

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

Dorothy Semenya,<sup>†</sup> Meir Touitou,<sup>†</sup> Camila Maringolo Ribeiro,<sup>§</sup> Fernando Rogerio Pavan,<sup>§</sup> Luca Pivano,<sup>†</sup> Vinayak Singh,<sup>⊥,Δ</sup> Kelly Chibale,<sup>⊥,Δ</sup> Georg Bano,<sup>†</sup> Anita Toscani,<sup>†</sup> Fabrizio Manetti,<sup>‡</sup> Beatrice Gianibbi,<sup>‡</sup> Daniele Castagnolo<sup>\*,†</sup>

<sup>†</sup>School of Cancer and Pharmaceutical Sciences, King's College London, 150 Stamford Street, London SE1 9NH, United Kingdom. <sup>§</sup>Tuberculosis Research Laboratory, School of Pharmaceutical Sciences, Sao Paulo State University (UNESP), Rod. Araraquara-Jau, km1, 14800-903 Araraquara, Brazil. <sup>⊥</sup>Drug Discovery and Development Centre (H3D), University of Cape Town, Rondebosch 7701, South Africa. <sup>Δ</sup>South African Medical Research Council Drug Discovery and Development Research Unit, Department of Chemistry and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa. <sup>‡</sup>Dipartimento di Biotecnologie, Chimica e Farmacia, – Dipartimento di Eccellenza 2018-2022, via A. Moro 2, I-53100 Siena, Italy

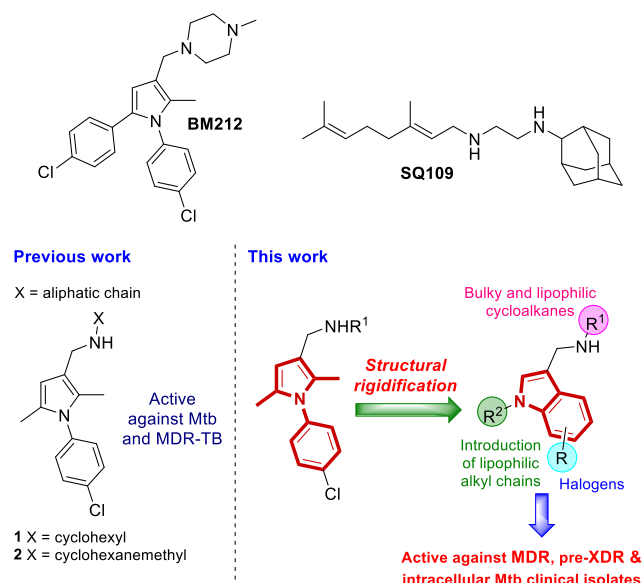
**ABSTRACT:** A series of indolyl-3-methyleneamines incorporating lipophilic side chains were designed through a structural rigidification approach and synthesized for investigation as new chemical entities against *Mycobacterium tuberculosis* (Mtb). The screening led to the identification of a 6-chloroindole analogue **7j**, bearing an *N*-octyl chain and a cycloheptyl moiety, which displayed potent *in vitro* activity against laboratory and clinical Mtb strains including a pre-extensively drug-resistant (pre-XDR) isolate. **7j** also demonstrated a marked ability to restrict the intracellular growth of Mtb in murine macrophages. Further assays geared toward mechanism of action elucidation have thus far ruled out the involvement of various known promiscuous targets thereby suggesting that the new indole **7j** may inhibit Mtb via a unique mechanism.

**KEYWORDS.** Tuberculosis, MDR-TB, XDR-TB, indole, pyrrole, antimicrobial resistance

Tuberculosis (TB) is an ancient scourge that blights millions of lives annually. In 1882, Robert Koch discovered *Mycobacterium tuberculosis* (Mtb) as the etiologic agent of TB heralding multitudinous advances against its pathogenesis.<sup>1</sup> TB has ranked above HIV/AIDS as the leading deadliest infectious disease since 2007 which spotlights its implacable threat to public health security; this burden is further aggravated by the rampant emergence of drug resistance in Mtb.<sup>2-4</sup> In 2019, an estimated 10 million people developed TB and 1.4 million died. It is also estimated that close to a quarter of the global population is latently infected with Mtb and the risk of progression to symptomatic TB disease is about 5-10% over a lifetime.<sup>2,5</sup> First-line treatment of active TB hinges on gruelling 6-month regimens comprising isoniazid, rifampicin, ethambutol and pyrazinamide, at around US\$ 40 per patient. MDR-TB that is resistant to isoniazid and rifampicin, requires a more protracted course of treatment which is not only inordinately expensive ( $\geq$ US\$ 1000 per person) but also involves more toxic drugs, and is vitiated by poor adherence.<sup>2,6,7</sup> Reports of more severe forms of TB, including pre-extensively drug-resistant (pre-XDR) TB that displays greater recalcitrance to the current anti-TB drug armamentarium and below par therapy success rates, paint a grim picture.<sup>8-11</sup> These exigencies necessitate intensified and sustained TB mitigation efforts, all the more so in light of the unprecedented COVID-19 pandemic which has cast a shadow over strides made thus far.

Drug discovery and development is central to TB research and innovation, but it is a laborious process that is faced with innumerable stumbling blocks including, inter alia, a paucity of research funds and investments, high drug attrition rates, biological complexities of Mtb in tandem with the lack of animal models that are representative of TB pathogenesis as seen in human hosts.<sup>2,12</sup> Mtb's inherent

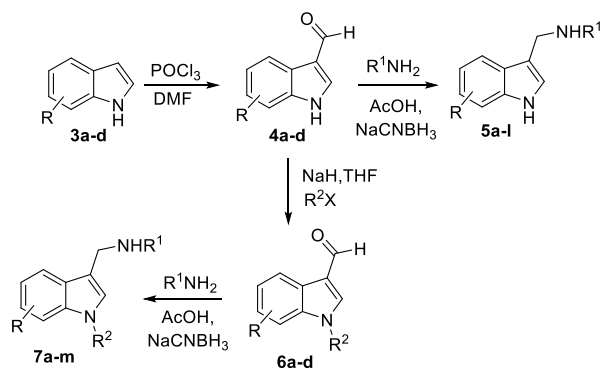
characteristics (e.g. the highly impermeable lipid-rich cell wall or the intrinsic activity of efflux pumps) in addition to the acquisition of mutations in genes encoding drug targets or drug-activating enzymes, confer resistance to antibiotics. Furthermore, Mtb is capable of altering its metabolic state from active growth to dormancy, effectuating phenotypic antibiotic resistance – this transition in itself is a challenge to the treatment of TB infection.<sup>13-15</sup> In spite of these challenges, 22 anti-TB drug candidates and various combination regimens were in clinical trials as of August 2020.<sup>2,16</sup>



**Figure 1.** Previously identified antitubercular pyrroles **1-2** and the rationale behind this study

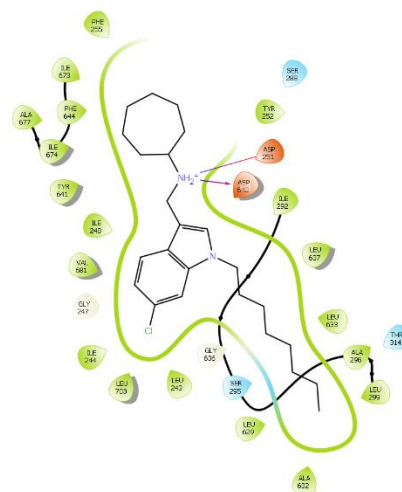
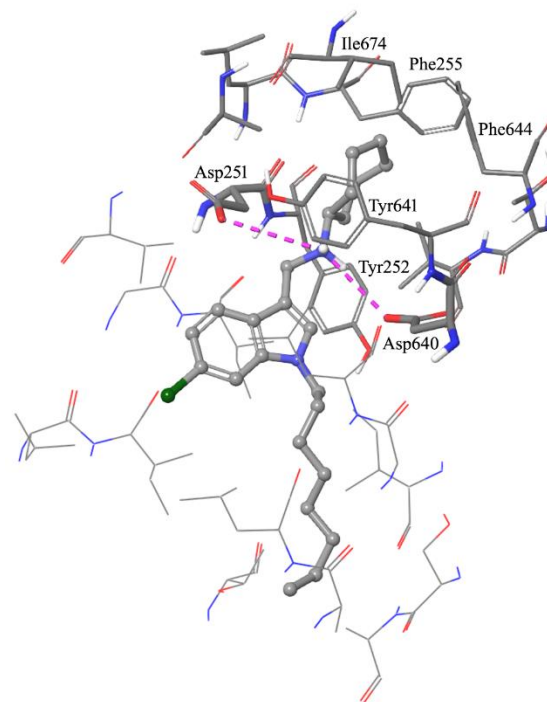
Some candidates in the development pipeline represent novel scaffolds and/or appealing drug targets e.g. oxaborole GSK-3036656 targeting leucyl-tRNA synthetase which is crucial for protein synthesis; benzothiazinones (BTZ043 & macozinone) and ethylenediamine SQ109 that interfere with cell wall assembly by blocking decaprenyl-phosphoribose-2'-epimerase (DprE1) and mycobacterial membrane protein large 3 (MmpL3), respectively. Moreover, three newly approved drugs have spurred renewed hope for tackling drug-resistant TB: bedaquiline (a diarylquinoline inhibiting ATP synthase) and two nitroimidazoles (delamanid and pretomanid) targeting cell wall biosynthesis and cell respiration.<sup>14,16-18</sup> Despite this ray of hope, there remains a need to ramp up TB drug discovery and development efforts so as to launch medicines that are not only safe and effective but also affordable and accessible.

Nitrogen-containing heterocycles are considered privileged and versatile scaffolds which constitute the core frameworks of many bioactive molecules and have thus garnered notable attention in library design of prospective drugs.<sup>19-21</sup> In the recent past, we reported the discovery of a series of antitubercular *N*-aryl-2,5-dimethylpyrroles including **1-2** (Figure 1), designed as molecular hybrids of the drugs BM212 and SQ109 and endowed with activity against drug-resistant mycobacteria.<sup>22-24</sup> In an endeavour to interrogate the structure-activity relationship (SAR) of our pyrroles more extensively, and with the aim to improve their properties or to find similar potent compounds that exist in novel chemical space, we adopted a structural rigidification strategy to design new antitubercular agents by substituting the *N*-aryl-2,5-dimethylpyrrole moiety of **1-2** with an indole nucleus. While our previous studies showed that replacement of the sole pyrrole ring of **1-2** with other heterocyclic cores was detrimental to the antitubercular activity,<sup>24</sup> indole motifs have recently attracted interest as valuable scaffolds in the discovery of effective antimycobacterial agents.<sup>25-30</sup> Herein, we describe the design, synthesis and biological evaluation of a series of structurally related indole compounds with potent activity against MDR-, pre-XDR- and intracellular Mtb clinical isolates. In this respect, we also provide a preliminary account of their mechanism of action (MoA) (Figure 1).



**Scheme 1.** Main synthetic routes followed for the synthesis of the desired indole derivatives **5a-l** and **7a-m**

We designed a new series of indoles **5a-l**, taking into consideration SAR deductions and molecular docking observations from our antimycobacterial pyrrole studies (Scheme 1, Table 1).<sup>22-24</sup> In line with our previous SAR considerations, the first series was designed by appending bulky and lipophilic amino-cycloalkanes to C3 of either unsubstituted or C5-/C6-substituted indole scaffolds to probe substituent effects. The synthesis of the derivatives is depicted in Scheme 1.<sup>23,24</sup> In brief, compounds **5a-l** were prepared via a facile two-step synthesis starting out with Vilsmeier-Haack formylation of commercially available indoles **3a-d** followed by reductive amination of aldehydes **4a-d** using NaCNBH<sub>3</sub> as the reducing agent (Scheme 1).



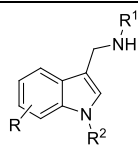
**Figure 2.** Best docked pose of *N*-alkyl indole **7j** into the Mtb MmpL3 homology model. Amino acids of the hydrophobic cage surrounding the heptylamino side chain are represented by thin tubes and some of them are also labeled; Asp251 and Asp640 responsible for two salt bridges with the basic amino group of **7j** 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.

In our previous works, we postulated an MmpL3 inhibition mechanism for our *N*-aryl-pyrroles based on their structural similarity to the known inhibitors BM212 and SQ109.<sup>31</sup> Based on this premise, we hypothesized a similar MoA for the indole compounds and thus adopted a molecular docking-aided approach in generating a second series of derivatives bearing substituents at the N1 position with the aim to improve their activity.

**Table 1.** Structures of the indole derivatives **5a-l** and **7a-m** and activity against Mtb H37Rv

Cmpd	R	R <sup>2</sup>	R <sup>1</sup>	MIC (µg/mL)	
				Mean	± SD
5a	6-Cl	H	cyclohexyl	>25	
5b	5-Cl	H	cyclohexyl	16.33	± 0.01
5c	5-Me	H	cyclohexyl	>25	
5d	6-Cl	H	cyclohexanemethyl	24.20	± 0.93
5e	H	H	cyclohexanemethyl	>25	
5f	6-Cl	H	cycloheptyl	17.14	± 5.30
5g	5-Me	H	cycloheptyl	24.72	± 0.32
5h	H	H	cycloheptyl	>25	
5i	6-Cl	H	cyclooctyl	>25	
5j	H	H	cyclooctyl	>25	
5k	6-Cl	H	2-adamantyl	6.07	± 0.22
5l	6-Cl	H	benzyl	22.83	± 2.41
7a	6-Cl	Me	cyclohexyl	>25	
7b	6-Cl	Me	cyclohexanemethyl	19.09	± 4.91
7c	6-Cl	Me	cyclooctyl	23.25	± 1.75
7d	6-Cl	Me	2-adamantyl	10.93	± 1.38
7e	6-Cl	Me	benzyl	>25	
7f	6-Cl	iPr	cyclohexyl	23.87	± 0.36
7g	6-Cl	iPr	cyclohexanemethyl	6.09	± 0.28
7h	6-Cl	iPr	cycloheptyl	16.32	± 4.98



7i	6-Cl	iPr	cyclooctyl	14.00	± 3.23
7j	6-Cl	Octyl	cycloheptyl	0.96	± 0.22
7k	6-Cl	Octyl	benzyl	19.73	± 0.09
7l	6-Cl	Octyl	cyclohexanemethyl	1.56	± 0.24
7m	6-Cl	Geranyl	cycloheptyl	2.75	± 0.79
1	-	-	-	0.2 <sup>22</sup>	
2	-	-	-	0.3 <sup>24</sup>	

The results are mean ± standard deviation of three independent tests.

The *N*-substituted indoles were docked into an Mtb MmpL3 homology model<sup>24,32,33</sup> and compared to the binding poses of pyrrole **2** and SQ109 (a comparison of the best scoring binding pose of both SQ109 and **7j** is reported in Figure S1, while the best scoring binding pose of **5f**, **7a**, **7h** and **7m** are reported in Figure S3). The molecular docking suggested that the introduction of lipophilic aliphatic groups of varying sizes at the N1 position could lead to better interaction of the compounds with the hydrophobic binding pocket of MmpL3 (Figure 2). Thus, a series of *N*-substituted indoles **7a-m** were synthesised by alkylation of 6-chloro-1*H*-indole-3-carbaldehyde **4a** with the appropriate alkylating agents in the presence of NaH, and subsequent reductive amination as aforementioned (Scheme 1).

First, we evaluated the minimal inhibitory concentration (MIC) of the indole derivatives **5a-l** and **7a-m** against drug-sensitive Mtb H37Rv using a resazurin-based microtiter assay in 96-well plates (Table 1).<sup>34,35</sup> As a general trend, most of the compounds exhibited good antimycobacterial activity. Indole **5k** bearing a 2-adamantyl moiety on the methyleneamine side chain at C3 was the most active among compounds **5a-l** lacking alkyl substituents at the N1 position of the scaffold.

The presence of a chloro substituent instead of a methyl group on the indole core appeared advantageous, as exemplified by compounds **5b-5c** and **5f-5g**. Interestingly, the derivative **5d**, bearing the same functionalities of the pyrrole hit **2**, displayed poorer activity. On the other hand, enhancement of the lipophilic character of the analogues by introducing methyl, isopropyl, octyl or geranyl groups on the N1 position proved to be favourable leading to a pronounced improvement in activity.

An analysis of the biological data allowed us to define several SAR rules. Compounds with an 8-carbon long hydrophobic side chain showed the best activity and the saturated octyl appendage of **7j** was preferred to the unsaturated chain of the same length present in **7m** (0.96 µg/mL and 2.75 µg/mL, respectively; the best docked pose of **7m** is represented in Figure S2). Among N1-octyl derivatives, the cycloalkyl analogues **7j** and **7l** showed similar activity (0.96 µg/mL and 1.56 µg/mL, respectively), while the benzyl analogue **7k** showed a significant decrease in activity (19.7 µg/mL). This suggests that an aromatic portion is not tolerated in such a position, as also confirmed by **5l**

and **7e** that showed activity values higher than 20. Reduction of the size of the N1 substituent led to low-active or inactive compounds. As an example, among the C3-cyclohexanemethyl analogues, shortening the octyl chain of **7i** to isopropyl, methyl, or hydrogen (as in **7g**, **7b**, and **5d**) led to about 4-15-fold reduction in activity (from 1.56 µg/mL to 6.09 µg/mL, 19.1 µg/mL, and 24.2 µg/mL, respectively). A very similar trend was observed among the cycloheptyl analogues, with activity ranging from 0.96 µg/mL (found for the octyl derivative **7j**) to 16.3 µg/mL (for the isopropyl analogue **7h**), and to 17.1 µg/mL (for the N1 unsubstituted analogue **5f**). Finally, the adamantyl derivatives **5k** and **7d** showed an interesting antimycobacterial activity (6 µg/mL and 10 µg/mL, respectively), independently from the presence of a long alkyl chain at N1.

The four derivatives with the higher *in vitro* antimycobacterial activity for each N1-substituted group, namely **5k**, **7d**, **7g** and **7j**, were selected for additional screening. The four compounds were first screened against murine macrophages (J774A.1 cell line) to assess their cytotoxicity profiles and to determine their selectivity indices (SI) (Table 2). Again, the most active compound **7j** showed low cytotoxicity ( $IC_{50} = 10.62$  µg/mL) and a SI greater than 10, thus turning out to be the best hit of the whole series.

**Table 2.** Cytotoxicity of select derivatives against murine macrophages

Cmpd	CLogP	$IC_{50}$ (µg/mL)	MIC (µg/mL)	SI
5k	6.08	7.51±2.58	6.07	1.24
7d	6.42	16.19±4.26	10.93	1.48
7g	6.21	14.57±3.50	6.09	2.39
7j	9.01	10.62±4.02	0.96	11.06

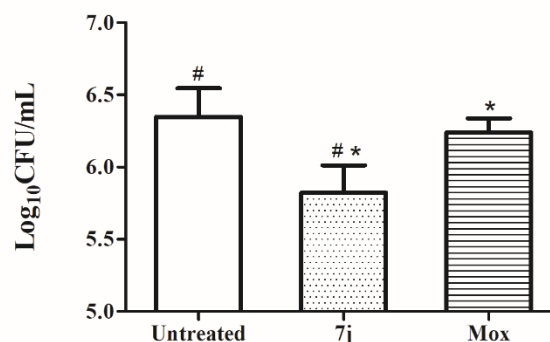
The results are mean ± standard deviation of three independent tests. The selectivity index is calculated as a ratio between the  $IC_{50}$  and the MIC values observed against Mtb H37Rv

Encouraged by the potent *in vitro* activity of **7j**, it was further assayed against a panel of MDR and pre-XDR mycobacterial clinical isolates. The compound **7j** displayed excellent activity against all the drug-resistant clinical isolates CI1-4 with MICs below 2 µg/mL and in some respects, a superior profile in comparison to the reference clinical antibiotics (Table 3). In particular, **7j** showed potent activity against the more recalcitrant pre-XDR Mtb CI3, with an MIC = 0.86 µg/mL similar to that against the drug-susceptible Mtb H37Rv strain (0.96 µg/mL) and >10 fold more potent than that of the second-line drug moxifloxacin.

Countering Mtb's persistence, partly attributable to its ability to subvert host immune responses and survive in macrophages, is a priority of myriad anti-TB drug discovery campaigns in their infancy.<sup>36,37</sup> In this regard, indole **7j**

was also assayed against murine macrophages infected with Mtb, where previously an incubation of 72 h ensured 100% macrophage growth at the concentration tested, to assess its ability to traverse cell membranes and inhibit the replication of intracellular bacilli.

The activity against intracellular Mtb is presented as  $\text{Log}_{10}$  colony forming unit (CFU)/mL and the results of **7j** and the reference antibiotic moxifloxacin are compared with an untreated control. Interestingly, **7j** showed a reduction of 0.53  $\text{Log}_{10}$  CFU/mL at a concentration 1 x MIC, and moxifloxacin only inhibited 0.11  $\text{Log}_{10}$  CFU/mL under the same conditions (1 x MIC). (Figure 3).



**Figure 3.** Intra-macrophagic activity of **7j** and Moxifloxacin (Mox) against Mtb-infected murine macrophages (J774A.1 cell line). Macrophages grew in the presence of **7j** and Mox at the concentration tested to ensure 100% cell growth. Concentration was 1 x MIC (Mox = 0.17 µg/mL and **7j** = 0.96 µg/mL). #,\* = significantly different according to one-way ANOVA with Newman-Keuls's post-test ( $p < 0.05$ ). The results are the mean ± standard deviation of at least two independent assays.

The indole derivatives **5** and **7** were designed as analogues of **1-2**, in turn synthesized as hybrids of the MmpL3 inhibitors BM212 and SQ109. Thus, a similar MoA was initially hypothesized for compound **7j**. However, with the aim to demonstrate experimentally the mechanism by which **7j** inhibits Mtb, we embarked on target deconvolution studies to elucidate its mechanistic profile. As a starting point, **7j** was tested in biology triage assays to assess whether known promiscuous targets are implicated in its MoA (Table S1). The compound **7j** did not show a positive signal in two standard bioluminescence reporter assays, namely the PiniB-LUX which detects modulation in *iniB* expression if a test compound is implicated in Mtb cell wall damage and the PrecA-LUX which detects modulation in *recA* expression, an indicator of compounds that disrupt DNA integrity. Moreover, it did not show MIC modulation against mutant strains including MmpL3 (G253E), DprE1 (C387S), CydA-KO, QcrB (A317T), and mono-resistant strains of standard anti-TB drugs (isoniazid, rifampicin, ethionamide, and moxifloxacin).

**Table 3.** MICs ( $\mu\text{g/mL}$ ) of **7j** and clinical antibiotics against Mtb H37Rv and four clinical isolates

Cmpd	H37Rv	Clinical isolate CI1	Clinical isolate CI2	Clinical isolate CI3	Clinical isolate CI4
7j	0.96 $\pm$ 0.22	1.97 $\pm$ 0.44	1.87 $\pm$ 0.68	0.86 $\pm$ 0.20	0.69 $\pm$ 0.04
Rifampicin (RIF)	<0.10	>25	0.65 $\pm$ 0.16	>25	<0.10
Isoniazid (INH)	0.30 $\pm$ 0.07	>25	>25	>25	0.25 $\pm$ 0.07
Amikacin (AMK)	0.27 $\pm$ 0.06	0.32 $\pm$ 0.08	23.13 $\pm$ 2.49	>25	0.14 $\pm$ 0.05
Moxifloxacin (MFX)	0.17 $\pm$ 0.08	0.20 $\pm$ 0.06	0.19 $\pm$ 0.09	10.26 $\pm$ 1.83	< 0.10
Classification	Susceptible laboratory strain	MDR-TB	Resistant to INH and AMK	Pre-XDR-TB	Susceptible clinical isolate

The results are mean  $\pm$  standard deviation of three independent tests

Altogether, these results seemingly suggest that **7j** exerts its antimycobacterial action via a novel mechanism. However, given the good binding affinity of **7j** to MmpL3 observed in computational studies, it cannot be excluded that MmpL3 may play a role in the mechanism of the indole compounds, such as being involved in the transport of molecules from extracellular space to an intracellular target. Mutagenesis and genome sequencing studies are currently in progress in our laboratories to ascertain the MoA of the newly identified antitubercular compound **7j**.

In conclusion, a new series of indoles (**5a-l** and **7a-k**) were designed through a structural rigidification approach as analogues of the antitubercular *N*-aryl-2,5-dimethylpyrroles **1-2** in efforts to explore their chemical space more exhaustively. The set of derivatives were synthesized and screened against Mtb which led to the identification of compound **7j** with outstanding *in vitro* potency against the drug-sensitive strain H37Rv as well as MDR and pre-XDR clinical isolates. **7j** also proved to be effective against intracellular Mtb bacilli showing approximately 60% growth inhibition at 1  $\times$  MIC. Finally, preliminary MoA studies have suggested that known promiscuous targets such as DprE1 are likely not implicated in the mechanism of **7j**. Further investigations are currently underway to fully elucidate its MoA and/or ascertain its biological target.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. General procedures for the synthesis and biological evaluation of the compounds are reported. Full characterization of compounds **5** and **7** is reported.

## AUTHOR INFORMATION

### Corresponding Author

\* Corresponding author. [daniele.castagnolo@kcl.ac.uk](mailto:daniele.castagnolo@kcl.ac.uk)

### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Funding Sources

EPSRC (Global Challenges Competition King's College London)  
South African National Research Foundation-SARChI

## ACKNOWLEDGMENT

We gratefully acknowledge EPSRC (Global Challenges Competition King's College London). DS acknowledges the South African National Research Foundation-SARChI for financial support. MT acknowledges King's College London for a period of leave. DC and AT acknowledge the University of London for the Maplethorpe Fellowship to AT. The South African Medical Research Council (SAMRC), South African Department of Science and Innovation and the South African National Research Foundation are gratefully acknowledged for research funding (KC and VS).

## ABBREVIATIONS

TB, tuberculosis; Mtb, *Mycobacterium tuberculosis*; MDR, multidrug resistant; XDR, extensively drug-resistant; MIC, minimum inhibitory concentration.

## REFERENCES

- (1) Daniel, T. M. The History of Tuberculosis. *Respir. Med.* **2006**, *100* (11), 1862–1870.
- (2) World Health Organization: Global Tuberculosis Report 2020  
<https://www.who.int/publications/i/item/9789240013131>.
- (3) Mabhula, A.; Singh, V. Drug-Resistance in Mycobacterium Tuberculosis: Where We Stand. *Medchemcomm* **2019**, *10* (8), 1342–1360.
- (4) Sheikh, B. A.; Bhat, B. A.; Mehraj, U.; Mir, W.; Hamadani, S.; Mir, M. A. Development of New Therapeutics to Meet the Current Challenge of Drug Resistant Tuberculosis. *Curr. Pharm. Biotechnol.* **2020**, *22* (4), 480–500.
- (5) Chakaya, J.; Khan, M.; Ntoumi, F.; Aklillu, E.; Fatima, R.; Mwaba, P.; Kapata, N.; Mfinanga, S.; Hasnain, S. E.; Katoto, P. D. M. C.; Bulabula, A. N. H.; Sam-Agudu, N. A.; Nachege, J. B.; Tiberi, S.; McHugh, T. D.; Abubakar, I.; Zumla, A. Global Tuberculosis Report 2020 – Reflections on the Global TB Burden, Treatment and Prevention Efforts. *Int. J. Infect. Dis.* **2021**, 4–9.
- (6) Tiberi, S.; Scardigli, A.; Centis, R.; D'Ambrosio, L.; Muñoz-Torrico, M.; Salazar-Lezama, M. Á.; Spanevello, A.; Visca, D.; Zumla, A.; Migliori, G. B.; Caminero Luna, J. A. Classifying New Anti-Tuberculosis Drugs: Rationale and Future Perspectives. *Int. J. Infect. Dis.* **2017**, *56*, 181–184.

- (7) Silva, D. R.; Mello, F. C. de Q.; Migliori, G. B. Shortened Tuberculosis Treatment Regimens: What Is New? *J. Bras. Pneumol.* **2020**, *46* (2), 1–8.
- (8) Sharma, A.; De Rosa, M.; Singla, N.; Singh, G.; Barnwal, R. P.; Pandey, A. Tuberculosis: An Overview of the Immunogenic Response, Disease Progression, and Medicinal Chemistry Efforts in the Last Decade toward the Development of Potential Drugs for Extensively Drug-Resistant Tuberculosis Strains. *J. Med. Chem.* **2021**, *64* (8), 4359–4395.
- (9) Santos-Lazaro, D.; Gavilan, R. G.; Solari, L.; Vigo, A. N.; Puyen, Z. M. Whole Genome Analysis of Extensively Drug Resistant Mycobacterium Tuberculosis Strains in Peru. *Sci. Rep.* **2021**, *11* (1), 1–12.
- (10) Abubakar, M.; Ahmad, N.; Ghafoor, A.; Latif, A.; Ahmad, I.; Atif, M.; Saleem, F.; Khan, S.; Khan, A.; Khan, A. H. Treatment Outcomes of Extensively Drug-Resistant Tuberculosis in Pakistan: A Countrywide Retrospective Record Review. *Front. Pharmacol.* **2021**, *12*, 1–9.
- (11) World Health Organization 2020 Meeting Report of the WHO Expert Consultation on the Definition of Extensively Drug-Resistant Tuberculosis <https://www.who.int/publications/i/item/meeting-report-of-the-who-expert-consultation-on-the-definition-of-extensively-drug-resistant-tuberculosis>.
- (12) Evangelopoulos, D.; McHugh, T. D. Improving the Tuberculosis Drug Development Pipeline. *Chem. Biol. Drug Des.* **2015**, *86* (5), 951–960.
- (13) Kolyva, A. S.; Karakousis, P. C. Old and New TB Drugs: Mechanisms of Action and Resistance. In *Understanding Tuberculosis—New Approaches to Fighting against Drug Resistance*; 2012; pp 209–232.
- (14) Semanya, M. M. D. Recent Advances in Repositioning Non-Antibiotics against Tuberculosis and Other Neglected Tropical Diseases. In *Medicinal Chemistry of Neglected and Tropical Diseases: Advances in the Design and Synthesis of Antimicrobial Agents*; Jayaprakash, V., Castagnolo, D., Özkay, Y., Eds.; CRC Press, 2019.
- (15) Nguyen, L. Antibiotic Resistance Mechanisms in Mtb: An Update. *Arch Toxicol* **2017**, *90* (7), 1585–1604.
- (16) Stop TB Partnership Working Group on New TB Drugs <https://www.newtbdrugs.org/>.
- (17) Huszár, S.; Chibale, K.; Singh, V. The Quest for the Holy Grail: New Antitubercular Chemical Entities, Targets and Strategies. *Drug Discov. Today* **2020**, *25* (4), 772–780.
- (18) Tiberi, S.; du Plessis, N.; Walzl, G.; Vjecha, M. J.; Rao, M.; Ntoumi, F.; Mfinanga, S.; Kapata, N.; Mwaba, P.; McHugh, T. D.; Ippolito, G.; Migliori, G. B.; Maeurer, M. J.; Zumla, A. Tuberculosis: Progress and Advances in Development of New Drugs, Treatment Regimens, and Host-Directed Therapies. *Lancet Infect. Dis.* **2018**, *18* (7), e183–e198.
- (19) Kumari, A.; Singh, R. K. Medicinal Chemistry of Indole Derivatives: Current to Future Therapeutic Prospectives. *Bioorg. Chem.* **2019**, *89*, 103021.
- (20) Thanikachalam, P. V.; Maurya, R. K.; Garg, V.; Monga, V. An Insight into the Medicinal Perspective of Synthetic Analogs of Indole: A Review. *Eur. J. Med. Chem.* **2019**, *180*, 562–612.
- (21) Ahmad, S.; Alam, O.; Naim, M. J.; Shaquiquzzaman, M.; Alam, M. M.; Iqbal, M. *Pyrrole: An Insight into Recent Pharmacological Advances with Structure Activity Relationship*; Elsevier Masson SAS, 2018; Vol. 157.
- (22) Bhakta, S.; Scalacci, N.; Maitra, A.; Brown, A. K.; Dasugari, S.; Evangelopoulos, D.; McHugh, T. D.; Mortazavi, P. N.; Twist, A.; Petricci, E.; Manetti, F.; Castagnolo, D. Design and Synthesis of 1-((1,5-Bis(4-Chlorophenyl)-2-Methyl-1H-Pyrrol-3-Yl)methyl)-4-Methylpiperazine (BM212) and N-Adamantan-2-Yl-N'-((E)-3,7-Dimethylocta-2,6-Dienyl)Ethane-1,2-Diamine (SQ109) Pyrrole Hybrid Derivatives: Discovery of Potent Antitubercul. *J. Med. Chem.* **2016**, *59* (6), 2780–2793.
- (23) Masci, D.; Hind, C.; Islam, M. K.; Toscani, A.; Clifford, M.; Coluccia, A.; Conforti, I.; Touitou, M.; Memdouh, S.; Wei, X.; La Regina, G.; Silvestri, R.; Sutton, J. M.; Castagnolo, D. Switching on the Activity of 1,5-Diaryl-Pyrrole Derivatives against Drug-Resistant ESKAPE Bacteria: Structure-Activity Relationships and Mode of Action Studies. *Eur. J. Med. Chem.* **2019**, *178*, 500–514.
- (24) Touitou, M.; Manetti, F.; Ribeiro, C. M.; Pavan, F. R.; Scalacci, N.; Zrebna, K.; Begum, N.; Semanya, D.; Gupta, A.; Bhakta, S.; Mchugh, T. D.; Senderowitz, H.; Kyriazi, M.; Castagnolo, D. Improving the Potency of N-Aryl-2,5-Dimethylpyrroles against Multidrug-Resistant and Intracellular Mycobacteria. *ACS Med. Chem. Lett.* **2020**, *11* (5), 638–644.
- (25) Onajole, O. K.; Pieroni, M.; Tipparaju, S. K.; Lun, S.; Stec, J.; Chen, G.; Gunosewoyo, H.; Guo, H.; Ammerman, N. C.; Bishai, W. R.; Kozikowski, A. P. Preliminary Structure–Activity Relationships and Biological Evaluation of Novel Antitubercular Indolecarboxamide Derivatives Against Drug-Susceptible and Drug-Resistant Mycobacterium Tuberculosis Strains. *J. Med. Chem.* **2013**, *56*, 4093–4103.
- (26) Stec, J.; Onajole, O. K.; Lun, S.; Guo, H.; Merenbloom, B.; Vistoli, G.; Bishai, W. R.; Kozikowski, A. P. Indole-2-Carboxamide-Based MmpL3 Inhibitors Show Exceptional Antitubercular Activity in an Animal Model of Tuberculosis Infection. *J. Med. Chem.* **2016**, *59*, 6232–6247.
- (27) Nyantakyi, S. A.; Li, M.; Gopal, P.; Zimmerman, M.; Dartois, V.; Gengenbacher, M.; Dick, T.; Go, M. L. Indolyl Azaspiroketal Mannich Bases Are Potent Antimycobacterial Agents with Selective Membrane Permeabilizing Effects and in Vivo Activity. *J. Med. Chem.* **2018**, *61* (13), 5733–5750.
- (28) Li, M.; Phua, Z. Y.; Xi, Y.; Xu, Z.; Nyantakyi, S. A.; Li, W.; Jackson, M.; Wong, M. W.; Lam, Y.; Chng, S. S.; Go, M. L.; Dick, T. Potency Increase of Spiroketal Analogs of Membrane Inserting Indolyl Mannich Base Antimycobacterials Is Due to Acquisition of MmpL3 Inhibition. *ACS Infect. Dis.* **2020**, *6* (7), 1882–1893.
- (29) Tan, Y. J.; Li, M.; Gunawan, G. A.; Nyantakyi, S. A.; Dick, T.; Go, M.-L.; Lam, Y. Amide–Amine Replacement in Indole-2-carboxamides Yields Potent Mycobactericidal Agents with Improved Water Solubility. *ACS Med. Chem. Lett.* **2021**, *12*, (5) 704–712.
- (30) Li, M.; Nyantakyi, S. A.; Gopal, P.; Aziz, D. B.; Dick, T.; Go, M.-L. Indolylalkyltriphenylphosphonium Analogues Are Membrane-Depolarizing Mycobactericidal Agents. *ACS Medicinal Chemistry Letters* **2017**, *8* (11) 1165–1170.
- (31) Maggiora, G.; Vogt, M.; Stumpfe, D.; Bajorath, J. Molecular Similarity in Medicinal Chemistry. *J. Med. Chem.* **2014**, *57* (8) 3186–3204.

(32) The structure of Mtb MmpL3 was disclosed in 2021 when the present manuscript was in preparation. Thus, the homology model previously described by us (ref. 24) was used for this study. A comparison of the homology model used in this work with the structure of Mtb MmpL3 recently reported, is presented in the Supporting Information (Figure S4).

(33) Adams, O.; Deme, J. C.; Parker, J. L.; the CRYPTIC Consortium, Fowler, P. W.; Lea, S. M.; Newstead, S. Cryo-EM structure and resistance landscape of *M. tuberculosis* MmpL3: An emergent therapeutic target. *Structure*, **2021**, *29*, 1–10.

(34) Palomino, J.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. Resazurin Microtiter Assay Plate: Simple and Inexpensive Method for Detection of Drug Resistance in Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **46AD**, 2002, 2720–2722.

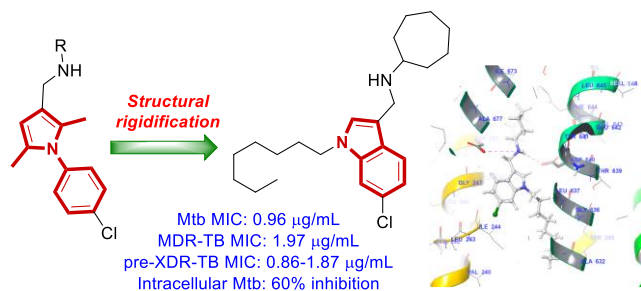
(35) Dos Santos Fernandes, G. F.; De Souza, P. C.; Moreno-Viguri, E.; Santivañez-Veliz, M.; Paucar, R.; Pérez-Silanes, S.; Chegaev, K.; Guglielmo, S.; Lazzarato, L.; Fruttero, R.; Man Chin, C.; Da Silva, P. B.; Chorilli, M.; Solcia, M. C.; Ribeiro, C. M.; Silva, C. S. P.;

Marino, L. B.; Bosquesi, P. L.; Hunt, D. M.; De Carvalho, L. P. S.; De Souza Costa, C. A.; Cho, S. H.; Wang, Y.; Franzblau, S. G.; Pavan, F. R.; Dos Santos, J. L. Design, Synthesis, and Characterization of N-Oxide-Containing Heterocycles with in Vivo Sterilizing Antitubercular Activity. *J. Med. Chem.* **2017**, *60* (20), 8647–8660.

(36) Martins, M. Targeting the Human Macrophage with Combinations of Drugs and Inhibitors of Ca<sup>2+</sup> and K<sup>+</sup> Transport to Enhance the Killing of Intracellular Multi-Drug Resistant Mycobacterium Tuberculosis (MDR-TB) - a Novel, Patentable Approach to Limit the Emergence of XDR. *Recent Pat. Antiinfect. Drug Discov.* **2011**, *6* (2), 110–117.

(37) Gawad, J.; Bonde, C. Current Affairs, Future Perspectives of Tuberculosis and Antitubercular Agents. *Indian J. Tuberc.* **2018**, *65* (1), 15–22.

SYNOPSIS TOC (Word Style "SN\_Synopsis\_TOC").



Insert Table of Contents artwork here