s, 2H), 2.84 (tt, *J* = 8.9, 4.3 Hz, 1H), 2.47 (s, 3H), 1.71 (m, 3H), 1.59 (m, 2H), 1.54 (br. s., 5H), 1.23 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.5, 128.9, 127.3, 123.8, 120.0, 118.2, 113.6, 111.0, 58.3, 41.4, 33.9, 28.2, 24.4, 21.6 ppm; HRMS (ESI): *m/z* calcd for $C_{17}H_{25}N_2$ $[M + H]^+$ 257.2012, found 257.2014.

N-((1H-Indol-3-yl)methyl)cycloheptanamine (5h). Yield: 46%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.28 (br. s., 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.23 (s, 1H), 7.10 (t, *J* = 7.0 Hz, 1H), 7.02 (t, *J* = 7.49 Hz, 1H), 3.97 (s, 2H), 2.81 (tt, *J* = 8.2, 4.1 Hz, 1H), 1.91 (m, 2H), 1.69 (m, 2H), 1.49 (m, 8H) ppm; ^{13}C NMR (101 MHz, Acetone-d_6) δ 137.7, 128.3, 123.7, 122.1, 119.8, 119.4, 115.5, 112.1, 58.9, 43.3, 35.4, 25.0 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{23}N_2$ $[M + H]^+$ 243.1861, found 243.1859.

N-((6-Chloro-1H-indol-3-yl)methyl)cyclooctanamine (5i).Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (br. s., 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.32 (s, 1H), 7.16 (s, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 3.97 (s, 2H), 2.90 (m, 1H), 1.92 (br. s., 1H), 1.62 (m, 14H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.6, 128.2, 125.4, 124.4, 120.5, 119.4, 112.3, 111.4, 58.0, 41.5, 31.8, 27.0, 25.7, 24.1 ppm; HRMS (ESI): *m/z* calcd for $C_{17}H_{24}ClN_2$ $[M + H]^+$ 291.1628, found 291.1624.

N-((1H-Indol-3-yl)methyl)cyclooctanamine (5j). Yield: 36%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.22 (br. s., 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.23 (s, 1H), 7.08 (m, 1H), 7.01 (m, 1H), 3.96 (s, 2H), 2.81 (s, 1H), 1.50 (m, 14H) ppm; ^{13}C NMR (101 MHz, Acetone-d_6) δ 128.3, 123.7, 122.1, 119.8, 119.4, 112.1, 57.9, 43.2, 32.7, 28.3, 26.4, 24.6 ppm; HRMS (ESI): *m/z* calcd for $C_{17}H_{25}N_2$ $[M + H]^+$ 257.2017, found 257.2014.

N-((6-Chloro-1H-indol-3-yl)methyl)adamantyl-2-amine (5k). Yield: 29%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (br. s., 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.31 (s, 1H), 7.10 (m, 2H), 3.95 (s, 2H), 2.88 (br. s., 1H), 1.91 (m, 13H), 1.54 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.8, 128.0, 125.8, 123.1, 120.2, 119.9, 115.7, 111.1, 61.7, 42.0, 38.0, 37.6, 32.1, 31.5, 27.9, 27.7 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{24}ClN_2$ $[M + H]^+$ 315.1628, found 315.1630.

N-Benzyl-1-(6-chloro-1H-indol-3-yl)methanamine (5l). Yield: 13%.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.4 Hz, 1H), 7.27 (m, 7H), 7.00 (d, *J* = 8.5 Hz, 1H), 3.87 (s, 2H), 3.77 (s, 2H), 2.80 (br. s., 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.7, 128.4, 127.6, 123.9,

120.5, 119.5, 113.2, 111.3, 53.0, 43.6 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{16}ClN_2$ $[M + H]^+$ 271.1002, found 271.0989.

N-((6-Chloro-1-methyl-1H-indol-3-yl)methyl)cyclohexanamine (7a). Yield: 21%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.4 Hz, 1H), 7.20 (m, 1H), 7.01 (dd, *J* = 8.4, 1.7 Hz, 1H), 6.93 (d, *J* = 9.9 Hz, 1H), 3.89 (s, 2H), 3.64 (s, 3H), 2.52 (m, 1H), 2.23 (br. s., 1H), 1.88 (m, 2H), 1.68 (m, 2H), 1.56 (m, 1H), 1.13 (m, 5H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5, 128.2, 127.9, 126.0, 119.8, 119.7, 113.0, 109.4, 56.5, 41.3, 33.1, 32.8, 26.0, 25.0 ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{22}ClN_2$ $[M + H]^+$ 277.1471, found 277.1472

***1-(6-Chloro-1-methyl-1H-indol-3-yl)-N-(cyclohexylmethyl)methanamine (7b)*.** Yield: 22%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 1.3 Hz, 1H), 7.08 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.02 (s, 1H), 3.92 (s, 2H), 3.72 (s, 3H), 2.53 (d, *J* = 6.7 Hz, 2H), 2.36 (br. s., 1H), 1.69 (m, 4H), 1.49 (m, 1H), 1.18 (m, 4H), 0.90 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5, 128.3, 127.9, 126.1, 119.8, 119.8, 112.9, 109.4, 56.1, 44.5, 37.6, 32.8, 31.4, 29.7, 26.6, 26.0 ppm; HRMS (ESI): *m/z* calcd for $C_{17}H_{24}ClN_2$ $[M + H]^+$ 291.1628, found 291.1624.

***N-((6-Chloro-1-methyl-1H-indol-3-yl)methyl)cyclooctanamine (7c)*.** Yield: 34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 1.4 Hz, 1H), 7.08 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.00 (s, 1H), 3.92 (s, 2H), 3.71 (s, 3H), 2.80 (m, 1H), 2.25 (br. s., 1H), 1.77 (m, 4H), 1.52 (m, 10H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5, 128.1, 127.8, 126.1, 119.8, 119.7, 113.5, 109.4, 57.6, 41.9, 32.8, 32.3, 27.3, 25.8, 24.1 ppm; HRMS (ESI): *m/z* calcd for $C_{18}H_{26}ClN_2$ $[M + H]^+$ 305.1784, found 305.1785.

***N-((6-Chloro-1-methyl-1H-indol-3-yl)methyl)adamantyl-2-amine (7d)*.** Yield: 21%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 1.4 Hz, 1H), 7.09 (m, 2H), 3.99 (s, 2H), 3.73 (s, 3H), 2.65 (br. s., 1H), 2.01 (m, 4H), 1.87 (m, 5H), 1.72 (m, 4H), 1.57 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5, 128.6, 128.0, 126.1, 120.0, 119.7, 112.4, 109.5, 61.7, 41.6, 37.8, 37.4, 32.9, 31.6, 31.3, 27.6, 27.5 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{26}ClN_2$ $[M + H]^+$ 329.1784, found 329.1785.

***N-Benzyl-1-(6-chloro-1-methyl-1H-indol-3-yl)methanamine (7e)*.** Yield: 20%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.4 Hz, 1H), 7.33 (m, 4H), 7.28 (m, 2H), 7.07 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.01 (s, 1H), 3.95 (s, 2H), 3.86 (s, 2H), 3.72 (s, 3H), 2.14 (br. s., 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.9, 137.5, 128.5, 128.3, 128.2, 127.8, 127.1, 126.1, 120.0,

119.7, 113.3, 109.3, 53.2, 43.7, 32.8 ppm; HRMS (ESI): *m/z* calcd for $C_{17}H_{18}ClN_2$ $[M + H]^+$ 285.1158, found 285.1181.

N-((6-Chloro-1-isopropyl-1H-indol-3-yl)methyl)cyclohexanamine (7f). Yield: 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.4 Hz, 1H), 7.34 (s, 1H), 7.19 (s, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 4.55 (m, 1H), 2.58 (m, 2H), 1.97 (m, 5H), 1.70 (m, 1H), 1.48 (m,6H), 1.24 (m, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 127.5, 126.1, 122.8, 119.8, 119.7, 114.0, 109.6, 56.9, 47.2, 41.9, 33.4, 26.2, 25.1, 22.7 ppm; HRMS (ESI): *m/z* calcd for $C_{18}H_{26}ClN_2$ $[M + H]^+$ 305.1784, found 305.1785.

***1-(6-Chloro-1-isopropyl-1H-indol-3-yl)-N-(cyclohexylmethyl)methanamine (7g)*.** Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 8.1, 3.2 Hz, 1H), 7.34 (s, 1H), 7.21 (d, *J* = 2.8 Hz, 1H), 7.07 (dd, *J* = 6.2, 2.1 Hz, 1H), 4.55 (m, 1H), 3.93 (s, 2H), 2.77 (br. s., 1H), 2.54 (s, 2H), 1.72 (m, 5H), 1.50 (m, 6H), 1.19 (m, 4H), 0.93 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 127.6, 126.1, 123.2, 119.8, 113.0, 109.6, 56.2, 47.3, 44.8, 37.6, 31.4, 29.7, 26.6, 26.0, 22.7 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{28}ClN_2$ $[M + H]^+$ 319.1941, found 319.1967.

***N-((6-Chloro-1-isopropyl-1H-indol-3-yl)methyl)cycloheptanamine (7h)*.** Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.4 Hz, 1H), 7.33 (d, *J* = 1.2 Hz, 1H), 7.17 (s, 1H), 7.07 (dd, *J* = 8.4, 1.6 Hz, 1H), 4.55 (m, 1H), 3.94 (s, 2H), 2.77 (m, 1H), 1.99 (br. s., 1H), 1.91 (m, 2H), 1.70 (m, 2H), 1.54 (m, 6H), 1.48 (m, 8H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 127.5, 126.2, 122.7, 119.8, 119.6, 114.2, 109.5, 59.0, 47.2, 42.5, 34.8, 34.7, 24.5, 22.7 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{28}ClN_2$ $[M + H]^+$ 319.1941, found 319.1941.

***N-((6-Chloro-1-isopropyl-1H-indol-3-yl)methyl)cyclooctanamine (7i)*.** Yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.24 (s, 1H), 7.07 (s, 1H), 6.97 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.46 (m, 1H), 3.84 (s, 2H), 2.70 (m, 1H), 1.70 (m, 4H), 1.44 (m, 17H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 127.5, 126.1, 122.6, 119.9, 119.6, 114.3, 109.6, 57.9, 47.2, 42.5, 32.7, 27.4, 25.9, 24.2, 22.7 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{30}ClN_2$ $[M + H]^+$ 333.2097, found 333.2097.

***N-((6-Chloro-1-octyl-1H-indol-3-yl)methyl)cycloheptanamine (7j)*.** Yield: 24%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 1.5 Hz, 1H), 7.05 (m, 1H), 7.03 (s, 1H), 4.00 (t, *J* = 7.2 Hz, 2H), 3.92 (s, 2H), 2.75 (m, 1H), 1.89 (m, 2H), 1.81 (m, 2H), 1.69 (m, 3H), 1.55 (m, 4H), 1.44 (m, 4H), 1.28 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (101

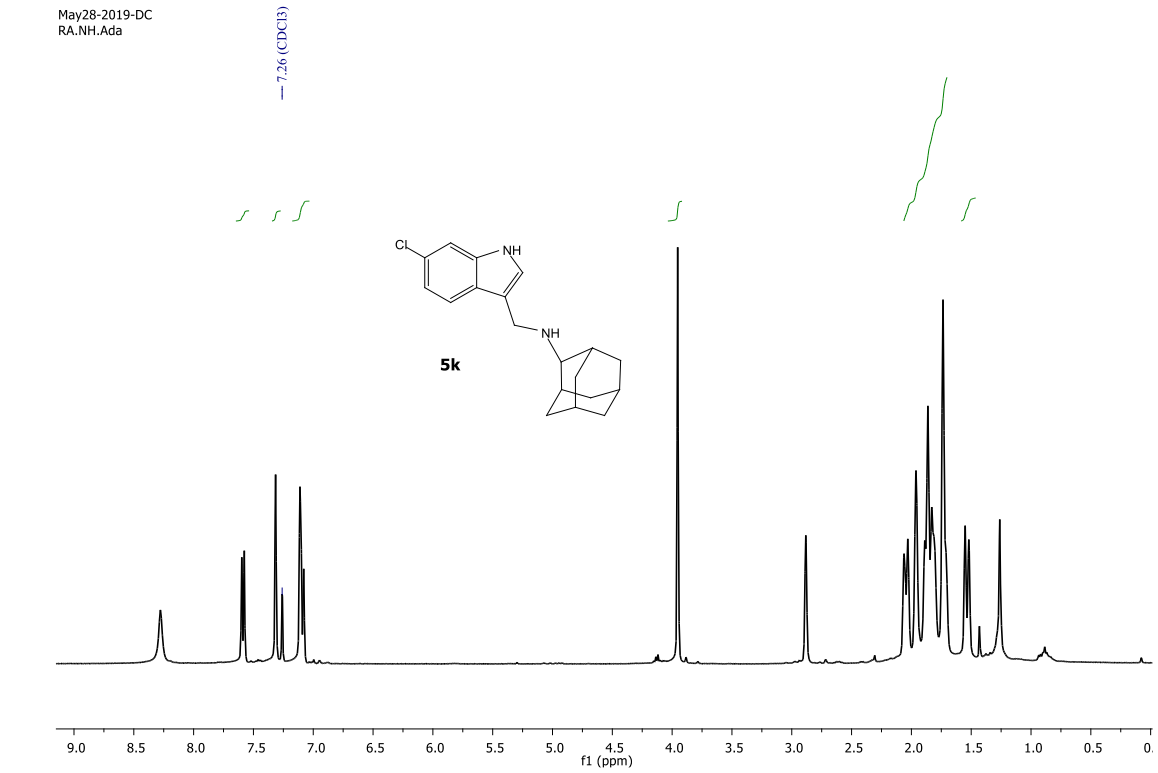
MHz, $CDCl_3$) δ 136.8, 127.6, 126.7, 126.1, 119.8, 119.5, 114.0, 109.5, 58.8, 46.4, 42.3, 34.8, 31.8, 30.2, 29.2, 29.1, 28.3, 27.0, 24.4, 22.6, 14.0 ppm; HRMS (ESI): *m/z* calcd for $C_{24}H_{38}ClN_2$ $[M + H]^+$ 389.2723, found 389.2723.

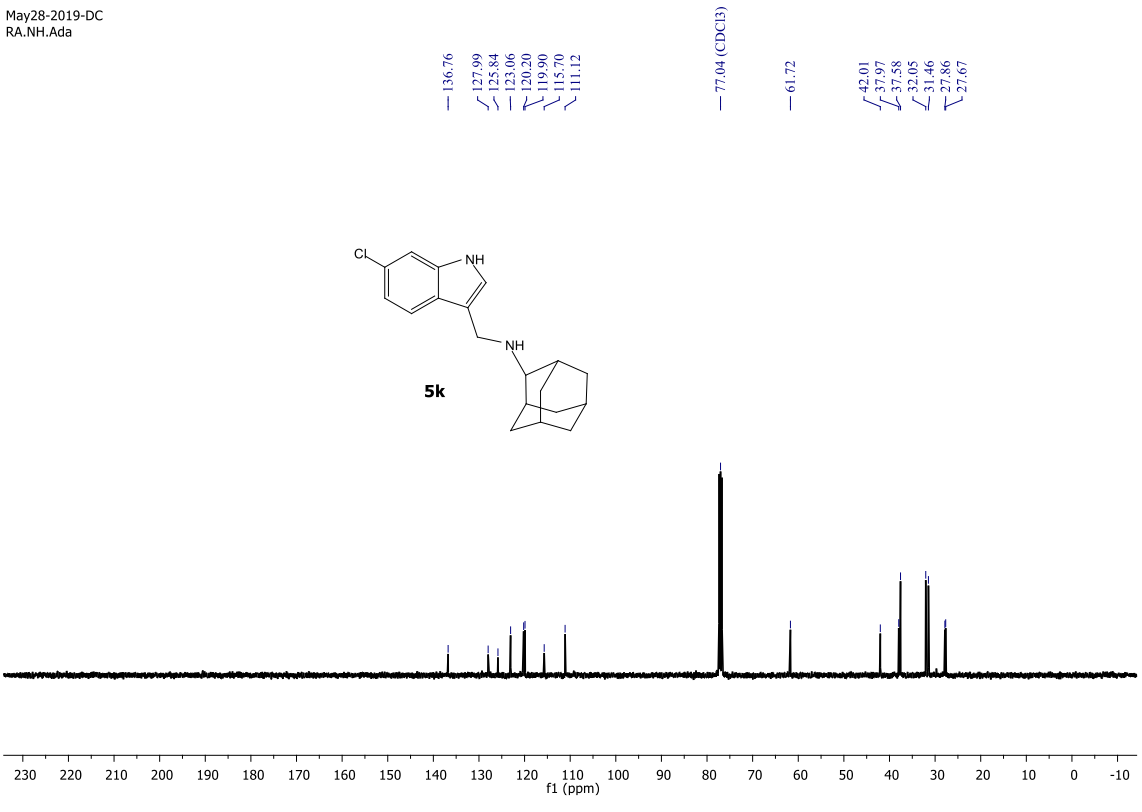
N-Benzyl-1-(6-chloro-1-octyl-1H-indol-3-yl)methanamine (7k). Yield: 4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.4 Hz, 1H), 7.31 (m, 6H), 7.05 (d, *J* = 13.5 Hz, 2H), 4.01 (t, *J* = 7.2 Hz, 2H), 3.95 (s, 2H), 3.86 (s, 2H), 1.80 (m, 2H), 1.61 (s, 2H), 1.36 (br. s., 1H), 1.28 (m, 8H), 0.86 (m, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.4, 128.21, 128.2, 127.6, 126.9, 126.8, 126.2, 120.0, 119.5, 113.7, 109.4, 53.4, 46.4, 44.01, 31.8, 30.2, 29.7, 29.2, 29.1, 27.0, 22.6, 14.1 ppm. HRMS (ESI): *m/z* calcd for $C_{24}H_{32}ClN_2$ $[M + H]^+$ 383.2249, found 383.2265.

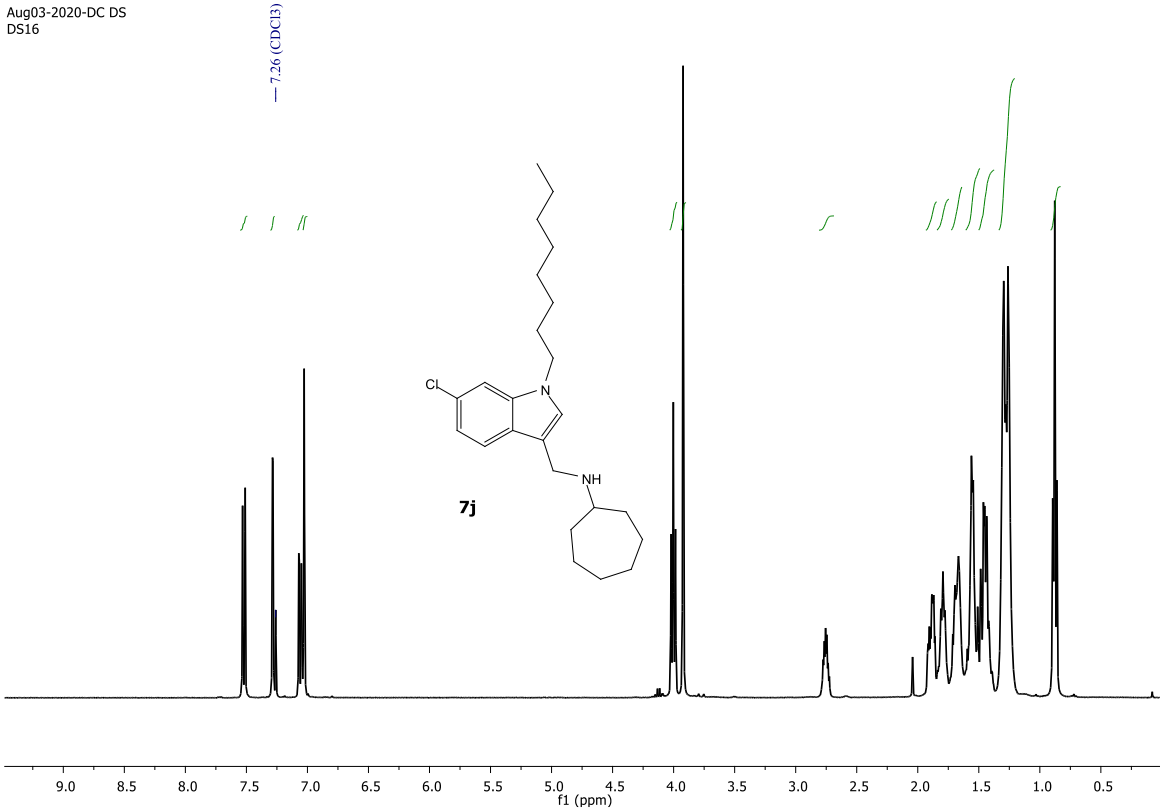
***1-(6-Chloro-1-octyl-1H-indol-3-yl)-N-(cyclohexylmethyl)methanamine (7l)*.** Yield: 15%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.5 Hz, 1H), 7.29 (d, *J* = 1.7 Hz, 1H), 7.06 (m, 2H), 4.04 (t, *J* = 7.1 Hz, 2H), 3.74 (s, 2H), 3.57 (t, *J* = 5.1 Hz, 2H), 2.42 (t, *J* = 5.5 Hz, 2H), 2.21 (m, 2H), 1.79 (m, 4H), 1.64 (m, 6H), 1.25 (m, 10H), 0.87 (t, *J* = 6.8 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.4, 128.5, 127.4, 127.3, 120.2, 119.6, 110.4, 109.4, 55.2, 48.6, 46.4, 32.1, 32.0, 31.8, 30.2, 29.2, 29.2, 27.0, 26.7, 26.2, 22.6, 14.1 ppm. HRMS (ESI): *m/z* calcd for $C_{24}H_{38}ClN_2$ $[M + H]^+$ 389.2718, found 389.2722.

***(E)-N-((6-Chloro-1-(3,7-dimethylocta-2,6-dienyl)-1H-indol-3-yl)methyl)cycloheptan-amine (7m)*.** Yield: 75%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 1.5 Hz, 1H), 7.06 (m, 2H), 5.33 (m, 1H), 5.05 (m, 1H), 4.60 (d, *J* = 6.7 Hz, 2H), 3.91 (s, 2H), 2.74 (m, 1H), 2.08 (m, 4H), 1.88 (m, 3H), 1.80 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.48 (m, 10H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.2, 136.8, 131.9, 127.5, 126.4, 126.3, 123.7, 119.8, 119.6, 119.4, 114.1, 109.7, 58.8, 44.1, 42.3, 39.5, 34.8, 28.4, 26.3, 25.6, 24.5, 17.7, 16.4 ppm. HRMS (ESI): *m/z* calcd for $C_{26}H_{38}ClN_2$ $[M + H]^+$ 413.2718, found 413.2726.

^{1}H & ^{13}C NMR spectra of 5k & 7j *(the most active compounds of the **5a-l** & **7a-m** series)*



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6.0
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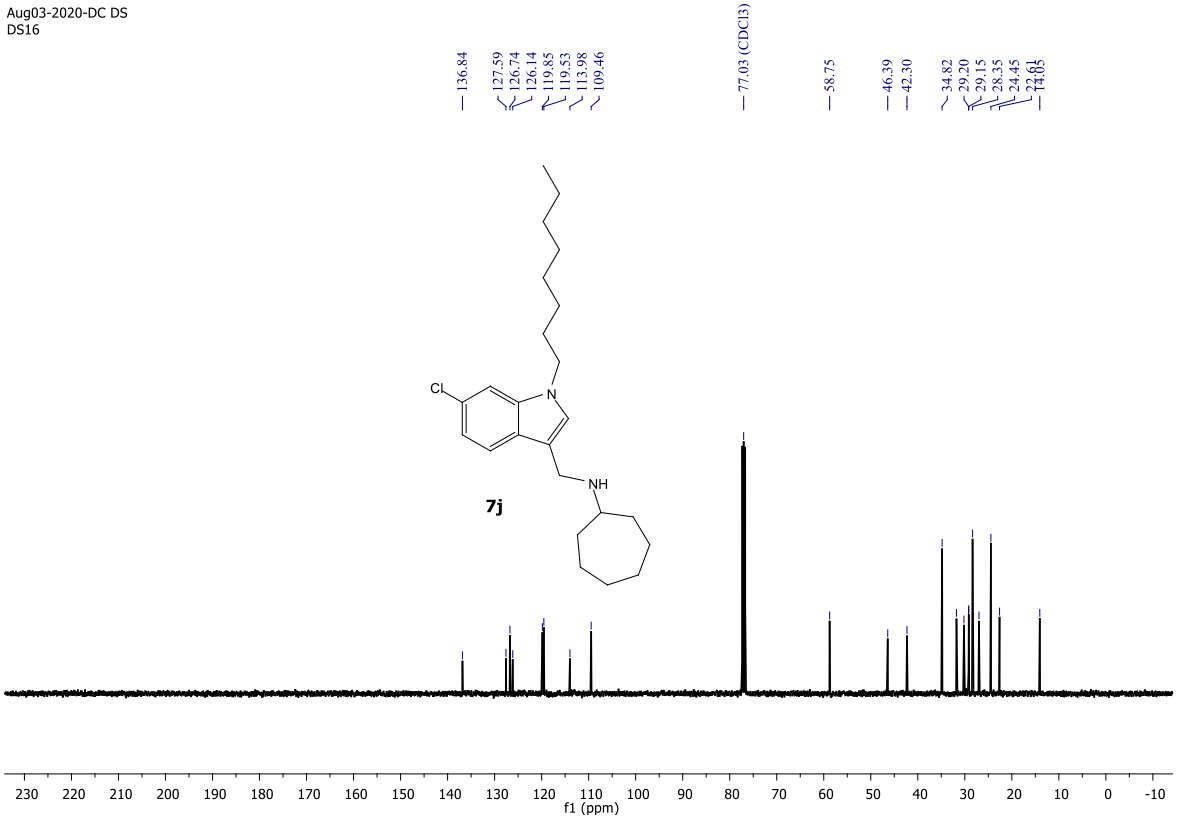
0.5
f1 (ppm)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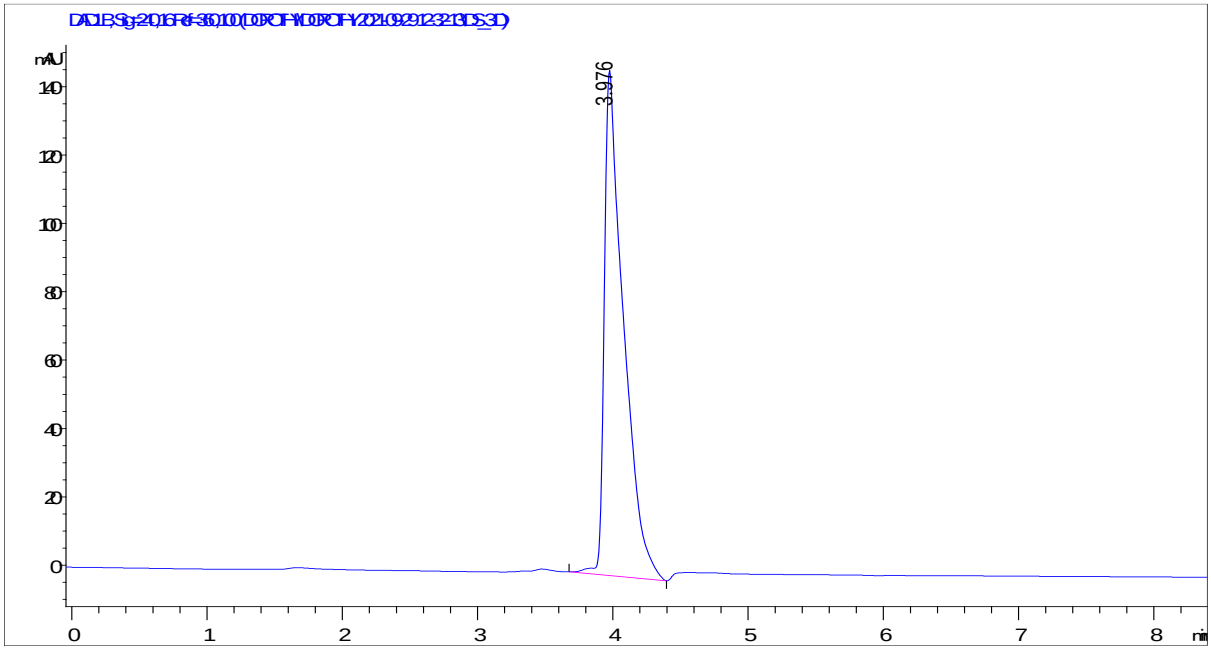
46.39
42.30
34.82
29.20
29.15
28.35
24.45
27.61
14.83
Cl
N
NH
7j
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

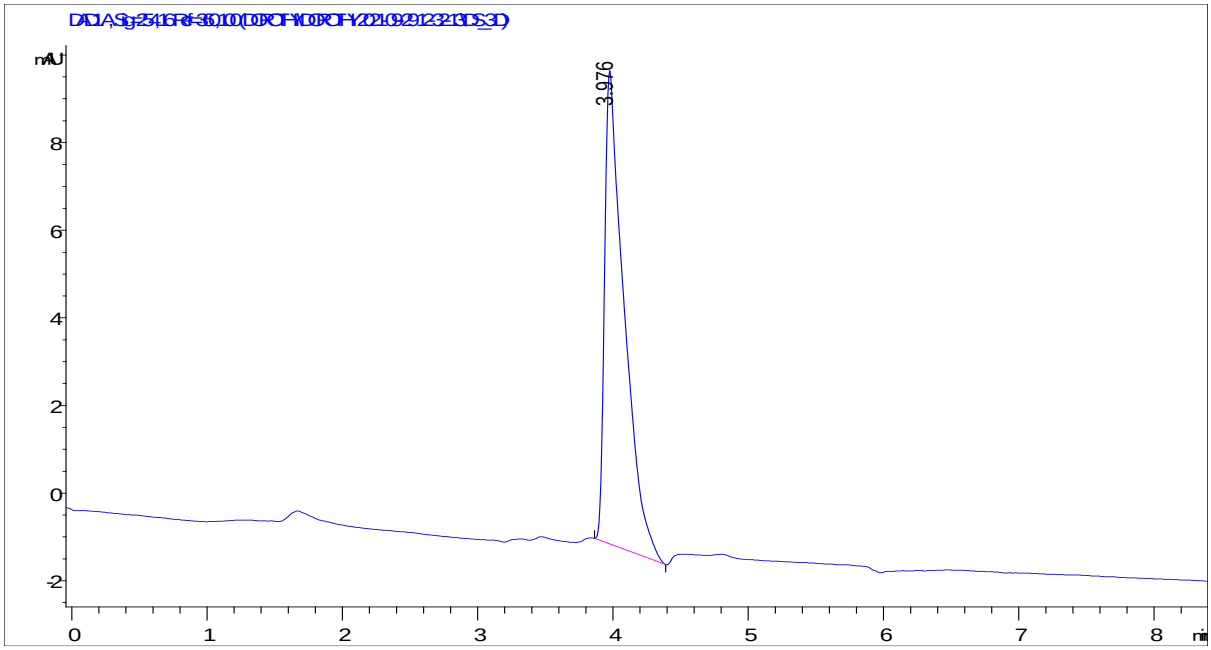
HPLC spectrum of pure 7j

Column: ChiralPak® IC
Eluent: Hex/EtOH (0.1% DEA was added in EtOH) = 60/40
Flow rate: 1mL/min

240nm



254nm



C. Biological evaluation

- **MIC determination**

Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294) and the clinical isolates were cultured in 7H9 medium supplemented with 10% OADC (Oleic acid, albumin, dextrose, and catalase). In a 96-well plate, the compounds and the drugs were serially diluted in the range 25 - 0.10 µg/mL (100 µL) and the mycobacterial suspension was added (100 µL) at approximately 10^5 colony forming unit (CFU)/mL. The plates were incubated at 37°C and 5% CO_2 for seven days and after that the resazurin solution (0.01% - 30µL) was added. After one more day, the fluorescence was read (Excitement / Emission: 530/590 nm). With the fluorescence results and considering a 100% growth control as one that did not receive any treatment, a growth curve was determined according to the concentrations of compounds or drugs tested and we indicated the minimal inhibitory concentration (MIC) as the concentration with 90% of mycobacterial growth inhibition using MS Excel[4,5]. The experiment was performed three times to consider biological replicates and the results are the mean ± standard deviation.

- **Cytotoxicity**

The murine macrophages (Cell line J774A.1, ATCC TIB-67) were cultivated in RPMI media containing 10% Fetal Bovine Serum (FBS) at 37°C and 5% CO_2.

In a 96-well plate, the volume of 100 µL of cell suspension (concentration of $5x10^5$ cells/mL) was added. The plate was incubated for 24h and after that the compounds were added at 100 µg/mL and diluted serially until 0.4 µg/mL. The plate was incubated for another 24h and then 50 µL of 0.01% freshly prepared resazurin solution was added to the wells and this plate was incubated for additional 2-3h to fluorescence reading (Excitement / Emission: 530/590 nm).

Based on the untreated control values, the IC_{50} represents the concentration at which 50% of the cells are viable. The experiment was performed three times to consider biological replicates and the results are the mean ± standard deviation[6].

- **Selective index (SI)**

SI was calculated by the ratio of the IC_{50} and the MIC and represent how much a compound could eliminate the mycobacteria without cell damage; SI greater than 10 is considered promising.[7]

- **Intramacrophagic activity**

Murine macrophages (lineage J774A.1) were cultured in RPMI medium (37°C and 5% CO_2). The cell concentration was adjusted to $5x10^4$ cells/well and 1 mL was plated in a 24-well plate. After 24h for macrophages attachment, 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 (previously determined) and it remained in contact with the infected cells for 72h to ensure 100% cell growth. After this time, the supernatant was discarded, the cells washed with PBS to remove treatment residue and the macrophages had their membranes ruptured (Triton 0,1%). The intramacrophagic content was diluted and seeded onto solid media plates (7H11 with 10% OADC). The colonies forming units (CFU) were counted and the action of the compound was exhibited by the Log_{10} CFU/mL and compared with an untreated control[1].

MIC testing and hit-triage assays.

Unless otherwise indicated, an Alamar Blue fluorescence-based broth microdilution assay was used to assess minimum inhibitory concentration (MIC) of compounds against Mtb strains, as described previously.[8] Briefly, a 10 mL culture of Mtb H37RvMa was grown to an OD600 of 0.6 - 0.7 in standard Middlebrook 7H9 medium supplemented with glycerol or glucose, albumin-dextrose-catalase (ADC), and tween-80. The culture was then diluted in standard Middlebrook 7H9 medium to get an inoculum of ~5 x 10^6 CFU/mL. A two-fold serial dilution of test compounds were prepared in a 96-well microtiter plate containing 50 μL of the growth medium. Finally, 50 μL of the diluted Mtb culture was added. Controls included media only, 5% DMSO, and Rifampicin. The microtiter plate was stored in a secondary container and incubated at 37°C for 7-days with humidifier to prevent evaporation of liquid. The lowest concentration of drug that inhibited > 90% growth of the bacterial population was considered to be the MIC. Biology triage assays were carried out as described earlier.[9–11]

Table S1. Activity of **7j** against bioluminescent reporter strains and mutant strains

	wild type Mtb	Reporter strains		Mutant strains								
	MIC* (µM)	PiniB-LUX	PrecA-LUX	MmpL3 (G253E)	DprE1 (C387S)	CydA-KO	QcrB (A317T)	H37Ra- INH-R	Rif-R	EthA (C253R)	GyrA (G88C)	
7j	1.95	-	-	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	
Controls												
Moxifloxacin	0.39	-	+	ND	ND	ND	ND	0.39	0.39	0.39	>6.25	Controls behaving as expected
Isoniazid	7.8	+	-	ND	ND	ND	ND	>62.5	7.8	7.8	7.8	
Rifampicin	0.001	ND	ND	ND	ND	ND	ND	0.001	>0.1	0.003	0.001	
NITDS8 (known QcrB inhibitor)	0.06	ND	ND	ND	ND	ND	0.48	ND	ND	ND	ND	
H3D-004415 (MmpL3-inhibitor)	1.9	ND	ND	7.8	ND	ND	ND	ND	ND	ND	ND	

*Alamar Blue Assay: Mtb H37RvMA; Media, Middlebrook 7H9. Glucose. Albumin-Dextrose-Catalase. Tween-80; (-) = no signal, (+) = positive signal; ND, not determined.

D. References

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