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Design, synthesis and biological evaluation of indole-2-carboxamides, a promising class of anti-tuberculosis agents

Ravinder Reddy Kondreddi,[†] Jan Jiricek,[†] Srinivasa P S Rao,[†] Suresh B. Lakshminarayana,[†] Luis R. Camacho,[†] Ranga Rao,[†] Maxime Herve,[†] Pablo Bifani,[†] Ngai Ling Ma,[†] Kelli Kuhen,[‡] Anne Goh,[†] Arnab K. Chatterjee,[‡, §] Thomas Dick,[†#] Thierry T. Diagana,[†] Ujjini H. Manjunatha [†] and Paul W. Smith[†]*

[†]Novartis Institute for Tropical Diseases, 10 Biopolis Road #05-01 Chromos Singapore 138670

[‡]Genomics Institute of the Novartis Research Foundation, 10675 John J. Hopkins Drive, San Diego, California 92121

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ABSTRACT. Indole-2-carboxamides have been identified as a promising class of anti-tuberculosis agents from phenotypic screening against mycobacteria. One of the hits indole-2-carboxamide analog (**1)** had low μM potency against *Mycobacterium tuberculosis* (Mtb), high mouse liver microsomal clearance and low aqueous solubility. Structure activity relationship studies revealed that attaching alkyl groups to the cyclohexyl ring significantly improved Mtb activity but reduced solubility. Further, chloro or fluoro or cyano substitutions on the 4- and 6-positions of the indole ring and methyl substitution on the cyclohexyl ring significantly improved metabolic stability. **39** and **41**, the lead candidates displayed improved *in vitro* activity compared to most of the current standard TB drugs. The low aqueous solubility could not be mitigated due to the positive correlation of lipophilicity with Mtb potency. However, both compounds displayed favorable oral pharmacokinetic properties in rodentsand demonstrated *in vivo* efficacy. Thus, indole-2-carboxamides represent a promising new class of anti-TB agents.

INTRODUCTION

Mycobacterium tuberculosis (Mtb), causative agent of tuberculosis (TB) infects one third of the world population and 5-10% of those infected will eventually develop active disease in their lifetime[1]. In 2011, 8.7 million new TB cases were reported with 1.4 million deaths, including 430,000 HIV positive patients[2]. Currently, drug susceptible TB is treated using drugs discovered more than 50 years ago. There are increasing reports on multi-drug resistant (MDR) and extensively drug resistant (XDR) tuberculosis in recent years[2]. Some strains of Mtb are becoming resistant to most of the currently available anti-mycobacterial drugs rendering them to be totally drug resistant (TDR)[3]. Hence, there is an urgent need to develop novel drugs with new modes of action in order to fight all the different forms of TB - MDR, XDR and TDR. Recently, drug discovery initiatives have yielded a few new interesting compounds that are being evaluated

for the treatment of TB namely TMC207 (**42**)[4], OPC-67683 (**43**)[5] and PA-824 (**44**)[6] (Figure S1). Recently, **42** was approved by U S Food and Drug Administration in 2012 for treatment of MDR TB patients as a part of new combination therapy when other alternatives have failed[7].

In order to find new lead compounds, both academic institutions and pharmaceutical companies have conducted phenotypic screens on compound libraries and made the hits publicly available (GSK, SRI, Novartis)[8-10]. These efforts will help many academic researchers to select starting points for further optimization using medicinal chemistry approaches to reach pre-clinical stage. In a similar effort the Novartis in-house compound library was subjected to whole cell based screening against *M. bovis* BCG using ATP content as a surrogate marker for bacterial growth[11]. Novel chemical entities identified were further re-confirmed for their activity against virulent MtbH37Rv and chemically clustered. The hit reconfirmation process resulted in a few interesting scaffolds for hit to lead optimization. Our criteria used for hit selection were based on potency, bactericidal activity and low cytotoxicity against mammalian cells. New anti-tubercular drugs need to have high chemical stability and be cost effective for TB treatment due to high burden disease in underdeveloped and developing countries. Hence, ease of synthesis and chemical stability were also taken into consideration for choosing the hit molecule. One of the hits, indole-2-carboxamide (**1**), showed promising anti-mycobacterial activity (minimum inhibitory concentration, MIC = 0.8 μM). Recently, Ballell *et al*. reported indole 2-carboxamides as a hit in anti-mycobacterial phenotypic high throughput screening assay[9, 12] and Onajole *et al*.[13] published preliminary structure activity relationship (SAR) of related indole-2-carboxamides against Mtb. In the present study, we describe SAR, structure property relationship (SPR), *in vivo* pharmacokinetic (PK) and mouse efficacy of indole-2-carboxamides. We primarily focused on modifying the indole and cyclohexyl moieties to simultaneously

improve Mtb activity and optimize PK properties. The molecular target of indole-2-carboxamides has been identified as mmpl3 protein using whole genome sequencing of resistant mutants and lipid profiling to be described elsewhere[14]. In this study, we have successfully identified the advanced lead candidates **39** and **41** with good PK properties and excellent *in vivo* efficacy in an acute mouse model of Mtb.

CHEMISTRY

Indole-2-carboxamides were synthesized in a straight-forward way by coupling of substituted indole-2-carboxylic acids with the corresponding aromatic, alkyl or cycloalkyl amines (Scheme 1) in the presence of 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) in dimethylformamide (DMF). The starting materials *i.e.,* indole-2-carboxylic acids were either commercially available or prepared by standard methods such as the Fisher indole synthesis or Hemetsberger–Knittel indole synthesis following published procedures[15-17]. The alkylamines used were commercially available except (1*R*,2*S*)-2-methylcyclohexanamine that was prepared as previously described[18, 19]. The tetrahydro indole-2-carboxamide **16** was synthesized as shown in scheme 1 by reduction of **9** with sodium cyano borohydride. 4,6-dimethyl-*N*-1H-benzo[d]imidazole-2-carboxamide **17** was obtained in an efficient one-pot synthesis using 3,5-dimethylbenzene-1,2-diamine and sodium 2-(((1*R*,2*S*)-2-methylcyclohexyl)amino)-2-oxoacetate in the presence of $POCl_3$ as previously reported[20]. The synthesis of **19** was performed analogously to the procedure described in the literature[21] followed by HATU coupling.

RESULTS AND DISCUSSION

A promising hit from cell based screening, **1**, had displayed attractive features, sub μM potency, mycobactericidal activity and easy synthetic feasibility. However, **1** has low aq solubility (<4 μM at pH 6.8), high lipophilicity (cLogP = 4.48) and high *in vitro* clearance in mouse liver microsomes (CL_{int} = 693 μL/min/mg). We hypothesized that the electron rich 4,6-dimethyl indole and cyclohexyl group of **1** are metabolically susceptible (Figure 1) leading to high turnover. Therefore we reasoned that blocking or modifying these positions should help to improve metabolic stability. Additionally, attaching polar groups to lower lipophilicity should improve aqueous solubility. Medicinal chemistry efforts focused on modifying the indole ring and cyclohexyl group are shown in tables 1-3. All compounds synthesized were analyzed in a whole cell assay for *in vitro* MIC against Mtb H37Rv[11] and screened for cytotoxicity against THP-1 and HepG2 mammalian cell lines. A few selected compounds were subjected to *in vitro* metabolic stability and solubility assays to understand SAR and SPR. The lead compounds **39** and **41** were further profiled *in vivo* to assess the PK properties and *in vivo* efficacy in a mouse model of TB infection.

SAR to improve potency and aqueous solubility. Our initial efforts to reduce lipophilicity of **1** by introducing a basic nitrogen in the cyclohexane ring (**2**, cLogP = 2.5) resulted in significant increase in aqueous solubility but Mtb activity was lost by >25 fold (Table 1). A basic amine is not tolerated in the amide side chain of the indolcarboxamide and loss of Mtb activity could be attributed to reduced lipophilicity and/or presence of ionisable group. In order to understand the importance of the hydrophobic interaction of the cyclohexyl group, different alkyl and cycloalkyl groups were explored on the amide. Replacing the cyclohexane ring of **1** with an isopropyl group (**3**) dramatically decreased its activity >25 fold, whereas increasing lipophilicity from sec-

butylamide (**4**, cLogP = 3.81) to cycloheptylamide (**8**, cLogP = 5.03) or (1R,2S)-2-methylcyclohexylamide (**9**, cLogP = 4.99) enhanced its activity against Mtb by 100 fold (Table 1). Further a positive correlation was observed between cLogP and MIC_{50}. Hit **1** and potent compound **9** dispersed along the same LiPE[22] line (Figure 2). The SAR of alkyl and cycloalkyl groups on the amide revealed that hydrophobic groups are important for Mtb activity and suggested a critical hydrophobic interaction with the molecular target. In addition, improved cellular activity may also be linked to an improved cell penetration of lipophilic analogues into highly waxy mycobacterial cell wall. Additional evidence of a lipophilic binding pocket was provided by the loss of Mtb activity with the introduction of other polar groups **10** (cLogP = 2.65), **11** (cLogP = 2.07) and **12** (cLogP = 3.28).

The replacement of the cyclohexyl group of **1** with a phenyl group (**13**) reduced potency approximately 20 fold. This is probably due to less lipophilic interaction of flat phenyl group with the three-dimensional active site of the potential molecular target as compared to the cyclohexyl ring. Our initial medicinal chemistry efforts on the cyclohexyl group led to the identification of a highly potent compound **9** (MIC = 79 nM) with 4 fold better activity against Mtb as compared to the standard TB drug isoniazid (MIC = 330 nM). We then investigated the indole ring and amide bonds to understand the pharmacophore.

SAR of indole and amide groups. The SAR on indole NH, amide NH and indole ring was investigated to understand the pharmacophore and the importance of both NHs for Mtb activity. The methylation of amide NH to NMe (**14**) reduced Mtb activity by more than 20 fold as compared to **1** (Table 2). Likewise, *N*-methyl indole derivative (**15**) was also significantly less potent against Mtb (>285 fold) compared to the corresponding analog **9**. The indoline (**16**) also displayed reduced Mtb activity (16 fold lower than **9)**, suggesting that indole ring planarity is

important for the potency. Introducing nitrogen atoms into the indole ring (**17**, **18** and **19**) reduced lipophilicity, but also resulted in significant reduction of Mtb activity. Improvement of aqueous solubility was observed for low liphophilic compound **19**. The SAR on indole ring and amide functionalities suggested that both NH and indole ring are important for Mtb activity. Thus, we identified the indole-2-carboxamides as a pharmacophore for potency against Mtb in the series. SAR on cyclohexyl group and indole core led to a highly potent compound **9** (MIC = 79 nM). However, it has a high *in vitro* mouse microsomal clearance (CLint = 433 μL/min/mg) and poor aqueous solubility (<4 μM) (Table 3). Therefore the SAR on **9** was focused on the improvement of the metabolic stability.

SAR to mitigate metabolic stability. *In silico* analysis of metabolic soft spots on **9** using commercial available software tool StarDrop[23] predicted both 4- and 6-methyl groups on the indole as potential sites for high metabolic clearance (Figure 1). In order to improve the metabolic stability of **9**, we modified the indole methyl groups by incorporating stable chloro/fluoro/cyano groups. Replacement of both indole methyl groups with chloro (**20**) significantly improved *in vitro* metabolic stability by 5 fold (Table 3) while retaining excellent potency (MIC = 0.053 μM). Repositioning of the methyl group on cyclohexyl in **20** further improved metabolic stability by 2 fold in **21**, while maintaining similar Mtb activity and removed the chirality. However the same modification with fluoro group (**22** and **23**) didnot improve metabolic stability. Other substitutions on indole with 6-chloro-4-fluoro (**24**), 4-chloro-6-fluoro (**25**) and dicyano (**26**) have improved metabolic stability similar to **20** with slightly reduced activity against Mtb. The high microsomal clearance of **9** was mitigated with metabolically stable **21** while retaining similar Mtb activity. The improvement of *in vitro* metabolic stability was also reflected in good *in vivo* PK properties (Table 6 and Figure 3). The

metabolically stable compound **21** is a mixture of *cis* and *trans* isomers (38:62). The pure *cis* (**27**) and *trans* (**28**) isomers displayed similar potency. However, a significant improvement in metabolic stability (>10 fold) was observed with the *trans* isomer (**28**). The replacement of both methyls on indole with other groups displayed the solubility similar to **9**. As predicted, metabolic soft spots were blocked with metabolically stable chloro groups on the indole ring. The SAR on cyclohexyl and indole ring resulted in the identification of lead compounds **27** and **28** based on *in vitro* potency and metabolic stability. The lead compound **28** was further optimized to improve Mtb activity by altering the chloro position on the indole ring.

SAR towards the optimum position of the chloro substituents on indole ring. Further investigation was focused on optimization of chloro substituents at different positions on the indole ring (Table 4). In the monochloroindole series, both **30** and **32** had similar Mtb activity compared to **21** with >10 fold reduced metabolic stability. The decreased potency of other two monochloroindole-2-carboxamides **31** and **33** indicated that chloro substitution on the 7-position and 5-position of the indole are less favorable for Mtb activity respectively. However, 5-chloro substitution as in **31** displayed improved metabolic stability over 4- and 6-positions. Overall SAR of mono chloro indole-2-carboxamides indicated that 4- and 6-positions are preferred for activity and 5-position is vulnerable for metabolic clearance. In fact monochloro indole-2-carboxamides are either less active or less metabolically stable compared to **21**. Therefore we moved on to investigate the effect of dichloro substitution on the indole ring.

Out of the six dichloroindole-2-carboxamide analogues, **35** showed significantly less activity, whilst **37** and **38** exhibited moderately reduced activity on Mtb. The chloro substitution on the 7-position of the indole alone or in combination with other positions is less favorable for Mtb activity. The other two compounds **34** and **36** had a comparable potency with **21**. Even though

mono-chloro analogues on the 4- or 6-position are metabolically less stable, dichloro substitutions at the 4,5- or 5,6-positions improved metabolic stability. The metabolic stability of **21** was superior to the 4,5- or 5,6-dichloro indoles. Overall 4,6-dichloro substitution on indole is found to be optimal for activity and metabolic stability.

Improving the potency of the lead compound. Additional optimization of lead compound **28** was focused on the improvement of potency against Mtb by exploring the hydrophobic space on the 4-position of the cyclohexane ring (Table 5). The introduction of *gem*-dimethyl group (**39**) increased cLogP (from 5.56 to 6.08) and also resulted in a 10 fold boost in potency as compared to **28**. The introduction of the *gem*-dimethyl group removed *cis* and *trans* isomerism. The difluoro analog **40** did not show any additional improvement in metabolic stability and has reduced potency by > 20 fold. The loss of Mtb activity of **40** was likely due to reduced cLogP. Likewise, the difluoro substitution on the indole ring (**41**) retained Mtb activity, however, metabolic stability was reduced by >5 fold. A similar trend in metabolic stability was observed between 4,6-difluoro (**29**) and 4,6-dichloro (**28**) derivatives. Although *in vitro* metabolic stability of **41** is less than **39,** this difference was not reflected *in vivo* where both compounds showed similar clearance in mouse and good exposure (Table 6).

Lead candidates **39** and **41** were selected for further profiling as they displayed excellent potency against Mtb and improved metabolic stability. Overall, optimization of potency and metabolic stability of the hit to the lead candidates also increased lipophilicity. The aqueous solubility of these lead candidates remained poor. The high lipophilicity of lead compounds **39** and **41** thus reduced "druglikeness"[24]. However, cLogPs of both indole-2-carboxamide lead compounds are still lower than the recently approved MDR TB drug **42**[7] (cLogP = 7.25) and

similar to **43**[25] (cLogP = 5.2) which is in phase II clinical trial. Indolcarboxamides **39** and **41** showed potent activity against all tested MDR TB clinical isolates to be described elsewhere[14].

***In vivo* PK and efficacy.** The *in vivo* pharmacokinetics of the potent analogues **21**, **39** and **41** were evaluated in mice by oral (*p.o.*) and intravenous (*i.v.*) routes at doses of 25 and 5 mg/kg respectively. Like other high lipophilic compounds[26], a special delivery microemulsion preconcentrate (MEPC) formulation was used to address the solubility limitation of **39** and **41** for oral dosing. The lead compounds **21**, **39** and **41** displayed favorable PK properties with low total systemic clearance and moderate to high volume of distribution with elimination half-lives ranging from 4.5 to 7 hours (Figure 3 and Table 6). Overall, these compounds achieved good oral exposure in systemic circulation that resulted in good oral bioavailability (48-53%).

Based on the potent *in vitro* activity and promising *in vivo* PK profile of **39** and **41**, we conducted *in vivo* efficacy studies in an acute mouse model of Mtb infection[11] along with the moderately potent analogue **28** (MIC 10 fold lower than **39**). Treatment was initiated 7 days post Mtb infection in Balb/c mice. The compounds were orally administered at 25 and 75 mg/kg once daily for 28 days. The lead candidate **39** showed a 2.6 and 4.8 log lung colony forming unit (CFU) reductions at 25 and 75 mg/kg respectively compared to the untreated control (Figure 4). In addition, the other lead candidate **41** displayed 2.4 and 4.6 log lung CFU reduction at 25 and 75 mg/kg respectively. However less potent analogue **28** achieved less than a log CFU reduction compared to the untreated control. A significantly better efficacy was observed with both lead candidates **39** and **41** at 75 mg/kg over the first-line TB drug ethambutol (100 mg/kg).

In summary, we have identified promising indole-2-carboxamides from whole cell phenotypic high-throughput screening and examined their SAR and SPR. The SAR clearly indicated that i) cyclohexyl group is interacting with a hydrophobic site of the potential molecular target, ii) a

strong relationship between Mtb activity and cLogP, iii) optimally positioned chloro and fluoro substituents on indole and *gem*-dimethyl functionality on the cyclohexyl ring improved metabolic stability, iv) hydrophilic groups improved aqueous solubility but lowers Mtb activity, v) indole planarity is important for activity and vi) 4,6-dichloro substitution is optimal for Mtb activity and metabolic stability. The lead candidates **39** and **41** displayed promising microbiological and pharmacological properties and also demonstrated potent oral efficacy in an acute TB mouse model. They also showed adequate safety profile with no cytotoxicity, mutagenicity, cardiotoxicity and phototoxicity, which will be described in detail elsewhere[14]. The data presented in this study suggest that **39** and **41** are promising lead candidates with a potential for MDR and XDR TB treatment, thus warranting further pre-clinical evaluation.

EXPERIMENTAL

Chemistry. Reagents and solvents were purchased from Aldrich, Acros, or other commercial sources and used without further purification. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F254 plates from Merck. Compounds were visualized under UV light, ninhydrin, or phosphomolybdic acid (PMA) stain. NMR spectra were obtained on a Varian 400 MHz Mercury plus NMR or Bruker 400 MHz Ultrashield spectrometer using $CDCl_3$ and DMSO-d_6 as solvents. Compound purity was determined by **method 1:** LC/MS using an Agilent UHPLC 1290 coupled with API 3200; Acquity UPLC BEH C18 column, 1.7µm, 2.1x50 mm; gradient of 98:2 H_2O (0.1% formic acid):CH_3CN to 2:98 H_2O (0.1% formic acid):CH_3CN for 2 minutes run time with 1.0 mL/min flow rate / **method 2**: Waters Acquity UPLC coupled with MS waters ZQ 2000; Acquity UPLC BEH C18 column, 1.7µm, 2.1x50mm; gradient of 98:2 H_2O (0.1% formic acid):CH_3CN to 2:98 H_2O (0.1% formic acid):CH_3CN for 2 minutes run time with 1.0 mL/min flow rate / **method 3**: Waters Quattro Micro UPLC-LCMS equipped with a Acquity

BEH C18 column, 1.7µm, 2.1x50mm using a gradient of 90:10 H_2O (0.025% trifluoro acetic acid):CH_3CN (0.025% trifluoro acetic acid) to 10:90 H_2O (0.025% trifluoro acetic acid):CH_3CN (0.025% trifluoro acetic acid) for 5 minutes run time with 0.4 mL/min flow rate and HPLC purity using **method 4**: Waters Acquity UPLC equipped with a Acquity UPLC HSS T3 column, 1.8µm, 2.1x50mm; gradient of 95:5 H_2O (0.1% formic acid):CH_3CN to 2:98 H_2O (0.1% formic acid):CH_3CN for 2 minutes run time with 1.0 mL/min flow rate / **method 5**: Waters UPLC equipped with a Acquity, BEH C18 column, 1.7µm, 2.1x100mm using a gradient (4 minutes) of 70:30 H_2O (0.025% trifluoro acetic acid):CH_3CN (0.025% trifluoro acetic acid) to 20:80 H_2O (0.025% trifluoro acetic acid):CH_3CN (0.025% trifluoro acetic acid) for 6 minutes run time with 0.3 mL/min flow rate. The purity of all compounds reported were > 95% measured at 214/254 nM. The *cis* and *trans* isomers ratio was measured by HPLC or NMR.

Minimum inhibitory concentration (MIC) determination. *M. tuberculosis* H37Rv (ATCC 27294) strain maintained in Middlebrook 7H9 broth medium supplemented with 0.05 % Tween 80 and 10 % ADS supplement was used for MIC determination. Compounds dissolved in 90 % DMSO were three fold serial-diluted in duplicates in 384-well clear plates. A volume of 50 µL of early log phase culture of Mtb diluted to OD_{600} 0.02 was added to each well, and the assay plates were incubated at 37 °C for 5 days. Absorbance was recorded using a Spectramax M2 spectrophotometer, and $MIC_{50,}$ a concentration required to inhibit growth by 50% was determined by plotting non-linear curves using GraphPad Prism 5 software. Isoniazid was used as internal control in all our assays, it showed an MIC_{50} of 0.33 µM.

Solubility. Solubility was measured using high through put equilibrium solubility (HT-Eq sol) assay using a novel miniaturized shake-flask approach and streamlined HPLC analysis. Briefly, transfer DMSO sample stock solution from source plate to MPV (mini-prep filer vials,

Whatman) chamber and subjected to centrifugal evaporator to remove DMSO. Add aqueous buffer to MPV chamber and insert MPV plunger till the membrane on the bottom of the plunger touches the surface of the solution in the chamber. Incubate the assembled MPV on orbital shaker for 24h and push the plunger to the bottom of the chamber followed by shaking for another 30 min and filtrate was analyzed using HPLC[27].

Mouse, Rat and Human *in vitro* metabolic stability. The metabolic stability of drug candidates was determined in mouse/rat/human liver microsomes using compound depletion approach and quantified by LC/MS/MS. The assay measured the rate and extent of metabolism of chemical compounds by measuring the compounds *in vitro* half-life ($T_{1/2}$) and hepatic extraction ratios (ER) and predicted metabolic clearance in all species[28, 29].

***In vivo* PK.** All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Novartis Institute for Tropical Diseases. Mice were allowed for acclimatization before initiation of pharmacokinetic (PK) experiments. Food and water were given *ad libitum*. Compounds were formulated at a concentration of 2.5 mg/mL for a dose of 25 mg/kg given orally (*p.o.*) and at 1 mg/mL concentration for a dose of 5 mg/kg given intravenously (*i.v.*). **21** was formulated using 20% 1,2-propandiol, 10% solutol HS15 and top up with pH 4.6 acetate buffer for both *i.v* and oral administration. **39** and **41** were formulated with 50% MEPC (containing 30% Capmul MCM, 50% Cremophor RH40, 20% Ethanol) diluted with 50% of 50 mM pH 6.8 phosphate buffer for *p.o.* administration and with 20% 1,2-Propylene Glycol, 10% Cremophor EL, 70% of 50 mM acetate buffer pH 4.65 for *i.v.* administration. Blood samples were collected at 0.08, 0.25, 0.75, 1.5, 3, 8, 16 and 24 hours following oral dosing. The sampling schedule was identical following *i.v.* dosing except the first time point was at 0.02 h.

Groups of three mice were used for each time point. Blood was centrifuged at 13,000 rpm for 7 min at 4 ºC, plasma harvested and stored at -20 ºC until analysis.

Extraction and LCMS analysis. Plasma samples were extracted with acetonitrile:methanol:acetic acid (90:9.8:0.2) containing warfarin as internal standard, using a 8.8 to 1 extractant to plasma ratio. Analyte quantitation was performed by LC/MS/MS. Liquid chromatography was performed using an Agilent 1200 HPLC system (Santa Clara, CA) coupled with a API4000 triple quadruple mass spectrometer (Applied Biosystems, Foster City, CA) using the Agilent Zorbax Phenyl (3.5µm, 4.6x75 mm) column at 45 ºC. Instrument control and data acquisition were performed using Applied Biosystems software Analyst, version 1.4.2. The mobile phases used were (A) 0.2% of acetic acid in water (99.8:0.2, v/v) and (B) 0.1% of acetic acid in methanol (99.9:0.1, v/v), using a gradient with flow rate of 1.0 mL/min and run time of 5 min. Compound detection on the mass spectrometer was performed in electrospray positive ionization mode for **41** and negative mode for **21**, **39** and using multiple reaction monitoring (MRM) for specificity (transitions: **21** 323.2 / 183.8, **39** 337.2 / 184.0 and **41** 307.4 / 197.1) together with their optimized MS parameters. The lower limit of quantification ranged between 1 and 3 ng/mL.

Pharmacokinetic analysis. The mean value from the three animals at each time point was plotted against time to give plasma concentration-time profile. Pharmacokinetic parameters were determined using Watson LIMS, version 7.2 (Thermo Electron Corporation, PA, U.S.), by non-compartmental analysis. The oral bioavailability (F) was calculated as the ratio between the area under the curve (AUC) following oral administration and the AUC following intravenous administration corrected for dose ($F = AUC_{p.o.} \times dose_{i.v.} / AUC_{i.v.} \times dose_{p.o.}$).

***In vivo* efficacy.** *In vivo* acute mouse efficacy studies were carried-out as described earlier[11]. Briefly, on day 0 animals were infected by intranasal route with 10^3 Mtb H37Rv bacilli. One week post-infection animals treated for one month (7 days a week) with 25 or 75 mg/kg of NCEs with ethambutol (100 mg/kg) as positive control. At indicated time points lungs were aseptically removed, homogenized and bacterial load estimated by serial dilution plating 7H11 agar plates. **39** and **41** were formulated using the formulation as described in PK section. **28** was formulated using 40% MEPC (micro emulsion pre concentrate containing Cremaphor RH40, Labrafil M-2125 CS, 1,2-propylene glycol and ethanol) diluted with 60% of water for *p.o.* administration. All procedures involving mice were reviewed and approved by the institutional animal care and use committee of the Novartis Institute for Tropical Diseases.

General procedure for library synthesis of amides. To 4,6-dimethyl indole-2-carboxylic acid (0.108 mmol, 500 µL of 0.2 M in anhydrous DMF) was added HATU (0.108 mmol, 250 µL of 0.4 M in anhydrous DMF) and Et_3N (0.378 mmol, 50 µL of 2.0 M in anhydrous DMF) and stirred for 5 minutes at room temperature. The amine (0.108 mmol in 520 µL anhydrous DMF) was added to the reaction mixture. The reaction mixture was stirred for two hours at 45°C and the solvent was evaporated *in vacuo* and the residue was purified via preparative Shimadzu HPLC UPLC equipped with a Bischoff C18 column, 10µm, 20x50mm; a gradient of H_2O (0.1% TFA) and Methanol.

The below compounds were prepared as a library using the above general procedure from corresponding indole-2-carboxylic acids and amines

*N-isopropyl-4,6-dimethyl-1H-indole-2-carboxamide (**3**)*. Yield 69%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (br. s, 1H), 8.13 (d, J = 7.82 Hz, 1H), 7.12 (d, J = 1.32 Hz, 1H), 7.02 (s, 1H),

6.66 (s, 1H), 4.18-4.05 (m, 1H), 2.44 (s, 3H), 2.34 (s, 3H), 1.18 (d, J = 6.60 Hz, 6H). LCMS (ESI): *m/z* 231.8 $[M + H]^+$. HPLC purity: >99% at 254 nM.

*N-(sec-butyl)-4,6-dimethyl-1H-indole-2-carboxamide (**4**)*. Yield 69%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 8.04 (t, J = 5.8 Hz, 1H), 7.14 (d, J = 1.6 Hz, 1H), 7.02 (s, 1H), 6.66 (s, 1H), 3.98-3.88 (m, 1H), 2.43 (s, 3H), 2.33 (s, 3H), 1.56-1.48 (m, 2H), 1.14 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 7.3 Hz, J = 7.4 Hz, 3H). LCMS (ESI): *m/z* 243.0 $[M - H]^-$. HPLC purity: > 99.0% at 254 nM.

*N-isobutyl-4,6-dimethyl-1H-indole-2-carboxamide (**5**)*. Yield 24%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 8.34 (d, J = 8.0 Hz, 1H), 7.12 (d, J = 1.6 Hz, 1H), 7.02 (s, 1H), 6.66 (s, 1H), 3.11-3.08 (m, 2H), 2.43 (s, 3H), 2.33 (s, 3H), 1.91-1.78 (m, 1H), 0.90 (d, J = 6.6 Hz, 6H). LCMS (ESI): *m/z* 243.2 $[M - H]^-$. HPLC purity: 97.7% at 254 nM.

*4,6-dimethyl-N-(pentan-3-yl)-1H-indole-2-carboxamide (**6**)*. Yield 64%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.16 (d, J = 1.2 Hz, 1H), 7.02 (s, 1H), 6.66 (s, 1H), 3.84-3.75 (m, 1H), 2.44 (s, 3H), 2.34 (s, 3H), 1.59-1.52 (m, 4H), 0.87 (t, J = 7.4 Hz, 6H). LCMS (ESI): *m/z* 258.8 $[M + H]^+$. HPLC purity: 98.4% at 254 nM.

*N-isopentyl-4,6-dimethyl-1H-indole-2-carboxamide (**7**)*. Yield 25%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 8.29 (t, J = 5.6 Hz, 1H), 7.08 (d, J = 1.6 Hz, 1H), 7.02 (s, 1H), 6.66 (s, 1H), 3.28-3.26 (m, 2H), 2.43 (s, 3H), 2.33 (s, 3H), 1.69-1.59 (m, 1H), 1.45-1.44 (m, 2H), 0.91 (d, J = 6.6 Hz, 6H). LCMS (ESI): *m/z* 258.8 $[M + H]^+$. HPLC purity: 97.2% at 254 nM.

*N-cycloheptyl-4,6-dimethyl-1H-indole-2-carboxamide (**8**)*. Yield 31%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 1.6 Hz, 1H), 7.02 (s, 1H), 6.66

(s, 1H), 4.05-3.97 (m, 1H), 2.43 (s, 3H), 2.33 (s, 3H), 1.90-1.84 (m, 2H), 1.70-1.40 (m, 10H). LCMS (ESI): *m/z* 283.3 $[M - H]^-$. HPLC purity: >99% at 254 nM.

*4,6-dimethyl-N-phenyl-1H-indole-2-carboxamide (***13***)*. Yield 27%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 10.12 (s, 1H), 7.81 (d, J = 7.84 Hz, 2H), 7.41 (d, J = 1.6 Hz, 1H), 7.39-7.33 (m, 2H), 7.12-7.0 (m, 2H), 6.71 (s, 1H), 2.49 (s, 3H), 2.36 (s, 3H). LCMS (ESI): *m/z* 265.2 $[M + H]^+$. HPLC purity: >99% at 254 nM.

*N-cyclohexyl-N,4,6-trimethyl-1H-indole-2-carboxamide (**14**)*. Yield 42%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 7.02 (s, 1H), 6.72 (s,1H), 6.66 (s, 1H), 4.31 (t, J = 11.2 Hz, 1H), 3.07 (s, 3H), 2.43 (s, 3H), 2.34 (s, 3H), 1.82-1.55 (m, 7H), 1.37-1.15 (m, 3H). LCMS (ESI): *m/z* 283.4 $[M - H]^-$. HPLC purity: >99% at 254 nM.

*4,6-dichloro-N-(4,4-dimethylcyclohexyl)-1H-indole-2-carboxamide (**39**)*. To a stirred solution of 4,6-dichloro-1*H*-indole-2-carboxylic acid (50 mg, 0.217 mmol) in DMF (2 mL) were added HATU (99.5 mg, 0.26 mmol) and *N*,*N*-diisopropylethylamine (0.188 mL, 1.08 mmol) at room temperature. The reaction mixture was stirred for 10 min at the same temperature then added 4,4-dimethylcyclohexanamine.HCl (39 mg, 0.238 mmol). The resulting reaction mixture was stirred at room temparature for 16 h. H_2O (10 mL) was added to the reaction mixture and extracted with ethyl acetate (2 x 20 mL). The combined organic layers was washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel (100-200 mesh) using a solvent gradient of 30-40% ethyl acetate in petroleum ether as eluent to afford 30 mg (41%) of *4,6-dichloro-N-(4,4-dimethylcyclohexyl)-1H-indole-2-carboxamide* **39** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (br. s, 1H), 8.43 (d, J = 8.19 Hz, 1H), 7.42 (s, 1H), 7.30 (s, 1H), 7.22 (d, J =

1.59 Hz, 1H), 3.82-3.67 (m, 1H), 1.72-1.62 (m, 2H), 1.61-1.48 (m, 2H), 1.45-136 (m, 2H), 1.28 (dt, J = 3.61, 13.17 Hz, 2H), 0.95 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 159.7, 137.2, 134.3, 128.0, 126.7, 125.3, 119.8, 111.6, 101.0, 48.6, 38.1, 32.6, 29.8, 28.5, 24.6. LCMS (ESI): *m/z* 339.5 $[M + H]^+$. HPLC purity: 98.1% at 254 nm. HRMS calcd for $C_{17}H_{20}Cl_2N_2O$ $[M + H]^+$ 339.10, found 339.10.

The following compounds were synthesized using above procedure from corresponding substituted indole-2-carboxylic acids and amines

*N-cyclohexyl-4,6-dimethyl-1H-indole-2-carboxamide (**1**)*. Yield 98%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 1.6 Hz,1H), 7.02 (s, 1H), 6.66 (s, 1H), 3.78-3.76 (m, 1H), 2.43 (s, 3H), 2.33 (s, 3H), 1.84-1.83 (m, 4H),1.76-1.73 (m, 1H), 1.36-1.15 (m, 5H).

*4,6-dimethyl-N-(1-methylpiperidin-4-yl)-1H-indole-2-carboxamide (**2**)*. Yield 26.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 8.14 (d, J = 7.6 Hz, 1H), 7.14 (d, J = 1.2 Hz, 1H), 7.02 (s, 1H), 6.66 (s, 1H), 3.79-3.69 (m, 1H), 2.78 (d, J = 11.6 Hz, 2H), 2.43 (s, 3H), 2.33 (s, 3H), 2.17 (s, 3H), 1.95 (t, J = 11.6 Hz, 2H), 1.78-1.76 (m, 2H), 1.63-1.52 (m, 2H). LCMS (ESI): *m/z* 284.2 $[M - H]^-$. HPLC purity: 99.0% at 254 nM.

*4,6-dimethyl-N-((1R,2S)-2-methylcyclohexyl)-1H-indole-2-carboxamide (**9**)*. Yield 44%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (br. s, 1H), 7.76 (d, J = 8.44 Hz, 1H), 7.29 (d, J = 1.34 Hz, 1H), 7.03 (s, 1H), 6.67 (s, 1H), 4.12 (dt, J = 4.02, 7.98 Hz, 1H), 2.46 (s, 3H), 2.35 (s, 3H), 2.01-1.90 (m, 1H), 1.75-1.63 (m, 2H), 1.61-1.46 (m, 4H), 1.44-1.28 (m, 2H), 0.89 (d, J = 6.97 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 161.2, 137.1, 133.0, 131.0, 130.4, 125.7, 122.1, 109.8,

102.3, 49.9, 33.3, 30.3, 28.9, 23.2, 22.8, 22.0, 18.9, 15.7. LCMS (ESI): *m/z* 285.28 $[M + H]^+$. HPLC purity: 97.9%.

*N-(2-methoxyethyl)-4,6-dimethyl-1H-indole-2-carboxamide (**10**)*. Yield 48%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.35 (s, 1H), 8.41 (t, J = 4.84 Hz, 1H), 7.12 (d, J = 1.6 Hz, 1H), 7.02 (s, 1H), 6.67 (s, 1H), 3.65-3.41 (m, 4H), 3.29 (s, 3H), 2.43 (s, 3H), 2.33 (s, 3H). LCMS (ESI): *m/z* 247.6 $[M + H]^+$. HPLC purity: >99% at 254 nM.

*4,6-dimethyl-N-(tetrahydro-2H-pyran-4-yl)-1H-indole-2-carboxamide (**11**)*. Yield 70%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 8.22 (d, J = 7.6 Hz, 1H), 7.14 (d, J = 1.2 Hz,1H), 7.02 (s, 1H), 6.66 (s, 1H), 4.05-3.90 (m, 1H), 3.90-3.84 (m, 2H), 3.42 (dt, J = 2.0 Hz, 9.6 Hz, 2H), 2.43 (s, 3H), 2.33 (s, 3H), 1.78 (dd, J = 2.28, 13.0 Hz, 2H), 1.59 (dq, J = 4.39, 12.0 Hz, 2H). LCMS (ESI): *m/z* 270.9 $[M - H]^-$. HPLC purity: 98.7% at 254 nM.

*N-(2-hydroxycyclohexyl)-4,6-dimethyl-1H-indole-2-carboxamide (**12**)*. Yield 53%. 1H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 8.04 (d, J = 8.08 Hz, 1H), 7.13 (s, 1H), 7.02 (s, 1H), 6.6 (s, 1H), 4.65 (d, J = 5.16 Hz, 1H), 3.7-3.57 (m, 1H), 2.40 (s, 3H), 2.33 (s, 3H), 1.95-1.81 (m, 2H), 1.69-1.58 (m, 2H), 1.30-1.17 (m, 4H). LCMS (ESI): *m/z* 287.2 $[M + H]^+$. HPLC purity: >99.0% at 254 nM.

*1,4,6-trimethyl-N-((1R,2S)-2-methylcyclohexyl)-1H-indole-2-carboxamide (**15**)*. Yield 50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.94 (d, J = 8.35 Hz, 1H), 7.13 (s, 1H), 7.10 (s, 1H), 6.73 (s, 1H), 4.01-4.14 (m, 1H), 3.89 (s, 3H), 2.46 (s, 3H), 2.39 (s, 3H), 1.98-1.88 (m, 1H), 1.69-1.45 (m, 6H), 1.34-1.32 (m, 2H), 0.89 (d, J = 7.0 Hz, 3H). LCMS (ESI): *m/z* 299.21 $[M + H]^+$. HPLC purity: 97.4% at 214 nM.

*4,6-Dimethyl-N-((1R,2S)-2-methylcyclohexyl)-1H-pyrrolo[3,2-c]pyridine-2-carboxamide (**18**)*. Yield 6%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (s, 1H), 7.91 (d, J = 8.24 Hz, 1H), 7.43 (s, 1H), 7.00 (s, 1H), 4.15-4.07 (m, 1H), 2.61 (s, 3H), 2.45 (s, 3H), 1.99-1.89 (m, 1H), 1.70-1.61 (m, 2H), 1.53-1.23 (m, 6H), 0.87 (d, J = 6.97 Hz, 3H). LCMS (ESI): 286.18 $[M + H]^+$. HPLC purity: 96.5% at 254 nm.

*2,4-Dimethyl-N-((1R,2S)-2-methylcyclohexyl)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxamide (**19**)*. Yield 13%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (br. s, 1H), 6.80 (s, 1H), 6.16 (d, J = 8.24 Hz, 1H), 4.28-4.26 (m, 1H), 2.76 (s, 3H), 2.74 (s, 3H), 1.94-2.03 (m, 1H), 1.79-1.77 (m, 1H), 1.67-1.65 (m, 3H), 1.52-1.25 (m, 4H), 0.96 (d, J = 6.97 Hz, 3H). LCMS (ESI): 287.1 $[M + H]^+$. HPLC purity: 99% at 254 nm.

*4,6-dichloro-N-((1R,2S)-2-methylcyclohexyl)-1H-indole-2-carboxamide (**20**)*. Yield 35.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.14 (s, 1H), 7.39 (s, 1H), 7.17 (d, J = 1.6 Hz, 1H), 6.89 (d, J = 1.6 Hz, 1H), 6.25 (d, J = 8.88 Hz, 1H), 4.38-4.28 (m, 1H), 2.08-2.0 (m, 1H), 1.85-1.79 (m, 1H), 1.70-1.60 (m, 3H), 1.58-1.48 (m, 2H), 1.45-1.33 (m, 2H), 0.98 (d, J = 6.98 Hz, 3H). LCMS (ESI): *m/z* 325.17 $[M + H]^+$. HPLC purity: 97% at 254 nM.

*4,6-dichloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**21**)*. Yield 25.5%. Mixture of *cis* and *trans* isomers in 38:62 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (br. s, 1H), 8.43 (d, J = 8.0 Hz, 0.43H), 8.29 (d, J = 7.46 Hz, 0.58H), 7.42 (s, 1H), 7.38 (s, 0.57H), 7.31 (s, 0.43H), 7.24-7.20 (m, 1H), 3.99-3.89 (m, 0.58H), 3.81-3.68 (m, 0.44H), 1.91-1.80 (m, 1H), 1.76-1.64 (m, 2H), 1.63-1.30 (m, 4H), 1.39-1.31 (m, 1H), 0.99-1.10 (m, 1H), 0.96 (d, J = 6.85 Hz, 1.72H), 0.90 (d, J = 6.48 Hz, 1.30H). LCMS (ESI): *m/z* 325.1 $[M + H]^+$. HPLC purity: >99% at 214 nM.

*4,6-difluoro-N-((1R,2S)-2-methylcyclohexyl)-1H-indole-2-carboxamide (**22**)*. Yield 36%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1H), 7.97 (d, J = 8.25 Hz, 1H), 7.46 (s, 1H), 7.02 (d, J= 8.25 Hz, 1H), 6.89 (t, J = 10.15 Hz, 1H), 4.2-4.05 (m, 1H), 2.0-1.9 (m, 1H), 1.7-1.25 (m, 8H), 0.87 (d, J = 6.98 Hz, 3H). LCMS (ESI): *m/z* 293.16 $[M + H]^+$. HPLC purity: 97.1% at 214 nM.

*4,6-difluoro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**23**)*. Yield 54%. Mixture of *cis* and *trans* isomers in 39:61 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (br. s, 1H), 8.28 (d, J = 8.07 Hz, 0.40H), 8.13 (d, J=7.46 Hz, 0.59H), 7.35 (d, J = Hz, 0.61H), 7.26 (s, 0.41H), 7.02 (td, J = 2.38, 9.41 Hz, 1H), 6.92-6.82 (m, 1H), 3.94 (m, 0.61H), 3.67-3.80 (m, 0.43H), 1.85 (m, 1H), 1.63-1.77 (m, 2H), 1.28-1.62 (m, 5H), 0.99-1.10 (m, 1H), 0.96 (d, J = 6.85 Hz, 1.84H), 0.90 (d, J = 6.48 Hz, 1.23H). LCMS (ESI): *m/z* 293.2 $[M + H]^+$. HPLC purity: >99% at 214 nM.

*4-chloro-6-fluoro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**24**)*. Yield 55%. Mixture of *cis* and *trans* isomers in 40:60 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.36 (d, J = 8.0 Hz, 0.42H), 8.22 (d, J = 7.4 Hz, 0.61H), 7.35 (s, 0.66H), 7.27 (s, 0.42H), 7.17-7.10 (m, 2H), 3.94-3.87 (m, 0.66H), 3.80-3.67 (m, 0.43H), 1.89-1.79 (m, 1H), 1.76-1.63 (m, 2H), 1.61-1.26 (m, 5H), 1.08-0.97 (m, 1H), 0.95 (d, J = 6.84 Hz, 1.87H), 0.89 (d, J = 6.48 Hz, 1.2H). LCMS (ESI): *m/z* 307.4 $[M - H]^-$. HPLC purity: >99% 254 nM.

*6-chloro-4-fluoro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**25**)*. Yield 41.5%. Mixture of *cis* and *trans* isomers in 40:60 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H), 8.33 (d, J = 8.0 Hz, 0.4H), 8.18 (d, J = 7.2 Hz, 0.61H), 7.37 (s, 0.61H), 7.32-7.24 (m, 1.4H), 6.97-6.92 (m, 1H), 3.94 - 3.89 (m, 0.62H), 3.80 - 3.70 (m, 0.39H), 1.86-1.81 (m, 1H), 1.74-1.62 (m, 2H), 1.61-1.28 (m, 5H),1.08-0.97 (m, 1H), 0.95 (d, J = 6.84 Hz, 1.82H), 0.88 (d, J = 6.48 Hz, 1.18H). LCMS (ESI): *m/z* 308.9 $[M + H]^+$. HPLC purity: >99% at 254 nM.

*4-cyano-6-isocyano-N-(4-methyl-cyclohexyl)-1H-indole-2-carboxamide (**26**)*. Yield 53%. Mixture of *cis* and *trans* isomers in 45:55 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.76 (br. s, 1H), 8.65 (d, J = 7.70 Hz, 0.44H), 8.50 (d, J = 7.09 Hz, 0.55H), 8.16 (s, 1H), 8.11 (s, 1H), 7.64 (s, 0.55H), 7.56 (s, 0.43H), 3.91-4.03 (m, 0.58H), 3.84-3.70 (m, 0.46H), 1.92-1.84 (m, 1H), 1.79-1.65 (m, 2H), 1.65-1.30 (m, 5H), 1.11-1.00 (m, 1H), 0.97 (d, J = 6.85 Hz, 1.64H), 0.91 (d, J = 6.48 Hz, 1.35H). LCMS (ESI): *m/z* 307.9 $[M + H]^+$. HPLC purity: 98.9% at 254 nM.

*4,6-dichloro-N-(cis-4-methylcyclohexyl)-1H-indole-2-carboxamide (**27**)*. Yield 50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (br. s, 1H), 8.29 (d, J = 7.46 Hz, 1H), 7.43-7.40 (m, 1H), 7.38 (d, J = 1.2 Hz, 1H), 7.22 (d, J = 1.59 Hz, 1H), 3.94 (td, J = 3.62, 7.31 Hz, 1H), 1.76-1.63 (m, 3H), 1.63-1.49 (m, 4H), 1.49-1.38 (m, 2H), 0.96 (d, J = 6.85 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 159.5, 136.7, 133.6, 127.5, 126.2, 124.8, 119.3, 111.0, 100.9, 46.5, 29.7, 28.5, 27.9, 19.5. LCMS (ESI): *m/z* 325.23 $[M + H]^+$ & 327.15 $[M + 2H]^+$. HPLC purity: 98.05% at 235 nM. HRMS calcd for $C_{16}H_{18}Cl_2N_2O$ $[M - H]^-$ 323.08, found 323.07.

*4,6-dichloro-N-(trans-4-methylcyclohexyl)-1H-indole-2-carboxamide (**28**)*. Yield 66%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.03 (br. s, 1H), 8.43 (d, J = 8.07 Hz, 1H), 7.42 (dd, J = 0.79, 1.53 Hz, 1H), 7.28-7.33 (m, 1H), 7.21 (d, J = 1.71 Hz, 1H), 3.81-3.67 (m, 1H), 1.85 (d, J = 10.03 Hz, 2H), 1.71 (d, J = 11.98 Hz, 2H), 1.36 (dq, J = 3.06, 12.39 Hz, 3H), 0.96-1.10 (m, 2H), 0.89 (d, J = 6.6 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 159.2, 136.7, 133.7, 127.5, 126.2, 124.8, 119.3, 111.0, 100.5, 48.1, 33.6, 32.2, 31.5, 22.1. LCMS (ESI): *m/z* 325.17 $[M + H]^+$ & 327.15 $[M + 2H]^+$. HPLC purity: 99% at 254 nM. HRMS calcd for $C_{16}H_{18}Cl_2N_2O$ $[M + H]^+$ 325.08, found 325.09.

*4,6-difluoro-N-(trans-4-methylcyclohexyl)-1H-indole-2-carboxamide (**29**)*. Yield 52%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.8 (s, 1H), 8.28 (d, J = 7.91 Hz, 1H), 7.26 (s, 1H), 7.02-7.0 (m, 1H), 6.89-6.84 (m, 1H), 3.76-3.69 (m, 1H), 1.86-1.83 (m, 2H), 1.72-1.69 (m, 2H), 1.38-1.30 (m, 3H), 1.07-0.98 (m, 2H), 0.89 (d, J = 6.6 Hz, 3H). LCMS (ESI): *m/z* 293.18 $[M + H]^+$. HPLC purity: 98.7% at 254 nM.

*4-chloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**30**)*. Yield: 43%. Mixture of *cis* and *trans* isomers in 45:55 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (br. s, 1H), 8.41 (d, J = 8.0 Hz, 0.46H), 8.28 (d, J = 7.46 Hz, 0.54H), 7.40 (dd, J = 3.48, 8.01 Hz, 1H), 7.33 (s, 0.55H), 7.26 (s, 0.45H), 7.20-7.14 (m, 1H), 7.13-7.09 (m, 1H), 4.00-3.90 (m, 0.56H), 3.81-3.68 (m, 0.48H), 1.86 (d, J = 9.90 Hz, 1H), 1.77-1.64 (m, 2H), 1.63-1.29 (m, 5H), 1.10-1.00 (m, 1H), 0.97 (d, J = 6.85 Hz, 1.68H), 0.90 (d, J = 6.48 Hz, 1.33H). LCMS (ESI): *m/z* 291.11 $[M + H]^+$. HPLC purity: >99% at 214 nM.

*5-chloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**31**)*. Yield 40%. Mixture of *cis* and *trans* isomers in 45:55 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.74 (d, J = 11.13 Hz, 1H), 8.28 (d, J = 8.04 Hz, 0.45H), 8.14 (d, J = 7.46 Hz, 0.55H), 7.74-7.64 (m, 1H), 7.43 (dd, J = 3.18, 8.68 Hz, 1H), 7.25-7.09 (m, 2H), 3.99-3.88 (m, 0.59H), 3.80-3.68 (m, 0.48H), 1.85 (d, J = 10.03 Hz, 1H), 1.75-1.65 (m, 2H), 1.62-1.29 (m, 5H), 1.1-0.98 (m, 1H), 0.96 (d, J = 6.8 Hz, 1.76 H), 0.90 (d, J = 6.52 Hz, 1.34H). LCMS (ESI): *m/z* 291.11 $[M + H]^+$. HPLC purity: >99% at 214 nM.

*6-chloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**32**)*. Yield 50%. Mixture of *cis* and *trans* isomers in 25:75 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.69 (br. s, 1H), 8.25 (d, J = 8.0 Hz, 0.26H), 8.11 (d, J = 7.46 Hz, 0.74H), 7.68-7.58 (m, 1H), 7.48-7.38 (m, 1H), 7.25 (s,

0.78H), 7.17 (s, 0.27H), 7.00-7.09 (m, 1H), 4.00-3.88 (m, 0.77H), 3.82-3.65 (m, 0.28H), 1.90-1.63 (m, 3H), 1.63-1.49 (m, 3H), 1.48-1.30 (m, 2.4H), 1.10-0.99 (m, 0.63H), 0.96 (d, J = 6.85 Hz, 2H), 0.90 (d, J = 6.48 Hz, 1H). LCMS (ESI): *m/z* 291.11 $[M + H]^+$. HPLC purity: >99% at 254 nM.

*7-chloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**33**)*. Yield 44%. Mixture of *cis* and *trans* isomers in 43:57 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.63 (br. s, 1H), 8.28 (d, J = 7.72 Hz, 1H), 8.16 (d, J = 6.97 Hz, 1H), 7.55-7.66 (m, 1H), 7.27-7.32 (m, 1H), 7.22 (s, 0.56H), 7.18 (s, 0.43H), 7.06 (dt, J = 2.63, 7.79 Hz, 1H), 3.91-4.00 (m, 0.58H), 3.68-3.80 (m, 0.45H), 1.90 (d, J = 9.90 Hz, 1H), 1.50-1.79 (m, 5H), 1.29-1.48 (m, 2H), 0.99-1.11 (m, 1H), 0.97 (d, J = 6.85 Hz, 1.70H), 0.91 (d, J = 6.48 Hz, 1.33H). LCMS (ESI): 291.11 $[M + H]^+$. HPLC purity: >99% at 214 nM.

*4,5-dichloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**34**)*. Yield 50%. Mixture of *cis* and *trans* isomers in 41:59 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (br. s, 1H), 8.44 (d, J = 7.92 Hz, 0.42H), 8.30 (d, J=7.46 Hz, 0.61H), 7.45-7.39 (m, 1H), 7.38-7.29 (m, 2H), 4.01-3.88 (m, 0.64H), 3.81-3.69 (m, 0.45H), 1.86 (d, J = 10.39 Hz, 1H), 1.78-1.64 (m, 2H), 1.63-1.27 (m, 5H), 1.11-1.00 (m, 1H), 0.97 (d, J = 6.72 Hz, 1.8H), 0.90 (d, J = 6.48 Hz, 1.2H). LCMS (ESI): *m/z* 324.98 $[M + H]^+$ & 327.02 $[M + 2H]^+$. HPLC purity: >99% at 254 nM.

*4,7-dichloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**35**)*. Yield 41%. Mixture of *cis* and *trans* isomers in 37:63 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 0.5H), 12.01 (s, 0.34H), 7.67-7.57 (m, 2H), 7.48 (s, 1H), 7.21-7.17 (m, 1H), 4.03-4.12 (m, 0.66H), 3.77-3.73 (m, 0.37H), 1.92-1.54 (m, 6H), 1.42-1.25 (m, 2H), 1.08-1.02 (m, 1H), 0.94 (d, J = 6.32 Hz, 1.97H),

0.89 (d, J = 6.54 Hz, 1.2H). LCMS (ESI): 324.9 $[M + H]^+$ & 327.0 $[M + 2H]^+$. HPLC purity: 97.0% at 214 nM.

*5,6-dichloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**36**)*. Yield 71%. Mixture of *cis* and *trans* isomers in 39:61 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.84 (br. s, 1H), 8.37 (d, J = 7.99 Hz, 0.41H), 8.23 (d, J = 7.38 Hz, 0.61H), 7.94 (d, J = 2.4 Hz, 1H), 7.60 (d, J = 2.8 Hz, 1H), 7.24 (s, 0.64H), 7.17 (s, 0.39H), 3.92-3.91 (m, 0.64H), 3.74-3.72 (m, 0.41H), 1.89-1.78 (m, 1H), 1.75-1.28 (m, 7H), 1.09-0.98 (m, 1H), 0.95 (d, J = 6.8 Hz, 1.84H), 0.89 (d, J = 6.44 Hz, 1.22H). LCMS (ESI): 325.15 $[M + H]^+$ & 327.10 $[M + 2H]^+$. HPLC purity: 99.0% at 254 nM.

*5,7-dichloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**37**)*. Yiled 10%. Mixture of *cis* and *trans* isomers in 34:66 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.88 (s, 0.67H), 11.83 (s, 0.35H), 8.32 (d, J = 7.6 Hz, 0.34H), 8.19 (d, J = 6.8 Hz, 0.66H), 7.72 (s, 1H), 7.38 (d, J = 1.32 Hz, 1H), 7.21-7.16 (m, 1H), 3.95 (m, 0.66H), 3.75-3.72 (m, 0.34H), 1.9-1.25 (m, 8H), 1.08-1.02 (m, 1H), 0.95 (d, J = 7.2 Hz, 2.01H), 0.89 (d, J = 6.4 Hz, 1.07H). LCMS (ESI): *m/z* 325.04 $[M + H]^+$. HPLC purity: >99% at 254 nM.

*6,7-dichloro-N-(4-methylcyclohexyl)-1H-indole-2-carboxamide (**38**)*. Yield 39%. Mixture of *cis* and *trans* isomers in 67:33 ratio. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (br. s, 1H), 8.32 (d, J = 7.82 Hz, 1H), 8.19 (d, J = 7.04 Hz, 1H), 7.67-7.60 (m, 1H), 7.29-7.23 (m, 1.33H), 7.21 (s, 0.67H), 4.0-3.90 (m, 0.35H), 3.80-3.67 (m, 0.72H), 1.95-1.85 (m, 1H), 1.78-1.50 (m, 4H), 1.48-1.22 (m, 3H), 1.13-0.99 (m, 1H), 0.96 (d, J = 6.8 Hz, 0.9H), 0.90 (d, J = 6.48 Hz, 2.16H). LCMS (ESI): 325.04 $[M + H]^+$ & 327.02 $[M + 2H]^+$. HPLC purity: 98.5% at 254 nM.

*4,6-dichloro-N-(4,4-difluorocyclohexyl)-1H-indole-2-carboxamide (**40**)*. Yield 39%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H), 8.51 (d, J = 7.50 Hz, 1H), 7.42 (s, 1H), 7.32 (d, J = 1.3

Hz, 1H), 7. 23 (d, *J* = 1.3 Hz, 1H), 4.04-4.02 (m, 1H), 2.07-1.90 (m, 6H), 1.69-1.61 (m, 2H). LCMS (ESI): *m/z* 347.0 $[M + H]^+$ & 349.0 $[M + 2H]^+$. HPLC purity: 98.8%.

*4,6-difluoro-N-(4,4-dimethylcyclohexyl)-1H-indole-2-carboxamide (**41**)*. Yield 66.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.96 (br. s, 1H), 8.29 (d, *J* = 8.07 Hz, 1H), 7.26 (d, *J* = 0.48 Hz, 1H), 7.02 (dd, *J* = 1.47, 9.41 Hz, 1H), 6.87 (dt, *J* = 2.08, 10.39 Hz, 1H), 3.63-3.81 (m, 1H), 1.73-1.62 (m, 2H), 1.60-1.46 (m, 2H), 1.45-1.36 (m, 2H), 1.28 (dt, *J* = 3.73, 13.17 Hz, 2H), 0.95 (s, 3H), 0.92 (s, 3H). LCMS (ESI): *m/z* 307.11 $[M + H]^+$. HPLC purity: 98.6%. HRMS calcd for $C_{17}H_{20}F_2N_2O$ $[M + H]^+$ 307.15, found 307.16.

*4,6-Dimethyl-N-((1R,2S)-2-methylcyclohexyl)indoline-2-carboxamide (**16**)*. To a stirred solution of **9** (0.10 g, 0.350 mmol) in TFA (8 mL) at 0 °C was added $NaCNBH_3$ (0.308 g, 4.901 mmol) slowly portion wise and stirred for 3 h at room temperature. $NaCNBH_3$ (0.308 g, 4.901 mmol) was added again at 0 °C and continued stirring for 8 h at room temperature. The reaction mixture was diluted with DCM and washed with aq. sat. $NaHCO_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel (100-200 mesh) using a solvent gradient of 50% ethyl acetate in petroleum ether followed by prep HPLC to afford 50 mg (50%) of diastereomeric mixture of *4,6-dimethyl-N-((1R,2S)-2-methylcyclohexyl)indoline-2-carboxamide* **16** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6.45 (s, 1H), 4.45 (br. s, 1H), 4.08 (br. s, 2H), 3.55-3.45 (m, 1H), 3.0-2.95 (m, 1H), 2.26 (s, 3H), 2.16 (s, 3H), 1.85 (br. s, 2H), 1.5-1.19 (m, 6H), 0.89-0.88 (m, 1H), 0.79 (d, *J* = 7.0 Hz, 3H). LCMS (ESI): *m/z* 287.26 $[M + H]^+$. HPLC purity: >99% at 214 nM.

*4,6-dimethyl-N-((1R,2S)-2-methylcyclohexyl)-1H-benzo[d]imidazole-2-carboxamide (**17**)*. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.28 (s, 1H), 6.92 (s, 1H), 6.68 (s, 1H), 6.23 (d, J = 8.24 Hz, 1H), 4.35-4.32 (m, 1H), 2.50 (s, 3H), 2.35 (s, 3H), 2.06 (br. s, 1H), 1.88 (br. s, 1H), 1.66-1.59 (m, 3H), 1.54-1.51 (m, 2H), 1.43-137 (m, 2H), 0.94 (d, J = 6.8 Hz, 3H). LCMS (ESI): 286.18 [M + H]$^+$. HPLC purity: 99% at 254 nm.

ASSOCIATED CONTENT

Supporting Information. Experimental details for synthesis of compound **17**, a table describing LCMS and HPLC methods for all compounds and structures of **42**, **43** and **44** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* Tel: +65-6722-2984. Fax: +65-6722-2918.

E-mail: ravinder.kondreddi@novartis.com

Present Addresses

§**Cal**ifornia **I**nstitute for **B**iomedical **R**esearch (Calibr)

11119 N. Torrey Pines Rd., Suite 100

La Jolla, CA 92037 USA.

Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597

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ABBREVIATIONS

Mtb: Mycobacterium tuberculosis; TB: Tuberculosis; MDR: multi-drug resistant; XDR: extensively drug resistant; TDR: totally drug resistant; DIPEA: *N*,*N*-diisopropylethylamine; DMF: dimethylformamide; HATU: 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; $POCl_3$: phosphorus oxychloride; MIC: minimum inhibitory concentration; SAR: structure–activity relationships; LiPE: Lipophilic Efficiency; SPR: structure–property relationships

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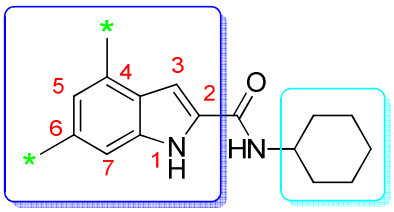


Figure 1. Structure of indole-2-carboxamide hit identified by Mtb phenotypic screening and potential metabolic soft spots are shown by asterix on indole ring and cyclohexyl group.

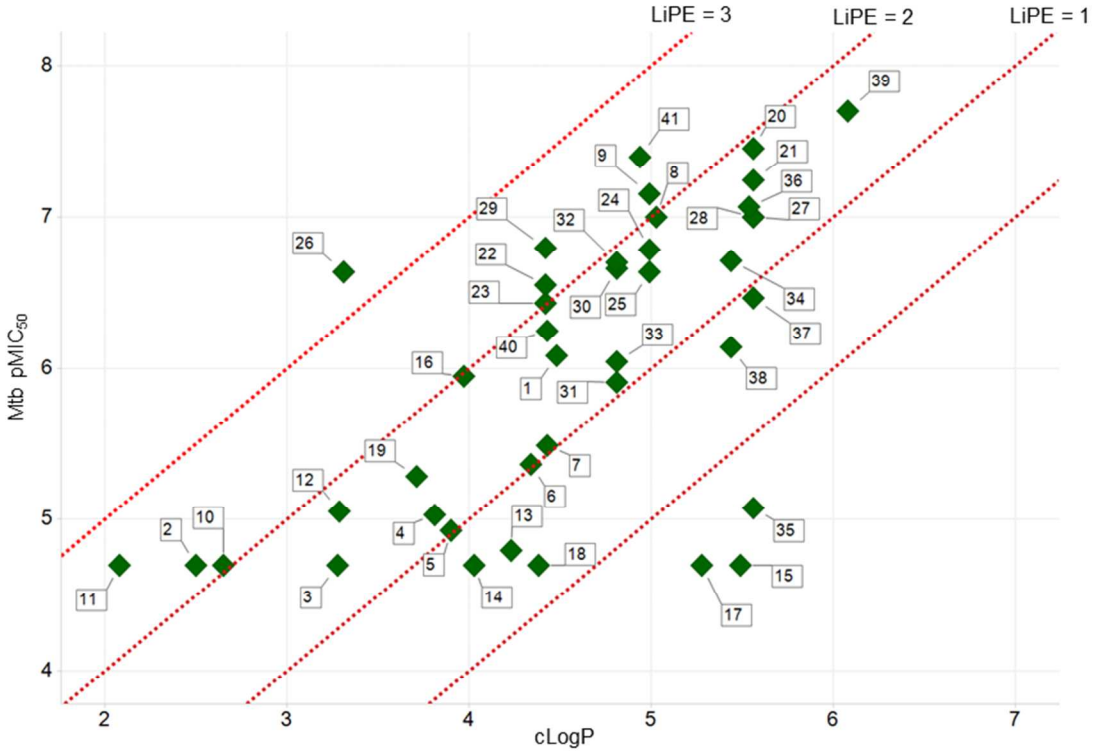


Figure 2: A correlation plot of indole-2-carboxamide analogue's Mtb $pMIC_{50}$ with cLogP.

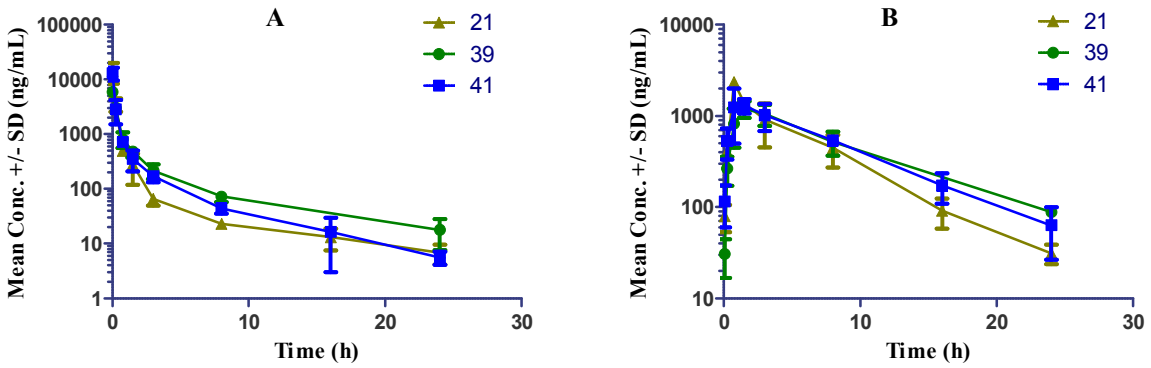


Fig 3: *In vivo* pharmacokinetic profile of selected compounds (A. *i.v.* PK at 5 mg/kg, B. *p.o* PK at 25 mg/kg)

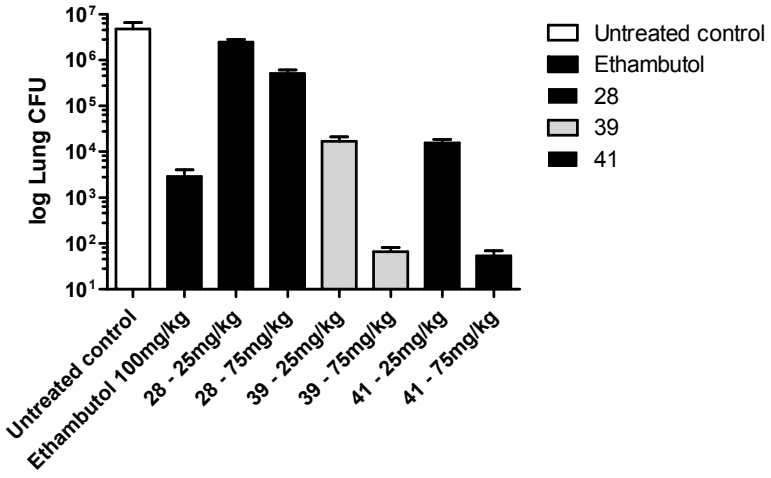
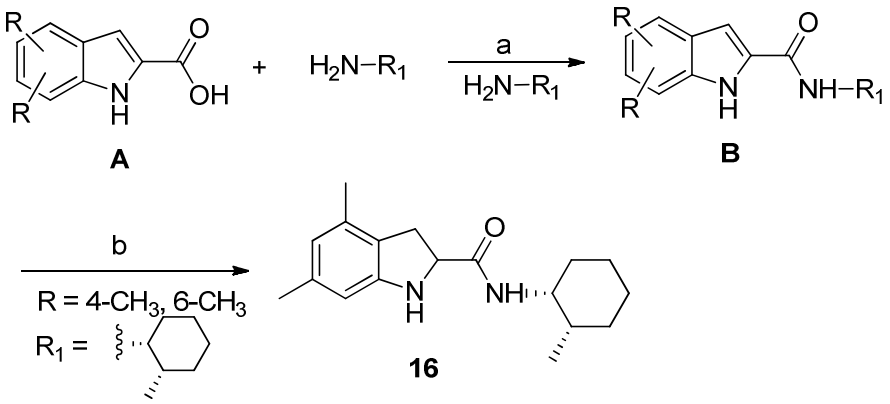


Fig 4: *In vivo* efficacy of lead compounds **28**, **39** and **41**

Scheme 1[a]



[a] Reagents and conditions: (a) HATU, DIPEA, DMF, RT, 24h (b) $NaCNBH_3$, TFA, 0°C to RT, 8h

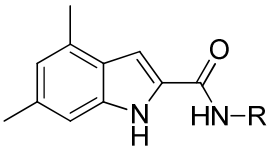
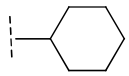
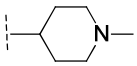
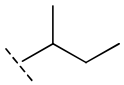
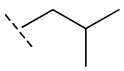
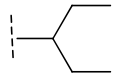
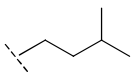
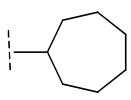


Table 1. Structure Activity Relationship of cyclohexyl group (RHS)

Compd	R	MIC_{50} µM	cLogP	aq. Solubility pH 6.8 (mM)
1		0.82	4.48	<0.004
2		>20	2.5	>1.0
3		>20	3.28	0.020
4		9.33	3.81	0.022
5		11.85	3.9	ND
6		4.33	4.34	<0.004
7		3.26	4.43	ND
8		0.1	5.03	<0.004

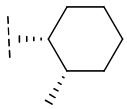
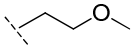
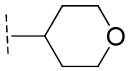
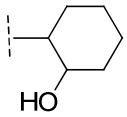
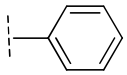
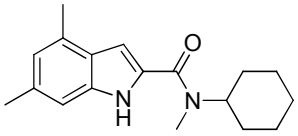
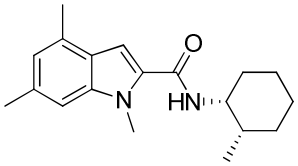
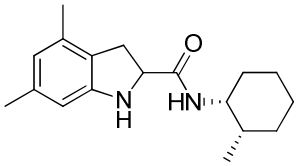
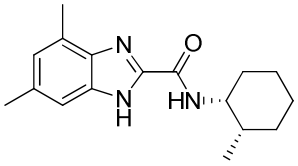
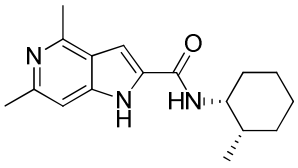
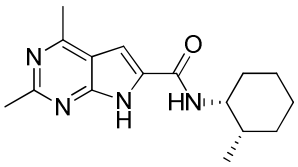
9		0.079	4.99	<0.004
10		>20	2.65	0.62
11		>20	2.07	0.017
12		8.9	3.28	0.056
13		16.0	4.23	<0.004

Table 2: Antitubercular activity, cLogP and solubility of amide, indole NH and indole core modifications

Compd	Structure	MIC_{50} μM	cLogP	aq. Solubility pH 6.8 (mM)
14		>20	4.03	ND
15		>20	5.48	ND
16		1.24	3.96	<0.004
17		>20	5.28	ND
18		>20	4.37	ND

19		>20	3.71	0.143

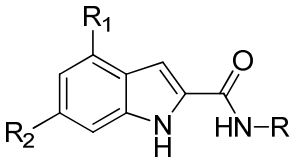
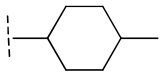
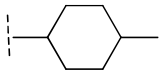
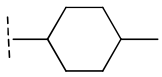
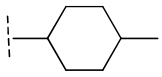
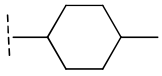
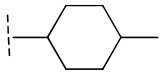
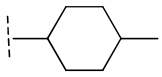
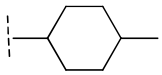
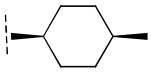
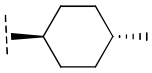
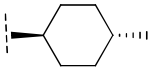


Table 3: Modification of indole and cyclohexyl to improve metabolic stability of potent compound **9**

Compd	R_1	R_2	R	MIC_{50} μM	cLogP	aq. Solubility pH 6.8 (mM)	Mice CL_{int} μL/min/mg
9	Me	Me		0.079	4.99	<0.004	433
20	Cl	Cl		0.053	5.56	<0.004	89
21	Cl	Cl		0.15	5.56	<0.004	37
22	F	F		0.28	4.42	<0.004	660
23	F	F		0.30	4.42	<0.004	866
24	Cl	F		0.18	4.99	<0.004	77
25	F	Cl		0.19	4.99	<0.004	28

26	CN	CN		0.27	3.30	<0.004	12
27	Cl	Cl		0.18	5.56	<0.004	47
28	Cl	Cl		0.14	5.56	<0.004	3.4
29	F	F		0.16	4.42	<0.004	149

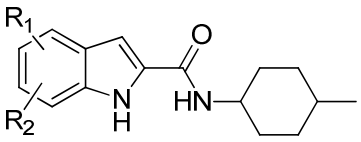


Table 4. Identification of optimal chloro substitutions on the indole ring

Compd	R_1	R_2	MIC_{50} μM	Mouse CL_{int} μL/ min/mg
21	4-Cl	6-Cl	0.15	37.0
30	4-Cl	H	0.19	462.0
31	5-Cl	H	1.29	121.6
32	6-Cl	H	0.23	433.1
33	7-Cl	H	1.49	1040
34	4-Cl	5-Cl	0.22	60.8
35	4-Cl	7-Cl	>20	40.4
36	5-Cl	6-Cl	0.16	73.3
37	5-Cl	7-Cl	0.41	187.3
38	6-Cl	7-Cl	0.97	308.0

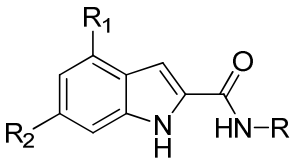


Table 5: Antitubercular activity, cLogP, solubility and metabolic stability of lead candidates **39** and **41**

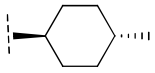
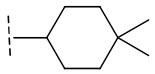
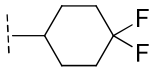
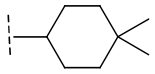
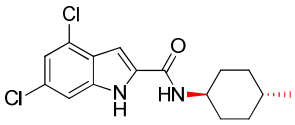
Compd	R_1	R_2	R	MIC_{50} µM	cLogP	aq. Solubility pH 6.8 (mM)	Mice/Human/Rat Cl_{int} µL/min/mg
28	Cl	Cl		0.14	5.56	<0.004	3.4/10/30.5
39	Cl	Cl		0.015	6.08	<0.004	13/59/139
40	Cl	Cl		0.85	4.43	<0.004	23/7.8/38.8
41	F	F		0.023	4.94	<0.004	132/3.4/87

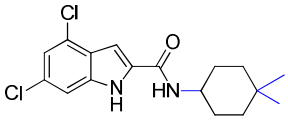
Table 6: *In vivo* pharmacokinetics of selected candidates

		i.v PK parameters[#]			*p.o.* PK parameters[$]			
Compd	MIC (μM)	V_{ss} (L/kg)	CL (mL/min/kg)	$T_{1/2}$ (h)	C_{max} (μM)	AUC_{0-24} (μM*h)	$T_{1/2}$ (h)	F%
21	0.150	2.2	20.15	6.89	7.23	30.35	4.2	48
39	0.015	4.11	18.10	5.25	3.51	33.90	5.96	53
41	0.023	2.24	18.74	4.47	4.21	35.48	5.18	51

[#]*i.v.* PK at 5 mg/kg, [$]*p.o* PK at 25 mg/kg

Table of Contents





	28	39
MIC H37Rv (μM)	0.14	0.015
AUC (μM*h) @ 75 mg/kg	89.07	70.27
T1/2 (h) @ 75 mg/kg	5.29	8.14