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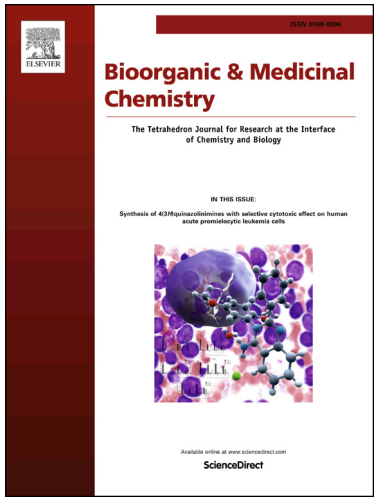

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Design, Synthesis and Evaluation of Indole-2-carboxamides with Pan Anti-mycobacterial Activity

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Abstract

Current treatment regimens for non-tuberculous mycobacteria (NTM) and tuberculosis (TB) generally require long duration of therapy with multiple drugs, some of which are broad spectrum antibiotics. Despite some advances in antimicrobial compounds, there remains a need in therapy for antibiotics with specific mycobacterial targets. It has been shown that MmpL3 is an essential transporter required for the translocation of mycolic acids to the mycobacterial cell envelope. Here, we synthesized a series of indole-2-carboxamides that inhibit MmpL3 and have potent pan-activity against mycobacterial species. The compounds were tested against several fast and slow-growing *Mycobacterium* species, including *M. abscessus*, *M. massiliense, M. bolletii, M. chelonae, M. tuberculosis, M. avium*, *M. xenopi* and *M. smegmatis.* The target of these indole compounds makes them selective for mycobacteria, while showing no clinically relevant bactericidal activity against *S. aureus* or *P. aeruginosa*. These compounds were tested against THP-1, a human-cell line, and showed minimal *in vitro* cytotoxicity and good selectivity indices. The data shown and discussed suggest that lead indole-2-carboxamides are strong contenders for further preclinical testing as NTM therapeutics.

1.1 - Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental pathogens that can cause a wide variety of infections, such as: progressive pulmonary disease, skin and soft tissue infections, lymphadenitis, and disseminated disease.[1, 2] The most common human pathogens are the species *M. avium* complex (MAC) and *M. kansasii*, however other clinically relevant species include the *M. abscessus* complex (MABSC, including the subspecies *M. abscessus*, *M. massiliense* and *M. bolletii*) and *M. fortuitum*.[3] NTM commonly cause pulmonary disease in older, immunocompromised patients who have underlying lung disease. The precise frequency of NTM disease is unknown because reporting for NTM is not mandatory in the United States and many other countries.[4, 5] Furthermore, current incidence and prevalence data are likely underestimated due to the indolent nature of NTM pulmonary disease, the most common form of NTM infections. A growing number of studies suggest that the number of NTM infections and mortality rates continue to increase.[4, 6] Growing prevalence of NTM isolates between 1997 (9.1/100,000) and 2003 (14.1/100,000) has been observed in Ontario, Canada.[7] More recently, Taiwan has reported increasing incidence of NTM disease, 10.2 cases per 100,000 persons in 2008 compared to 2.7 cases per 100,000 persons in 2000.[8, 9] In addition, estimates from 1999 to 2010 suggest the number of immediate NTM-related deaths in the United States rose significantly and are expected to increase over the next few years, given the increasing median age in North America.[10] Although NTM are opportunistic pathogens, they represent a matter of significant concern to health practitioners because of increasing incidence and mortality rates. As the number of patients with immune-compromising conditions continues to grow, both opportunistic and primary cases of NTM infection present a significant challenge for current antibiotic therapy.

In contrast to the insidious nature of NTM infections, *M. tuberculosis* is the cause of highly transmittable disease and infects about one-third of the world's population.[11] In 2015, tuberculosis (TB) became the leading infectious disease killer in the world and causes illness in approximately 9.6 million people, killing 1.5 million each year.[11] The global incidence of multi-drug-resistant tuberculosis (MDR-TB) remains unchanged at 3.3%, causing growing concern for healthcare professionals.[11] MDR-TB is classified as resistant to both rifampicin and isoniazid, two critical antibiotics used in standard treatment. This form of TB goes largely undetected, as the estimated number of cases of MDR-TB in 2014 was 480,000 people and the number of these patients who were started on appropriate treatment was only about 111,000.[11] Current treatment against mycobacterial infections are inadequate and this may be due, at least in part, to the absence of timely diagnostics and a paucity of narrow spectrum antimycobacterial antibiotics. The current treatment of NTM recommended by the American Thoracic

Society (ATS) and Infectious Diseases Society of America (IDSA) include regimens of multiple antibiotics, potentially including macrolides, aminoglycosides, fluoroquinolones, oxazolidinones, tigecycline, carbapenems, cephalosporins, sulfonamides, ethambutol, and rifampicin.[3] Many of these recommendations are made on the basis of preliminary *in vitro* efficacy studies due to the lack of a standardized animal model.[3] Depending on the organism and site of infection, two to four of these agents are often used in combination for a duration of at least 12 months after the first negative culture.[3] The use of multiple antibiotics for prolonged periods of time is challenging due to common complications such as drug interactions and noncompliance.[3]

Problems surrounding current anti-TB therapy are similar to that of NTM. The treatment of drug-susceptible TB continues to revolve around the same four-antibiotic regimen (isoniazid, rifampicin, ethambutol, and pyrazinamide) introduced more than 40 years ago.[11] Although the treatment of TB has been extensively studied, therapeutic regimens are still somewhat lacking in that: 1) they require the use of multiple antibiotics and 2) the duration of therapy is long. These are both factors contributing to non-compliance and treatment failure, which can lead to the emergence of MDR-TB and extensively drug-resistant tuberculosis (XDR-TB). The treatment of MDR-TB/XDR-TB is more difficult and requires multiple broad-spectrum antibiotics, which exposes patients to complications like *Clostridium difficile* infections and other resistant microorganisms.[12] Clinical practice guidelines for the treatment of drug-resistant TB are currently under development by the ATS and IDSA.[13] Depending on the susceptibilities of particular drug-resistant TB strains, second-line anti-TB drugs are typically used for a duration of 2 years and treatment success rates range from 30 to 80%, underscoring the need for newer anti-mycobacterial drugs and treatment regimens that maximize efficacy and shorten duration of treatment.[14-21]

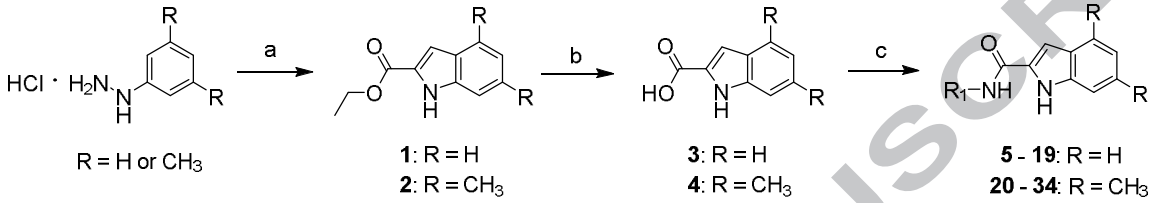
Current drugs that are in the pharmaceutical pipeline for mycobacterial infections are largely being used against *M. tuberculosis* and not specifically against NTM. Despite the number of new drugs being studied in pre-clinical and clinical trials for TB, they are being studied in combination drug regimens with other broad-spectrum antibiotics. Furthermore, these drugs, including DC-159a,[22] SQ-641,[23] CPZEN-45,[24] BTZ043,[25] bedaquiline,[26] and pretomanid[27], lack bactericidal activity against many NTM species. The development of narrow-spectrum anti-mycobacterial drugs could revolutionize the treatment of both TB and NTM.

Indole-2-carboxamides (IC) have been reported as novel antitubercular agents with activity against drug-resistant strains and in *in vivo* efficacy mouse models.[28-32] IC are bioisosteric isomers of published urea based *M. tuberculosis* inhibitors and have similar structure activity relationships.[33-35]

Herein, we report the expansion of i) the chemical space for the published antitubercular IC compounds and ii) the spectrum of antimycobacterial activity. In addition, lead compounds have demonstrated a safe pharmacological profile and are inhibitors of the mycolic acid biosynthetic pathway in both TB and NTM strains. Specifically, they inhibit the translocation of trehalose-monomycolate (TMM) to the outer membrane, suggestive of *Mycobacteria* membrane protein large 3 (MmpL3) inhibition.

2.1 - Results and Discussion

2.2 - Chemistry. Indole-2-carboxamide (IC) compounds were generated using published methods and is shown in scheme 1.[30] Briefly, the reaction of the arylhydrazine with ethyl pyruvate in the presence of p-toluenesulfonic acid (pTsOH) afforded ethyl indole-2-carboxylate. After NaOH-mediated saponification of the ester, commercially available amines were coupled to indole-2-carboxylic acid using standard coupling conditions.



Scheme 1. Synthetic pathway for the production of IC compounds. Reagents and conditions: (a) Ethyl pyruvate, pTsOH, EtOH, reflux, 6 hr; (b) NaOH, EtOH, reflux, 3 hr; (c) amine, DCC, DMAP, CH_2Cl_2, r.t., overnight.

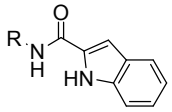
2.3 - Anti-Mycobacterial Activity. Two IC series were evaluated for their anti-mycobacterial activity against various mycobacterial pathogens, including *M. abscessus, M. massiliense, M. bolletii, M. chelonae, M. tuberculosis, and M. smegmatis*. The first series is an unsubstituted indole (R = H, compounds **5**-**19**) and the second was as 4,6-dimethyl indole (R = CH_3, compounds **20**-**34**). A variety of commercially available bulkyl aliphatic and aromatic head groups were incorporated into final compounds that are consistent with published SAR for previously evaluated urea-based compounds and IC.[28, 30, 32-35]

Table 1 shows the minimum inhibitory concentration (MIC) values for the synthesized unsubstituted IC compounds. Overall, these compounds have limited pan-antimycobacterial activity as demonstrated by inhibition from compounds **5** and **16**. Excluding *M. smegmatis,* compounds **5** and **16** show the best antimycobacterial activity with sub-µg/mL MIC values and compound **16** obtaining 2-4-fold increased activity over **5**. An adamantyl substitution in compound **5** confers activity, however the 3-ethyl adamantyl (**6**) or adamantyl with an ethyl or methyl linker (**7**, **8**) is not tolerated. Interestingly, changing the connection point from the 1-position (**5**) to the 2-position (**9**) on adamantine abolished NTM activity, however retained potent activity against *M. tuberculosis*. The methyl-linked adamantyl substitution on compound **8** elicits greater activity against NTM than the larger ethyl-linked compound **7**, suggesting an upper limit for linker bulk. Saturated cycloalkyl rings that are 8 carbons or smaller (**10, 11, 12,** and **13**) abolish all NTM activity. However, adding methyl components to smaller aliphatic rings

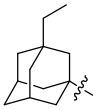
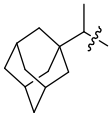
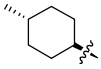
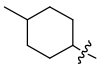
can increase activity as seen with the isopinocampheyl substituted (**16**)**,** which attains the highest pan-activity of the series.

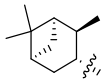
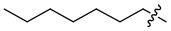
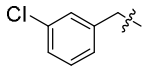
The antitubercular SAR is much looser, as the constraints on for the bulky aliphatic group are less restrictive. A cyclooctyl (**10**) substituent maintains antimicrobial activity against *M. tuberculosis* with an MIC of 0.39 µg/mL, despite being absent for NTM species. However, smaller rings like cycloheptyl, cyclohexyl, and cyclopentyl (**11, 12,** and **13**) are not tolerated for any species. There remains modest anti-TB activity with the 3-ethyl adamantyl (**6**) with an MIC of 1.25 µg/mL.

Table 1. Anti-NTM activity of unsubstituted indole-2-carboxamides. MIC values are in µg/mL.



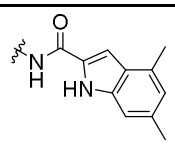
5 - 19

#	R	*M. abscessus*		*M. massiliense*		*M. bolletii*	*M. chelonae*	*M. tb*	*M. smeg.*
		ATCC 19977	DCI #21	CIP10 8297	CRM001 9	ATCC 14472	ATCC 35752	H37 Rv (mc2-6206)	mc^2155
5		0.25	0.25	0.5	0.25	0.25	0.25	0.2	1.56
6		>32	>32	>32	>32	>32	>32	1.25	>20
7		16	16	8	8	16	2	>5	>20
8		2	2	2	>32	2	1	>5	>20
9		>32	>32	>32	32	32	>32	0.39	>25
10		>32	>32	>32	32	32	>32	0.39	25
11		>32	>32	>32	>32	>32	>32	>5	>20
12		>32	>32	>32	>32	>32	>32	>10	>25
13		>32	>32	>32	>32	>32	>32	>10	>25
14		>32	>32	>32	>32	>32	>32	>10	>25
15		>32	>32	16	16	16	16	5	12.5

16		0.12	0.5	0.06	0.06	0.12	<0.06	0.05	0.78
17		>32	>32	>32	>32	>32	>32	>10	>25
18		>32	>32	>32	>32	>32	>32	>10	>25
19		>32	>32	>32	>32	>32	>32	>5	>20

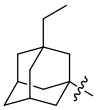
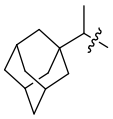
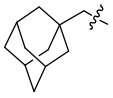
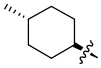
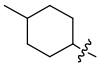
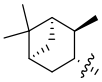
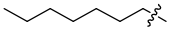
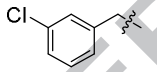
The second series evaluated for pan-antimycobacterial activity was the 4,6-dimethyl indole substitution and contributes to greater potency against the mycobacterial panel than with the unsubstituted indole compounds as demonstrated in table 2. The most potent pan-activity is seen with cyclooctyl (**25**) and cycloheptyl (**26**) head groups with MIC values ranging from 0.0039 to 0.625 µg/mL, depending on the species of *Mycobacterium*. The 4-methylcyclohexyl substituted compounds (**29** and **30**) also display significant anti-NTM and anti-TB activity with the exception of *M. smegmatis*. The pure trans isomer (**29**) and cis/trans mixture (**30**) achieved the same MIC values showing no preference for stereochemical orientation at the 4-position. The dimethyl indole series allow for smaller aliphatic substituents, presumably through the maintaining of lipophilicity from the dimethyl groups. Aliphatic rings smaller than cyclooctyl confer anti-mycobacterial activity that was not present in the unsubstituted indoles **11, 12, 13** and **14**. Substituents the size of the cyclooctyl (**25**) or cycloheptyl (**26**) have activity against *M. abscessus, M. massiliense, M. bolletii, M. chelonae, M. smegmatis, and M. tuberculosis* but begin to lose potency as the ring size decreases, until any clinically relevant activity is lost at a size of a cyclopentyl (**28**). Compounds with substitutions larger than adamantyl (**20, 21, 22, 23**) lose all MABSC NTM activity. Comparable to the unsubstituted indoles of table 1, activity against *M. tuberculosis* in the dimethyl series is less restrictive to substituent size than that of NTM species. The majority of these compounds (**20, 21, 22, 23, 24, 25, 26, 27, 29, 30,** and **31**) exhibit anti-tuberculous activity with MIC values ranging from 0.00195 to 0.625 µg/mL.

Table 2. Anti-NTM activity of substituted 4, 6-dimethylindole-2-carboxamides. MIC values are in µg/mL.



20 - 34

#	Structure	*M. abscessus*		*M. massiliense*		*M. bolletii*	*M. chelonae*	*M. tb*	*M. smeg.*
		ATCC 19977	DCI #21	CIP108297	CRM0019	ATCC 14472	ATCC 35752	H37 Rv (mc2-6206)	mc^2155

20		>32	32	>32	>32	>32	>32	0.0195	1.25-2.50
21		>32	>32	>32	>32	>32	>32	0.039	>20
22		16	>32	8	8	16	16	0.31	>20
23		>32	>32	>32	>32	>32	>32	0.16	>20
24		>32	>32	>32	>32	>32	>32	0.04	>20
25		0.063	0.0078	0.031	0.0078	0.0039	0.063	0.0195	0.313-0.625
26		≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.08	0.625
27		0.5	0.25	0.25	0.25	0.25	0.25	0.625	2.5
28		4	4	8	8	2	2	2.5	5
29		≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.31	>20
30		≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.31	>20
31		0.125	0.125	0.125	0.125	0.125	0.125	0.0195	1.25-2.50
32		>32	>32	>32	>32	32	>32	>5	5
33		>32	>32	>32	>32	>32	>32	>20	2.50-5.00
34		>32	>32	>32	>32	>32	>32	>5	>20

Regarding the unsubstituted ICs, only two compounds demonstrate sub-µg/mL pan-activity potencies compared to six total dimethyl ICs. Furthermore, preference for bulky aliphatic rings is shown, since potency is lost with decreasing size in both series, albeit more drastically in the unsubstituted series. It is clearly seen that the 4, 6-dimethylated indole is preferred due to the acquisition of previously absent anti-mycobacterial activity (**25, 26-29**), which is most likely due to the boost in lipophilicity from the two methyl groups. Supporting this observation is the attainment of anti-NTM activity for smaller saturated cycloalkyl substitutions (table 2, compounds **25, 26, 27, 29,** and **30**) when the indole ring is dimethylated. The anti-NTM activity seen with adamantyl (table 1, **5**) in the unsubstituted ICs is abolished when the indole is 4,6-dimethylated (table 2, **20**), however, this is not the case for the

isopinocampheyl substitution in either series (table 1, **16** and table 2, **31**). It seems when this minimum liphophilic threshold is not met, such as with the aliphatic rings smaller than an 8-carbon ring size in the unsubstituted series (table 1, **10, 11, 12,** and **13**), the compounds suffer a loss of activity. Lastly, no mycobacterial activity is observed in either series for uncyclized straight alkyl chains (table 1, compounds **17, 18**; table 2, compounds **32**, **33**).

To further evaluate the spectrum of anti-mycobacterial activity of the IC compounds, we also tested these compounds against MAC strains including *M. avium* and *M. xenopi* (table 3). Compounds that were not effective anti-mycobacterial agents were not tested. Overall, current IC compounds are more active against *M. xenopi* over *M. avium*. The best potential candidate for pan-activity against mycobacteria in the cyclooctyl substituted 4,6-dimethyl-indole (**25**), which also showed the most potent activity in this panel with MIC values ranging from 0.25-1 μg/mL. Compounds **26** and **31** also showed good activity against *M. avium* in addition to *M. xenopi* with MIC values between 2-4 μg/mL.

Table 3. Anti-NTM activity of substituted 3,5-dimethylindole-2-carboxamides. MIC values are in μg/mL.

#	*M. avium*		*M. xenopi* WT
	104	Intracellulare 1956	
5	8	8	0.5-1.0
6	>32	>32	1.0-2.0
7	>32	32	8
8	>32	>32	4
9	>32	>32	2
10	>32	>32	2
15	>32	>32	16
16	8	8	0.5-1.0
20	>32	>32	>32
21	>32	>32	>32
22	>32	>32	>32
23	>32	>32	1
24	>32	>32	0.5-1.0
25	0.25-0.05	1	0.25
26	2	4	0.06-0.12
27	>32	>32	1.0-2.0
28	>32	>32	8-16
29	>32	>32	0.25-1
30	>32	>32	0.5-1
31	2	2	0.25-1
32	>32	>32	>32
33	>32	>32	>32

2.4 - Indolecarboxamides are inactive against S. aureus and P. aeruginosa. Lead compounds in this IC series appear to be selective for mycobacteria, as IC compounds **5**, **16**, **25**, **26**, **30** and **31** were inactive against *P. aeruginosa* and *S. aureus* (table 4). All compounds tested showed no bacterial growth inhibition up to 160 μg/mL except for **16**, which inhibited *S. aureus* growth at 40 μg/mL.

Table 4. MIC values against *P. aeruginosa* and *S. aureus*

Compound #	MIC (μg/mL)	
	P. aeruginosa PAO-1	*S. aureus* ATCC25923
5	>160	>160
16	>160	40
25	>160	>160
26	>160	>160
30	>160	>160
31	>160	>160

2.5 - Indolecarboxamides are potent bactericidal agents. Minimum bactericidal concentration (MBC) values were determined for IC compounds **16**, **25**, **26** and **30**. Our results show that for these tested IC compounds, MBC_{90} values were highly potent and similar to determined MIC values. MBC_{90} values for IC **16**, **25**, **26** and **30** are 0.06, 0.015, 0.015 and 0.03 μg/mL, respectively.

2.6 - In vitro toxicity characterization. The cytotoxicity of compounds **5**, **16**, **25**, and **31** was tested against THP-1 cells, a human cell line (Table 5). With the exception of Compound **16** (TD_{50} of 11.3 μg/mL), the ICs were found not to be cytotoxic up to 50 μM. Across several *Mycobacterium* species, these compounds have exceptional selectivity indices, ranging from 22.6 to at least 3,800. Compound **25**, the most potent of the scaffold, shows the most promise against NTM, with selectivity indices of >1,910 for *M. abscessus, M. massiliense,* and *M. bolleti,* while compound **31** holds the most selectivity against *M. tuberculosis*.

Table 5. Cytotoxicity profiles of selected indole-carboxamides against THP-1 cells.

		Selectivity Indices (TD_{50}/MIC)				
Compound	TD_{50} (μg/mL)	*M. abscessus* DCI #21	*M. massiliense* CRM0019	*M. bolletii*	*M. chelonae* ATCC14472	*M. tb* H37Rv
5	>14.7	>58	>58	>58	>58	>73
16	11.3	22.6	188	92	>188	226
25	>14.9	>1910	>1910	>3820	>236	>764
31	>16.2	>130	>130	>130	>130	>830

2.7 - Indolecarboxamides inhibit the transfer of mycolic acids to their cell envelope acceptors in M. abscessus complex strains. IC compounds, including compound **5**, have been reported to target the

M. tuberculosis trehalose monomycolate (TMM) transporter, MmpL3, resulting in an inhibition of the transfer of mycolic acids to their cell envelope acceptors, arabinogalactan and trehalose dimycolates (TDM).[29, 31, 36] To determine whether the NTM inhibitors identified herein displayed a similar activity on *M. abscessus* complex strains, cultures of *M. massiliense* strain 1513 treated with different concentrations of compounds **5**, **16**, **25** and **31** were metabolically labeled with [1,2-^{14}C]acetate and their lipid and cell wall-bound mycolic acid contents analyzed by TLC. Inhibitor treatments resulted in a concentration-dependent build-up of TMM in the cells that accompanied a decrease in mycolic acid transfer onto cell wall arabinogalactan and TDM (figure 1). Inhibitor concentration-dependence was less marked in the case of compound **5**. Thus, similar to the situation in *M. tuberculosis*, the IC under study herein do not inhibit mycolic acid biosynthesis *per se* but rather the transfer of these products to their cell envelope acceptors. It is likely that the killing of *M. abscessus* complex strains that ensues results from the inhibition of the formation of their outer membrane.

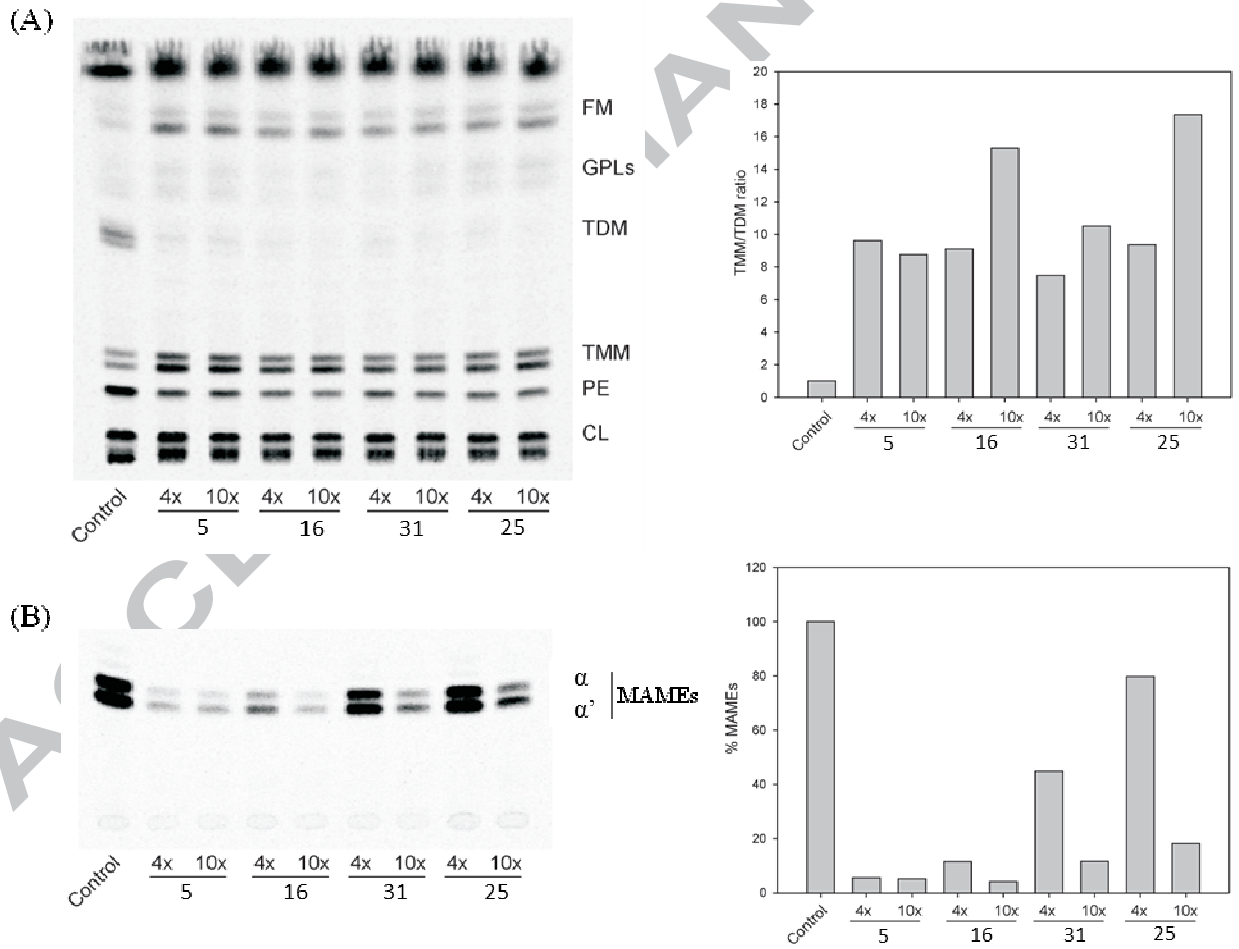


Figure 1. Effect of indolecarboxamides on the transfer of mycolic acids to their cell envelope acceptors. *M. massiliense* strain 1513 were either untreated or treated with compound **5** (4 µg/ml [4 x MIC] or 10 µg/ml [10 x MIC]), **16** (2 µg/ml [4 x MIC] or 5 µg/ml [10 x MIC]), **31** (2 µg/ml [4 x MIC] or 5 µg/ml [10 x MIC]) or **25** (0.5 µg/ml [4 x MIC] or 1.25 µg/ml [10 x MIC]) and labeled with [1,2-^{14}C]acetate as described under the experimental section. Panel (A): Analyses of lipids from untreated and treated cells. The same volume of [^{14}C]-labeled lipids from bacterial cells either treated or untreated were analyzed by TLC in the solvent system [$CHCl_3$:CH_3OH:H_2O, 20:4:0.5] and revealed by phosphorimaging. TMM/TDM ratios, expressed relative to the value measured in the untreated control (arbitrarily set to 1), are presented as histograms. PE, phosphatidylethanolamine; CL, cardiolipin; GPLs, glycopeptidolipids; FM, free mycolates. Panel (B): Analyses of cell wall-bound mycolic acid methyl esters (MAMEs) prepared from the same untreated and treated cells as in (A). TLC plates were developed thrice in the solvent system [*n*-hexane:ethyl acetate, 95:5] and revealed by phosphorimaging. The amount of radioactivity incorporated in MAMEs was semi-quantified using a PhosphorImager and the results, expressed as a percentage of the value measured in the untreated control, are presented as histograms.

3.1 - Conclusions

We have expanded the spectrum of activity of the IC class of antituberculars to include a panel of NTM species, including the most prevalent NTM strains MABSC and MAC. Against MABSC and *M. tuberculosis*, the IC class was found to be subdivided into three categories; 1) pan-active against the *M. abscessus* complex and *M. tuberculosis*; 2) pan-inactive against the *M. abscessus* complex and *M. tuberculosis*; 3) solely active against *M. tuberculosis*. *M. tuberculosis* was generally more susceptible to the IC class over the *M. abscessus* complex. Eight IC compounds showed sub-µg/mL potency against the *M. abscessus* complex. In addition, IC compounds were generally inactive against *M. avium*, however compound **25** also showed sub-µg/mL potency against *M. avium*. As seen in previous reports and our current data, this IC series has a safe pharmacological profile and are nontoxic against a human cell line with large selectivity indexes. The mechanism of action for NTM inhibition against *M. massiliense* suggests similar macromolecular activity to antitubercular MmpL3 inhibition, which is consistent with mycolic acid transport inhibition. The mechanism of action seems to be specific to mycobacteria as lead IC compounds were generally inactive against gram-positive and gram–negative bacteria. Recently, compound **25** was shown to achieve *in vivo* efficacy in TB infected mouse models, demonstrating its excellent pharmacokinetics/pharmacodynamics and is especially intriguing for evaluation in NTM mouse models.[29] Altogether, compounds **5**, **16**, **25** and **31** are promising lead candidates for treatment of MABSC and MAC strains and future preclinical studies are warranted.

4.1 - Experimental

4.2 - Materials and instrumentation. All chemicals and solvents were purchased from commercial sources. The chemical reactions were tracked by TLC using Biotage Silica Gel $60F_{254}$ plates and spots were visualized by UV lamp or I_2 condensation. Compounds were purified using a Biotage SP silica gel column on a Biotage Isolera One. ^{1}H and ^{13}C NMR were recorded on a 400 MHz Bruker NMR and chemical shifts were reported relative to solvent peak. Analytical RP-HPLC was determined on a Waters Acquity UPLC system equipped with an Acquity BEH C18 column (1.7 µm), flow rate of 0.5 mL/min and a gradient of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid): 0-0.25 min 97% A; 0.25-3.0 min 3-100% B (linear gradient); 3.0-4.5 min 100% B; 4.5-4.75 min 0-97% A (linear gradient); 4.75-5.0 min 97% A. UV absorbance at 254 nm was used as the detection method. All compounds were found to have >95% purity with the described analytical methods.

4.3 - Methyl 4,6-H-indole-2-carboxylate (2). Compound **2** was synthesized according to the published protocol of Onajole et al.[30] 1.75 mg (27.7%) of tan powder; ^{1}H NMR (DMSO-d_6) δ = 1.32 (3H, t, *J* = 8 Hz), 2.34 (3H, s), 2.43 (3H, s), 4.30 (2H, q, *J* = 8 Hz), 6.69 (1H, s), 7.03 (1H, s), 7.10 (1H, s), 11.67 (1H, s); ^{13}C NMR (DMSO-d_6) δ = 14.31, 18.24, 21.52, 60.17, 106.41, 109.53, 122.17, 124.96, 126.04, 130.73, 134.21, 137.69, 161.35; ESI-MS: $[M+H]^+$ calculated for $C_{13}H_{16}NO_2$: 218.1, found: 218.1.

4.4 - 4,6-H-indole-2-carboxylate (4). Compound **4** was synthesized according to the published protocol of Onajole et al.[30] 438 mg (>99%) of white powder; ^{1}H NMR (DMSO-d_6) δ = 2.34 (3H, s), 2.42 (3H, s), 6.68 (1H, s), 7.02 (1H, s), 7.04 (1H, s), 11.54, (1H, s), 12.74, (1H, s); ^{13}C NMR (DMSO-d_6) δ = 18.26, 21.51, 106.03, 109.49, 121.96, 125.07, 127.10, 130.56, 133.79, 137.53, 162.83: $[M+H]^+$ calculated for $C_{11}H_{10}NO_2$: 188.1, found: 187.8.

4.5 - General synthetic method for unsubstituted indole-2-caboxamide series. Indole-2-carboxamide (1.5 eq), DCC (1.2 eq), DMAP (0.1 eq) and amine (1.0 eq) were all dissolved in anhydrous CH_2Cl_2 (10 mL/1.0 mmol amine) and stirred at room temperature for 24 hours. The evolved ppt was filtered and the filtrate was removed under reduced pressure. The residue was purified by normal phase flash column chromatography using a hexane to ethyl acetate gradient.

4.5.1 - *N*-(1-adamantyl)-1H-indole-2-carboxamide (5). 197 mg (33.5%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.75 (6H, br), 2.18 (9H, br), 5.92 (1H, s), 6.76 (1H, s), 7.12 (1H, t, *J* = 8 Hz), 7.25 (1H, t, *J* = 8 Hz), 7.50 (1H, d, *J* = 8 Hz), 7.61 (1H, s, 8 Hz) 9.99 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 29.52, 36.36, 41.86, 52.50,

101.18, 112.14, 120.42, 121.69, 124.04, 127.68, 131.93, 136.41, 160.93; ESI-MS: $[M+H]^+$ calculated for $C_{19}H_{23}N_2O$: 295.2, found: 295.0.

4.5.2 - *N*-(3-ethyl-1-adamantyl)-1H-indole-2-carboxamide (6). 189 mg (29.3%) of white powder; ^1H NMR ($CDCl_3$) δ = 0.83 (3H, t, *J* = 8 Hz), 1.21 (2H, q, *J* = 8 Hz), 1.44-1.52 (4H, m), 1.60-1.73 (2H, m), 1.88 (2H, s), 2.07-2.15 (4H, m), 2.21 (2H, s), 5.92 (1H, s), 6.75 (1H, s), 7.12 (1H, t, *J* = 8 Hz), 7.26 (1H, t, *J* = 8 Hz), 7.50 (1H, d, *J* = 8 Hz), 7.61 (1H, d, *J* = 8 Hz), 9.94 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 7.05, 29.70, 34.61, 35.85, 35.95, 40.76, 41.46, 46.08, 53.37, 101.14, 112.06, 120.45, 121.70, 124.08, 127.68, 131.86, 136.27, 160.86; ESI-MS: $[M+H]^+$ calculated for $C_{21}H_{27}N_2O$: 323.2, found: 323.2.

4.5.3 - *N*-(1-(1-adamantyl)ethyl)-1H-indole-2-carboxamide (7). 198 mg (30.7%) of white powder; ^1H NMR ($CDCl_3$) δ = 1.17 (3H, d, *J* = 4 Hz), 1.58-1.74 (12H, m), 2.01 (3H, s), 4.03 (1H, t, *J* = 8 Hz), 6.13 (1H, d, *J* = 8 Hz), 6.86 (1H, s), 7.12 (1H, t, *J* = 8 Hz), 7.26 (1H, t, *J* = 8 Hz), 7.45 (1H, d, *J* = 8 Hz), 7.62 (1H, d, *J* = 8 Hz), 10.26 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 14.66, 28.29, 36.19, 37.01, 38.49, 53.18, 101.15, 111.95, 120.63, 121.74, 124.34, 127.63, 131.10, 136.18, 161.03; ESI-MS: $[M+H]^+$ calculated for $C_{21}H_{27}N_2O$: 323.4, found: 323.3.

4.5.4 - *N*-(1-(1-adamantyl)methyl)-1H-indole-2-carboxamide (8). 266 mg (43.1%) of white powder; ^1H NMR ($CDCl_3$) δ = 1.17 (3H, d, *J* = 4 Hz), 1.58-1.74 (13H, m), 4.03 (1H, quin, *J* = 8 Hz), 6.13 (1H, d, *J* = 4 Hz), 6.86 (1H, s), 7.12 (1H, t, *J* = 8 Hz), 7.26 (1H, t, *J* = 8 Hz), 7.45 (1H, d, *J* = 8 Hz), 7.62 (1H, d, *J* = 8 Hz), 10.26 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 28.19, 34.13, 36.88, 40.27, 50.98, 101.32, 111.90, 120.67, 121.80, 124.42, 129.51, 130.90, 136.12, 161.68; ESI-MS: $[M+H]^+$ calculated for $C_{20}H_{25}N_2O$: 309.4, found: 309.3

4.5.5 - *N*-(2-adamantyl)-1H-indole-2-carboxamide (9). 202 mg (34.3%) of white powder; ^1H NMR ($CDCl_3$) δ = 1.72 (1H, s), 1.75 (1H, s), 1.80 (1H, s), 1.88 (1H, s), 1.92 (8H, s), 2.09 (2H, s), 4.29 (1H, d, *J* = 8 Hz), 6.50 (1H, d, *J* = 8 Hz), 6.86 (1H, s), 7.14 (1H, t, *J* = 8 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.44 (1H, d, *J* = 8 Hz), 7.64 (1H, d, *J* = 8 Hz), 9.54 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 27.23, 32.04, 37.13, 27.50, 53.54, 101.33, 111.99, 120.62, 121.78, 124.34, 127.69, 131.23, 136.26, 160.76; ESI-MS: $[M+H]^+$ calculated for $C_{19}H_{23}N_2O$: 295.4, found: 295.3.

4.5.6 - *N*-cyclooctyl-1H-indole-2-carboxamide (10). 233 mg (43.1%) of white powder; ^1H NMR ($CDCl_3$) δ = 1.62-1.72 (13H, m), 1.96-2.01 (2H, m), 4.25 (1H, sep, *J* = 4 Hz), 6.10 (1H, d, *J* = 8 Hz), 6.80 (1H, s), 7.14 (1H, t, *J* = 4 Hz), 7.28 (1H, t, *J* = 4 Hz), 7.44 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.49 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 23.73, 27.19, 32.46, 49.71, 101.33, 111.96, 120.59, 121.80, 124.31, 127.69, 131.18, 136.21, 160.4; ESI-MS: $[M+H]^+$ calculated for $C_{17}H_{24}N_2O$: 271.4, found: 271.4.

4.5.7 - *N*-cycloheptyl-1H-indole-2-carboxamide (11). 231 mg (45.1%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.57-1.69 (8H, m), 1.71-1.94 (2H, m), 2.04-2.08 (2H, m), 4.19 (1H, quin, , *J* = 4 Hz), 6.09 (1H, d, *J* = 8 Hz), 6.80 (1H, s), 7.14 (1H, t, *J* = 4 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.43 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.41 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 24.14, 23.04, 31.05, 50.78, 101.36, 111.71, 111.94, 120.61, 121.82, 124.34, 127.69, 131.15, 136.19, 160.46; ESI-MS: $[M+H]^+$ calculated for $C_{16}H_{21}N_2O$: 257.3, found: 257.2.

4.5.8 - *N*-cyclohexyl-1H-indole-2-carboxamide (12). 233 mg (48.1%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.11 (1H, quin, *J* = 8 Hz), 1.18-1.47 (5H, m), 1.66 (1H, s), 1.76-1.95 (4H, m), 2.05 (1H, d, *J* = 8 Hz), 6.00 (1H, d, *J* = 8 Hz), 7.14 (1H, t, *J* = 8 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.43 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.29 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 24.60, 24.92, 25.54, 32.44, 33.32, 48.54, 101.39, 106.48, 111.89, 120.63, 121.85, 124.38, 127.71, 136.13, 160.64; ESI-MS: $[M+H]^+$ calculated for C15H19N2O: 243.3, found: 243.3.

4.5.9 - *N*-cyclopentyl-1H-indole-2-carboxamide (13). 277 mg (60.7%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.54 (2H, quin, *J* = 4 Hz), 1.66-1.77 (4H, m), 2.11 (2H, sex, *J* = 4 Hz), 4.43 (1H, sex, *J* = 4 Hz), 6.09 (1H, d, *J* = 4 Hz), 6.80 (1H, s), 7.13 (1H, t, *J* = 8 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.43 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.41 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 23.81, 26.16, 33.32, 51.49, 101.47, 111.94, 120.62, 121.85, 124.38, 127.70, 131.01, 136.19, 161.22; ESI-MS: $[M+H]^+$ calculated for C14H17N2O: 229.3, found: 229.2.

4.5.10 - *N*-(4-methylcyclohexyl)-1H-indole-2-carboxamide pure *trans* isomer (14). 277 mg (54.0%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.04-1.15 (4H, m), 1.33 (3H, q, *J* = 8 Hz), 1.56-1.67 (3H, m), 1.87 (5H, d, *J* = 4 Hz), 3.67-3.74 (1H, m), 4.39 (1H, s), 6.47 (1H, s), 7.02 (1H, s), 7.10 (1H, t, *J* = 8 Hz), 7.62 (1H, d, *J* = 8 Hz), 9.31 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 24.66, 25.37, 25.99, 32.43, 50.21, 106.53, 111.72, 120.70, 122.37, 129.50, 136.14, 154.03; ESI-MS: $[M+H]^+$ calculated for C16H21N2O: 257.3, found: 257.3.

4.5.11 - *N*-(4-methylcyclohexyl-1H-indole-2-carboxamide *cis*/*trans* mix (15). 280 mg (54.6%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.97 (3H, d, *J* = 4 Hz), 1.23-1.29 (2H, m), 1.64-1.85 (7H, m), 4.27 (1H, s), 6.27 (1H, d, *J* = 8 Hz), 6.85 (1H, s), 7.14 (1H, t, *J* = 8 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.44 (1H, d, *J* = 8 Hz), 7.64 (1H, d, *J* = 8 Hz), 9.77 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 21.13, 26.15, 29.41, 30.09, 30.48, 33.86, 45.80, 101.42, 112.05, 120.58, 121.77, 124.31, 127.66, 136.36, 160.96; ESI-MS: $[M+H]^+$ calculated for $C_{16}H_{21}N_2O$: 257.3, found: 256.9.

4.5.12 - *N*-((1R,2R,3R,5S)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-1H-indole-2-carboxamide (16). 378 mg (63.8%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.94 (1H, d, *J* = 8 Hz), 1.13 (3H, s), 1.19 (3H, d, *J* = 8 Hz), 1.27 (3H, s), 1.66-1.71 (1H, m), 1.88-1.98 (2H, m), 2.00-2.04 (1H, m), 2.45-2.51 (1H, m), 2.70-2.78 (1H,

m), 4.55-4.63 (1H, m), 6.11 (1H, d, *J* = 8 Hz), 6.86 (1H, d, *J* = 4 Hz), 7.13 (1H, t, *J* = 8 Hz), 7.27 (1H, t, *J* = 8 Hz), 7.47 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.95 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 20.83, 23.38, 28.05, 35.48, 38.50, 41.64, 46.49, 47.85, 48.21, 101.43, 112.12, 120.53, 121.77, 124.25, 127.65, 131.07, 136.45, 161.25; ESI-MS: $[M+H]^+$ calculated for $C_{19}H_{25}N_2O$: 297.4, found: 297.3.

4.5.13 - *N*-heptyl-1H-indole-2-carboxamide (17). 354 mg (68.5%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.89 (3H, t, *J* = 8 Hz), 1.28-1.41 (8H, m), 1.65 (2H, quin, *J* = 8 Hz), 3.49 (2H, q, *J* = 8 Hz), 6.19 (1H, s), 6.82 (1H, s), 7.14 (1H, t, *J* = 8 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.43 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.56 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 14.06, 22.60, 26.95, 28.99, 29.80, 31.76, 39.79, 101.51, 112.00, 120.61, 121.83, 124.38, 127.67, 130.91, 136.26, 161.63; ESI-MS: $[M+H]^+$ calculated for $C_{16}H_{23}N_2O$: 259.4, found: 259.1.

4.5.14 - *N*-octyl-1H-indole-2-carboxamide (18). 267 mg (49.0%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, *J* = 8 Hz), 1.28- 1.39 (10H, m), 1.64 (2H, quin, *J* = 8 Hz), 3.48 (2H, q, *J* = 8 Hz), 6.17 (1H, s), 6.81 (1H, s), 7.14 (1H, t, *J* = 4 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.43 (1H, d, *J* = 8 Hz), 7.63 (1H, d, *J* = 8 Hz), 9.46 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 14.09, 22.65, 26.99, 29.22, 29.79, 31.80, 39.79, 101.49, 111.97, 120.64, 121.84, 124.40, 127.88, 130.91, 136.22, 161.58; ESI-MS: $[M+H]^+$ calculated for $C_{17}H_{25}N_2O$: 273.3, found: 273.3.

4.5.15 - *N*-(3-chlorobenzyl)-1H-indole-2-carboxamide (19). 454 mg (79.7%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 2.97 (2H, s), 4.61 (2H, s), 6.99 (1H, s), 7.12 (1H, t, *J* = 4 Hz), 7.24-7.31 (4H, m), 7.35 (1H, s), 7.42 (1H, d, *J* = 12 Hz), 7.63 (1H, d, *J* = 8 Hz); ^{13}C NMR ($CDCl_3$) δ = 42.90, 48.92, 49.35, 49.57, 49.78, 103.21, 112.12, 120.60, 121.97, 124.61, 125.82, 127.71, 130.05, 134.59, 140.41, 162.27; ESI-MS: $[M+H]^+$ calculated for $C_{16}H_{14}ClN_2O$: 285.7, found: 285.0.

4.5.16 - *N*-1-adamantyl-4,6-dimethyl-1H-indole-2-carboxamide (20). 54 mg (31.0%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.75 (6H, s), 2.16 (9H, s), 2.42 (3H, s), 2.51 (3H, s), 5.85 (1H, s), 6.71 (1H, s), 6.77 (1H, s), 7.08 (1H, s), 9.23 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.62, 21.62, 29.52, 36.36, 41.90, 52.39, 99.67, 109.18, 122.67, 130.70, 130.86, 134.42, 136.39, 160.90; ESI-MS: $[M+H]^+$ calculated for $C_{21}H_{27}N_2O$: 323.4, found: 323.3.

4.5.17 - N-(3-ethyl-1-adamantyl)-4,6-dimethyl-1H-indole-2-carboxamide (21). 61 mg (32.2%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.81 (3H, t, *J* = 8 Hz), 1.20 (3H, q, *J* = 8 Hz), 1.46 (4H, s), 1.56 (1H, s), 1.84 (1H, s), 2.06 (4H, q, *J* = 12 Hz), 2.20 (3H, s), 2.42 (3H, s), 2.51 (3H, s), 5.85 (1H, s), 6.70 (1H, s), 6.77 (1H, s), 7.05 (1H, s), 9.02 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 7.05, 18.61, 21.62, 35.86, 40.79, 41.52, 46.23, 53.28, 99.68, 109.11, 122.72, 130.39, 134.49, 136.31, 160.89; ESI-MS: $[M+H]^+$ calculated for $C_{23}H_{31}N_2O$: 351.5, found: 351.7.

4.5.18 - *N*-(1-(1-adamantyl)ethyl)-4,6-dimethyl-1H-indole-2-carboxamide (22). 55 mg (29.1%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.16 (3H, d, *J* = 4 Hz), 1.57-1.74 (14H, m), 2.02 (3H, s), 2.42 (3H, s), 2.53 (3H, s), 3.94-4.02 (1H, m), 5.98 (1H, d, *J* = 8 Hz), 6.78 (2H, s), 7.06 (1H, s), 9.33 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 14.69, 18.64, 21.83, 28.3036.21, 37.01, 38.50, 53.08, 99.65, 109.17, 122.77, 125.65, 129.85, 130.86, 134.59, 136.47, 161.19; ESI-MS: $[M+H]^+$ calculated for $C_{23}H_{31}N_2O$: 351.5, found: 351.2.

4.5.19 - *N*-(1-(1-adamantyl)methyl)-4,6-dimethyl-1H-indole-2-carboxamide (23). 62 mg (34.1%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.60 (6H, s), 1.64 (1H, s), 1.67 (2H, s), 1.72 (2H, s), 1.75 (1H, s), 2.01 (3H, s), 2.42 (3H, s), 2.52 (3H, s), 3.19 (2H, d, *J* = 8 Hz), 6.24 (1H, s), 6.77 (1H, s), 6.81 (1H, s), 7.07 (1H, s), 9.53 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.62, 21.83, 28.22, 34.15, 36.90, 40.28, 50.97, 99.89, 109.18, 122.77, 125.67, 129.68, 130.90, 134.66, 136.49, 161.93; ESI-MS: $[M+H]^+$ calculated for $C_{22}H_{29}N_2O$: 337.4, found: 337.3.

4.5.20 - *N*-2-adamantyl-4,6-dimethyl-1H-indole-2-carboxamide (24). 83 mg (47.7%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.72 (1H, s), 1.75 (1H, s), 1.80 (2H, s), 1.93 (8H, s), 2.52 (4H, s), 2.52 (4H, s), 4.34 (1H, d, *J* = 8 Hz), 6.51 (1H, d, *J* = 8 Hz), 6.76 (2H, d, *J* = 20 Hz), 7.09 (1H, s), 10.01 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.58, 18.66, 21.87, 27.28, 32.07, 37.23, 37.53, 53.66, 99.90, 109.37, 109.46, 110.91, 122.65, 123.53, 130.79, 134.42, 136.88, 137.44, 138.86, 157.48, 161.21; ESI-MS: $[M+H]^+$ calculated for $C_{21}H_{27}N_2O$: 323.4, found: 323.3.

4.5.21 - *N*-cyclooctyl-4,6-dimethyl-1H-indole-2-carboxamide (25). 56 mg (34.8%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.57-1.75 (14H, m), 1.93-2.01 (2H, m), 2.42 (3H, s), 2.51 (3H, s), 4.21-4.30 (1H, m), 6.09 (1H, d, *J* = 8 Hz), 6.77 (2H, s), 7.07 (1H, s), 9.44 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.62, 21.83, 23.79, 27.19, 32.55, 49.63, 99.92, 109.23, 122.71, 125.71, 130.88, 134.53, 136.55, 160.65; ESI-MS: $[M+H]^+$ calculated for C19H27N2O: 299.4, found: 299.0.

4.5.22 - *N*-cycloheptyl-4,6-dimethyl-1H-indole-2-carboxamide (26). 65 mg (42.3%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.56-1.59 (6H, m), 1.61- 1.73 (4H, m), 2.04-2.09 (2H, m), 2.42 (3H, s), 2.51 (3H, s), 4.19 (1H, s), 6.06 (1H, d, *J* = 8 Hz), 6.77 (2H, s), 7.06 (1H, s), 9.25 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.62, 21.84, 24.17, 28.06, 31.03, 35.32, 50.68, 99.90, 109.17, 122.75, 125.72, 130.92, 134.59, 136.46, 160.61; ESI-MS: $[M+H]^+$ calculated for $C_{18}H_{25}N_2O$: 285.4, found: 285.3.

4.5.23 - *N*-cyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide (27). 69 mg (47.3%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.83-0.90 (1H, m), 1.21-1.32 (6H, m), 1.56 (2H, s), 1.75-1.80 (2H, m), 1.91 (2H, m), 2.03 (1H, d, *J* = 16 Hz), 2.43 (2H, s), 2.48 (2H, s), 6.77 (2H, d, *J* = 4 Hz), 7.05 (1H, s), 9.01 (1H, s); ^{13}C NMR

($CDCl_3$) δ = 18.62, 24.95, 25.57, 33.38, 34.94, 48.44, 99.94, 109.09, 122.80, 130.98, 134.68; ESI-MS: $[M+H]^+$ calculated for $C_{17}H_{23}N_2O$: 271.3, found: 271.2.

4.5.24 - *N*-cyclopentyl-4,6-dimethyl-1H-indole-2-carboxamide (28). 72 mg (52.0%) of white powder; ^1H NMR ($CDCl_3$) δ = 1.55 (2H, quin, *J* = 8 Hz), 1.63-1.79 (4H, m), 2.08-2.12 (2H, m), 2.42 (3H, s), 2.50 (3H, s), 4.44 (1H, q, *J* = 8 Hz), 6.11 (1H, d, *J* = 8 Hz), 6.77-6.79 (2H, m), 7.05 (1H, s), 9.39 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.61, 21.85, 33.32, 51.43, 100.11, 109.23, 122.72, 125.71, 134.60, 136.55, 161.46; ESI-MS: $[M+H]^+$ calculated for $C_{16}H_{21}N_2O$: 257.3, found: 257.1.

4.5.25 - *N*-4S-methylcyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide (29). 77 mg (50.1%) of white powder; ^1H NMR ($CDCl_3$) δ = 0.92 (2H, d, *J* = 8 Hz), 1.08-1.27 (6H, m), 1.60 (1H, s), 1.75 (1H, d, *J* = 16 Hz), 1.92 (2H, d, *J* = 16 Hz), 2.08 (1H, d, *J* = 12 Hz), 2,42 (3H, s), 2.51 (3H, s), 5.93 (1H, d, *J* = 8 Hz), 6.77 (2H, s), 7.05 (1H, s), 9.18 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.61, 21.84, 22.19, 32.01, 33.88, 34.93, 48.73, 99.98, 109.14, 122.75, 125.73, 130.94, 134.63, 136.43, 160.92; ESI-MS: $[M+H]^+$ calculated for $C_{18}H_{25}N_2O$: 285.4, found: 285.3.

4.5.26 - *N*-4-methylcyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide (30). 80 mg (52.1%) of white powder; ^1H NMR ($CDCl_3$) δ = 0.92 (1H, d, *J* = 4 Hz), 0.97 (1H, d, *J* = 8 Hz), 1.11- 1.28 (4H, m), 1.65- 1.80 (6H, m), 2.10 (1H, d, *J* = 12 Hz), 2.42-2.52 (6H, m), 6.02-6.27 (1H, m), 6.76-6.80 (2H, m), 7.06 (1H, s), 9.63 (1H, d, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$) δ = 18.62, 18.66, 21.95, 22.20, 29.40, 30.14, 33.92, 45.84, 48.82, 99.97, 100.06, 109.30, 122.70, 125.68, 130.67, 136.66, 161.14; ESI-MS: $[M+H]^+$ calculated for $C_{18}H_{25}N_2O$: 285.3, found: 285.3.

4.5.27 - *N*-((1R,2R,3R,5S)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)-4,6-dimethyl-1H-indole-2-carboxamide (31). 60 mg (34.2%) of white powder; ^1H NMR ($CDCl_3$) δ = 0.96 (1H, d, *J* = 8 Hz), 1.14 (3H, s), 1.19 (3H, d, *J* = 8 Hz), 1.27 (3H, s), 1.66-1.72 (1H, m), 1.88-1.97 (2H, m), 2.03 (1H, s), 2.42 (3H, s), 2.45-2.50 (1H, m), 2.52 (3H, s), 2.70-2.77 (1H, m), 2.88 (1H, d, *J* = 28 Hz), 4.57 (1H, t, *J* = 16 Hz), 6.06 (1H, d, *J* = 8 Hz), 6.77 (1H, s), 6.82 (1H, s), 7.10 (1H, s), 9.66 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.64, 20.86, 21.83, 23.48, 28.05, 37.42, 38.52, 41.66, 46.54, 47.87, 48.10, 99.98, 10933, 122.70, 125.71, 130.86, 134.52, 136.70, 161.34; ESI-MS: $[M+H]^+$ calculated for $C_{21}H_{29}N_2O$: 325.4, found: 325.1.

4.5.28 - *N*-heptyl-4,6-dimethyl-1H-indole-2-carboxamide (32). 99 mg (64.0%) of white powder; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, *J* = 4 Hz), 1.28- 1.39 (7H, m), 1.64 (3H, quin, *J* = 8 Hz), 1.92-1.95 (1H, m), 2.42 (2H, s), 2.47 (1H, s), 2.50 (3H, s), 3.47 (2H, q, *J* = 8 Hz), 6.19 (1H, s), 6.77 (2H, d, *J* = 8 Hz), 7.05 (1H, s), 9.38 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 14.07, 18.81, 21.84, 22.61, 26.98, 29.02, 31.76, 39.75, 100.14, 109.21,

122.75, 129.71, 130.93, 134.64, 136.54, 161.79; ESI-MS: $[M+H]^+$ calculated for $C_{18}H_{27}N_2O$: 287.4, found: 287.3.

4.5.29 - *N*-octyl-4,6-dimethyl-1H-indole-2-carboxamide (33). 101 mg (62.3%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, *J* = 8 Hz), 1.28-1.37 (9H, m), 1.62-1.65 (3H, m), 2.42 (3H, s), 2.50 (3H, s), 3.47 (2H, q, *J* = 8 Hz), 6.16 (1H, s), 6.77 (2H, d, *J* = 4 Hz), 7.05 (1H, s), 9.27 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 14.09, 18.60, 22.65, 27.02, 29.23, 29.85, 31.81, 37.75, 100.13, 109.19, 122.77, 125.73, 130.94, 134.68, 136.52, 161.77; ESI-MS: $[M+H]^+$ calculated for $C_{19}H_{29}N_2O$: 301.4, found: 301.4.

4.5.30 - *N*-(3-chlorobenzyl)-4,6-dimethyl-1H-indole-2-carboxamide (34). 103 mg (61.0%) of white powder; ^{1}H NMR ($CDCl_3$) δ = 1.58 (1H, s), 1.75 (6H, t, *J* = 12 Hz), 2.16 (8H, s), 2.42 (3H, s), 2.51 (3H, s), 5.85 (1H, s), 6.71 (1H, s), 6.77 (1H, s), 7.08 (1H, s), 9.23 (1H, s); ^{13}C NMR ($CDCl_3$) δ = 18.62, 21.82, 29.52, 36.36, 41.90, 52.39, 99.67, 109.18, 122.67, 130.86, 134,42, 136.39, 160.90; ESI-MS: $[M+H]^+$ calculated for $C_{18}H_{18}ClN_2O$: 313.7, found: 313.2.

4.6 - Inhibitor susceptibility determinations. MIC values against a number of clinical isolates of mycobacteria and other bacterial species were determined in 96-well microtiter plates using the colorimetric resazurin microtiter assay[37] and visually scanning for growth. MIC values against *M. tuberculosis* H37Rv mc^{2}6206 were determined in 7H9-ADC–0.05% tyloxapol medium supplemented with 0.2 % casaminoacids, 48 µg/ml pantothenate and 50 µg/ml L-leucine. MICs against *M. smegmatis* mc^{2}155 were determined in 7H9-ADC-0.05% Tween 80. MICs against *M. abscessus* ATCC 19977, *M. abscessus* 21, *M. abscessus* 103, *M. massiliense* CIP108297, *M. massiliense* 1513, *M. massiliense* CRM-0019, *M. massiliense* CRM-0270, *M. massiliense* 105, *M. bolletii* ATCC 14472, *M. bolletii* 88, *M. chelonae* ATCC 35752, *M. chelonae* 28, *M. chelonae* 49, *M. chelonae* 69, *M. kansasii* 662, *M. avium* 104, *M. intracellulare* 1956, *M. xenopi* 4042, *Pseudomonas aeruginosa* PAO-1 and *Staphylococcus aureus* ATCC 25923 were determined in Mueller Hinton II (BD). MBC_{90} values were determined by subculture from the MIC wells onto 7H11 agar and defined as the lowest concentration reducing CFU by 90 % relative to the inoculum.

4.7 - Cytotoxicity determination. The cytotoxicity of compounds was determined by measuring THP-1 cell viability after 3 days in the presence of test compounds. Compounds were prepared as 10-point serial dilutions in DMSO. The highest concentration of compound tested was 50 µM where compounds were soluble in DMSO at 10 mM. THP-1 cells were cultured in complete RPMI and differentiated into macrophage-like cells using 80 nM PMA overnight at 37°C, 5% CO_2. Cells were inoculated into assay

plates and cultured for 24h before compound dilutions were added to a final DMSO concentration of 0.5%. Each run included staurosporine as a control. Assay plates were incubated for 3 days at 37°C, 5% CO_2; growth was measured using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega) which uses ATP as an indicator of cell viability. Relative luminescent units (RLU) were measured using a Biotek Synergy 4 plate reader. The dose response curve was fitted using the Levenberg–Marquardt algorithm. The IC_{50} was defined as the compound concentration that resulted in 50% viability.

4.8 - Metabolic labeling and mycolic acid analysis. Cultures of *M. massiliense* 1513 grown to mid log-phase in Mueller-Hinton II broth were incubated with different concentrations of inhibitors for 16 hr at 37°C with shaking. 0.5 µCi/ml [1,2-^{14}C]acetic acid (specific activity, 52 Ci/mol, PerkinElmer Inc.) was added at the same time as the inhibitors. Total lipids and cell wall-bound mycolic acid methyl esters were prepared from treated and untreated cells and analyzed by TLC as described previously.[38] TLCs were revealed and semi-quantified using a PhosphorImager (Typhoon, GE Healthcare).

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Design, Synthesis and Evaluation of Indole-2-carboxamides with Pan Anti-mycobacterial Activity

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Graphical Abstract