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Design, Synthesis and biological evaluation of novel imidazo[*1,2-a*]pyridinecarboxamides as potent anti-tuberculosis agents

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ABSTRACT

Tuberculosis (TB) is a highly infectious disease that has been plaguing the human race for centuries. The emergence of multidrug resistant strains of TB has been detrimental to the fight against tuberculosis with very few safe therapeutic options available. As part of an ongoing effort to identify potent anti-tuberculosis agents,

we synthesized and screened a series of novel imidazo[1,2-*a*]pyridinecarboxamide derivatives for their anti-tuberculosis properties. These compounds were designed based on reported anti-tuberculosis properties of the indolecarboxamides (I2Cs) and imidazo[1,2-*a*]pyridinecarboxamides (IPAs). In this series, we identified compounds **15** and **16** with excellent anti-TB activity against H37Rv strain of tuberculosis (MIC = 0.10 – 0.19 μM); these compounds were further screened against selected clinical isolates of *Mtb*. Compounds **15** and **16** showed excellent activities against multidrug resistant (MDR) and extensively drug resistant (XDR) strains of TB (MIC range: 0.05 – 1.5 μM) with excellent selectivity indices. In addition, preliminary ADME studies on compound **16** showed favorable pharmacokinetic properties.

INTRODUCTION

Tuberculosis (TB) is an insidious and air-borne infectious disease caused by *Mycobacterium tuberculosis* that mainly affects the lungs. TB is one of the top 10 leading cause of mortality worldwide from a single infectious disease (above HIV/AIDS). In 2018, the World Health Organization (WHO) estimated about 10 million people fell ill with TB worldwide while 1.5 million died as a result of TB infection (0.25 million among people living with HIV).(1) An estimated 0.9 million new cases of TB among people living with HIV was reported in 2018, 72% of whom lived in Africa.(1) In addition, eight counties namely: India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh and South Africa, accounted for two third of new TB cases reported globally.(1) The global health burden of TB infection is further aggravated by HIV co-infection, thus making TB one of the leading cause of death among people living with HIV.(1, 2) Active drug-susceptible TB is treatable and curable using a standard 6 month course of 4 antimicrobial drugs (first-line anti-TB drugs namely isoniazid, rifampin, pyrazinamide and ethambutol), however the long duration of treatment, poor drug quality, inability to provide the required drug regimens for the duration of treatment and/or inappropriate drug use has given rise to the emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB.(2) The burden of TB is further exacerbated by the emergence of MDR-TB and XDR-TB and current TB drugs are inadequate in putting an end to this multidrug resistance epidemic.

In August 2019, the FDA approved PA-824 (pretomanid) in combination with TMC207 (bedaquiline) and Linezolid for the treatment of certain type of extremely treatment-resistant TB of the lungs.(3) Noteworthy, TMC207 (bedaquiline; approved 2012) was the first anti-TB drug approved in over four decades by the FDA for treatment of MDR-TB in adults when there are no other alternatives. However, bedaquiline (TMC207) is associated with side effect such as irregular heart rhythm,(4) which show that the search for newer, highly efficient, fast acting drug with low toxicity is far from over. Bedaquiline targets the membrane-bound adenosine triphosphate (ATP) synthase enzyme of *M. tuberculosis*,(5-7) while the exact target of pretomanid is

not yet known. Pretomanid was also reported to show activity against both replicating and hypoxic nonreplicating strains of tuberculosis.(7-9)

Our TB drug discovery campaign led to the identification of indole-2-carboxamide**s** which showed excellent activity against susceptible, MDR and XDR strains of *Mtb*.(10-12) The indole-2-carboxamides has been reported to inhibit *Mtb* growth by targeting the MmpL3 protein (which belongs to the resistance, nodulation and cell division (RND) family of membrane transporters), an essential membrane transporter.(10-14) In addition, we recently reported that indole-2-carboxamide (**1**) showed anti-M. abscessus activity by acting on the MmpL3 protein of this organism.(15, 16)

Another heterocyclic aromatic carboxamide, which has been extensively researched as a highly potent anti-TB scaffold, is the imidazopyridine-3-carboxamides (IPA).(17-24) Using genome sequencing technique, it was established that IPA inhibitor family exert its anti-TB properties by targeting *M. tuberculosis* QcrB, an essential component of the electron transport chain.(25)

Structural Activity Relationship (SAR) studies of the indole-2-carboxamides (I2C) showed the importance of cyclic aliphatic ring on the amide nitrogen(10, 12) in order to enhance and maintain activity, this motivated us to investigate the anti-TB activity of IPAs with varying size of cyclic aliphatic rings on the amide nitrogen at both positions 2 and 3 of the imidazopyridine ring. Herein, we report the design, synthesis, biological evaluation and *in vitro* pharmacokinetic (PK) of novel imidazo[1,2-*a*]pyridine carboxamide derivatives **3** – **19**, our goal is to design and investigate small molecules with potent anti-TB activity.

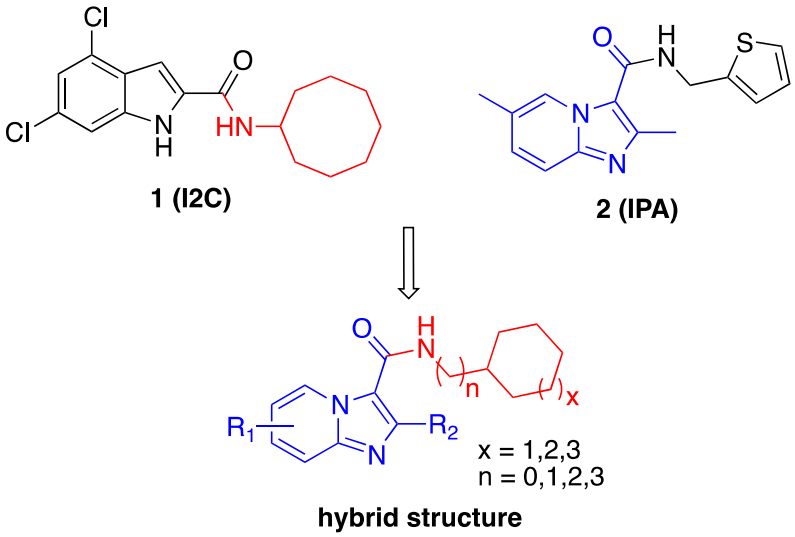


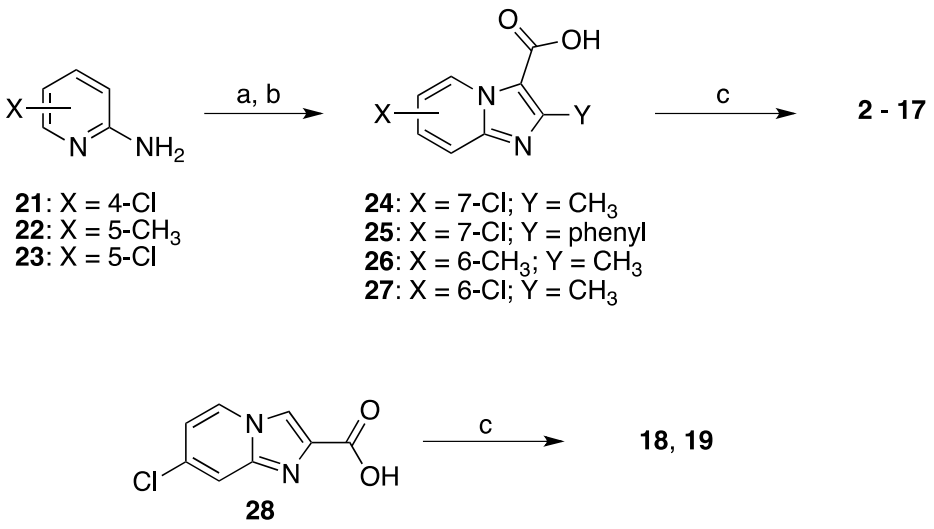
Figure **1**. Indole-2-carboxamide derivative **1**; Imidazo[*1,2-a*]pyridine-3-carboxamide derivative **2** and hybrid structure.

CHEMISTRY

Following efficient amide coupling protocol (Scheme 1), seventeen novel imidazopyridine carboxamide derivatives were generated. Briefly, ethyl acetoacetate or ethyl benzoylacetate was reacted with *N*-bromosuccinimide (NBS) to afford the corresponding ethyl-2-bromoacetoacetate or ethyl-2-bromobenzoylacetate, which was subsequently reacted with substituted 2-amino pyridine (**21-23**) to afford the various substituted imidazo[*1,2-a*]pyridine-3-carboxylates. Basic hydrolysis of the ester derivatives afforded the corresponding acids **24** - **27**.

The substituted imidazopyridine carboxylic acids (**24** – **28**) were reacted with their corresponding cycloalkyl amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and hydroxybenzotriazole (HOBt) as coupling agents and triethylamine as a base to obtain compounds **2**–**19** (Scheme 1).

Scheme 1[a]



[a]Reagents and conditions: (a) (i) ethyl acetoacetate or ethyl benzoylacetate, NBS, H_2O, reflux, 1.5 h (ii) substituted 2-aminopyridine, reflux, 3 h; (b) LiOH, EtOH, reflux, 3 h; (c) EDC·HCl, HOBt, corresponding amine, Et_3N, CH_2Cl_2, rt, 12–16 h, yield: 33 - 77%.

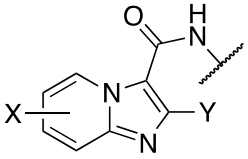
DISCUSSION

Structure-Activity Relationships: In general, the biological activity of the analogs was found to depend on the number of carbon spacers between the amide Nitrogen and the cyclic aliphatic ring. The reference compound **2** showed similar anti-TB activity (MIC = 0.2 μM) as reported in literature.(25) Replacement of the aromatic side chain on the amide nitrogen with cyclic aliphatic rings such as *N*-cyclohexyl, *N*-cycloheptyl and *N*-cyclooctyl groups (compounds **3** – **5**) resulted in a loss of activity against susceptible strain of *Mtb*. Furthermore, replacing the methyl group at position 6 of the imidazo[1,2-*a*]pyridine ring with a chloro group at positions 6 or 7 (compounds **6** and **7**) did not improve their activities against TB (MIC >200 μM). In addition, replacing the methyl group at 2-position of the imidazo[1,2-*a*]pyridine ring with a phenyl group (compound **8**) did not show any improved activity (MIC > 160 μM).

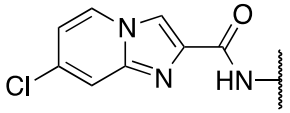
Compounds **9** - **11** bearing one carbon spacer between the amide nitrogen and the cycloalkyl moieties (i.e. cyclohexyl, cycloheptyl and cyclooctyl groups) turned out to be 267-, 133- and 133-fold more potent against *M.tb* compared to their counterparts compounds **3** - **5** with no carbon spacers. On the other hand, compound **12** bearing one carbon spacer between the amide nitrogen and a polycyclic group (1-adamantyl derivative) showed

poor anti-TB activity compared to its monocyclic counterparts **9** – **11**. To complete the investigation of the effect of the carbon spacer between amide nitrogen and cycloalkyl group, a two and three carbon spacers were installed and investigated. Compounds **13** (bearing a cyclohexyl group) and **14** (bearing a cycloheptyl group) with two carbon spacers showed similar activities as their one carbon spacer counterparts **9** and **10**. On the other hand, compound **15** (bearing a cyclohexyl group) with a three carbon spacers was 4-fold more active than compounds **9** (one carbon spacer) and **13** (two carbon spacers) respectively. Switching the methyl group at position 6 of compound **15** to a 6-chloro (compound **16**) resulted in approximately 2-fold drop in activity, while switching the methyl group at position 2 on the imidazopyridine ring to a phenyl group (compound **17**, with chloro substituent at position 7) resulted in a complete loss in activity, suggesting that a methyl or small group at the 2-position is essential for activity. Noteworthy, compounds **15** and **16** were approximately 1.5 - 3-fold more active than isoniazid against H37Rv strain of TB. Next, we examined the effect of the position of the amide group on the imidazopyridine ring. The amide group at position 2 of the imidazo[1,2-*a*]pyridine ring (**18** and **19**) proved to be less active than the amide group at position 3, implying that amide substituent at the position 3 is essential for anti-TB activity.

Table 1. MIC Values of Imidazo[*1,2-a*]pyridine carboxamide analogs

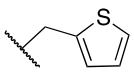
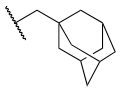
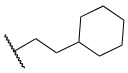
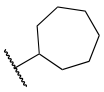
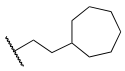
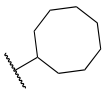
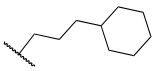
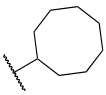
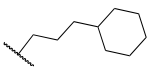
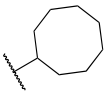
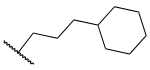
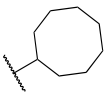
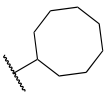
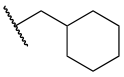
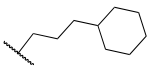
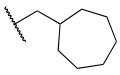
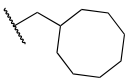


2 - 17



18, 19

Compd	X	Y	R	MIC[a] (μM)	Compd	X	Y	R	MIC[a] (μM)

2	6-methyl	methyl		0.22	**12**	6-methyl	methyl		47.41
3	6-methyl	methyl		117.91	**13**	6-methyl	methyl		0.42
4	6-methyl	methyl		56.06	**14**	6-methyl	methyl		0.40
5	6-methyl	methyl		106.88	**15**	6-methyl	methyl		0.10
6	6-chloro	methyl		>200.13	**16**	6-chloro	methyl		0.19
7	7-chloro	methyl		≥200	**17**	7-chloro	phenyl		>161.66
8	7-chloro	phenyl		≥167.58	**18**	7-chloro	-		≥209.29
9	6-methyl	methyl		0.44	**19**	7-chloro	-		>200.13
10	6-methyl	methyl		0.42	**INH**[b]				0.29
11	6-methyl	methyl		0.80					

[a]The lowest concentration of drug leading to at least a 90% reduction of bacterial growth signal by the microplate AlamarBlue assay (MABA). MIC values are reported as an average of three individual measurements; [b]INH = Isoniazid.

Based on our preliminary results, the most active compounds (**15** and **16**) were selected for further screening against a panel of clinical isolates(26) of *Mtb* originally obtained from pulmonary TB patients (Table 2). Among those strains are one drug-sensitive (DS) (V4207), two MDR *Mtb* (KZN494 and V2475), two XDR *Mtb* (TF274, R506) respectively. In addition, the toxicity of these compounds against mammalian cells was evaluated on Vero cells, and their respective selectivity indices (SI) were calculated (Table 2). Compounds **15** and **16** maintained their excellent activity or showed only a moderate drop in activity (up to 2- fold for **15** and up to 8-fold for **16**) against the tested clinical isolates of *Mtb*. Noteworthy is the fact that both compounds (**15** and **16**) showed a 2-fold increase in activity against the MDR *Mtb* strain KZN494 while compounds **15** maintained its excellent activity against XDR *Mtb* strain R506. The high activity of these compounds against MDR and XDR strains of TB indicates their potential use to treat drug-resistant *Mtb* strains. In addition, compounds **15** and **16** displayed high IC_{50} values against Vero cells resulting in high SI indices suggesting their potential lack of toxicity towards mammalian cells.

As for the mode of action, the imidazo[1,2-a]pyridinecarboxamides (IPAs) are well known QcrB inhibitors of M. tuberculosis,(18, 22, 25, 27) thus we propose that these novel imidazo[1,2-a]pyridinecarboxamide compounds could potentially target and bind to QcrB, which encodes the beta subunit of the cytochrome bc1 complex of Mycobacterium tuberculosis. The cytochrome bc1 facilitates electron transfer and proton [H^+] translocation, which is the driving force for ATP synthesis and essential for Mycobacterium tuberculosis.

Table 2. Activity of imidazo[*1,2-a*]pyridine-3-carboxamide**s** against Selected Clinical Isolates of *Mtb* [MIC (μM)] and Vero Cells [IC_{50} (μM)].

	Mtb MIC (μM)							
Compd	**H37Rv/DS**	**V4207/DS**	**KZN494/MDR[a]**	**V2475/MDR[a]**	**R506/XDR[b]**	**TF274/XDR[b]**	**Vero cells IC_{50} (μM)**	**SI[c]**
15	0.10	0.10	0.05	0.20	0.10	0.20	102.07	1024
16	0.19	0.37	0.09	0.37	0.75	1.5	≥191.67	≥1024

[a]Resistance to isoniazid and rifampin. [b]Resistance to isoniazid, rifampin, levofloxacin, and kanamycin. [c]Selectivity index (SI) = IC_{50} (Vero)/MIC.

Preliminary ADME Studies on Compound 16.

Based on the encouraging biological results described above, we conducted some selected ADME studies on compound **16** to assess its drug-like properties (Table 3). The compound exhibited high plasma protein binding in human (99.89%) and mouse (99.64%). As shown in Table 3, in the presence of compound **16** (10 μM) none of the tested CYP isoforms (with the exception of CYP3A4) showed greater than 48% inhibition.

Table 3. ADME data for imidazo[1,2-*a*]pyridine-3-carboxamide 16.

Assay	**16**
Plasma protein binding (%), human/mouse	99.89/99.64
CYP inhibition (% inhibition at 10 μM)	
CYP1A2	26.3
CYP2C9	32.2
CYP2C19	45.2
CYP2D6	16.4
CY3A4[a]	52.2
hERG IC_{50} (μM)	>10
Hepatocytes, % remaining at 2 hrs, starting concentration 1 μM	0.19 (human)
Liver microsomes, % remaining after 1 hr, starting concentration 1 μM	1.51 (human, 60 mins), 0.15 (mouse, 60 mins)

[a]Midazolam is used as substrate

The potential inhibitory effect of compound **16** on human Ether-à-go-go related gene (hERG) channel was evaluated using manual patch-clamp system. Based on the result, compound **16** is suggested to be ranked as a

weak inhibitor on hERG channel (low inhibition of hERG (IC_{50} > 10 μM); thus implying low potential risk for blocking the cardiac calcium channel. Noteworthy, in the Ether-hERG analysis we found that compound **16** may encounter solubility problem in hERG assay buffer at the highest concentration, 30 μM. The IC_{50} is therefore only estimated to be > 10 μM, as percentage hERG inhibition tested at 10 μM was found less than 50% (29.10% inhibition at 10 μM).

When incubated with human hepatocyte, over 95% of compound **16** degraded after 2 hours incubation using a starting concentration of 1 μM and the human hepatocytes. Compound **16** was unstable in liver microsomes with approximately 1.5% (human) and 0.15% (mouse) remaining unchanged after 1 hour using a starting concentration of 1 μM.

In summary, we describe the synthesis, structure-activity relationships and preliminary ADME analysis of novel imidazo[1,2-*a*]pyridinecarboxamide derivatives as anti-TB agents. Modifications to the *N*-methylthiophen-2-yl group at position 3 of the imidazo[1,2-*a*]pyridine ring in the lead compound **2** have been explored to create compounds with improved biological activities. Compounds **15** and **16** were identified as the most potent compounds in the series against susceptible strain of tuberculosis, and further screening of these compounds (**15** and **16**) showed excellent activity against MDR and XDR clinical isolates of TB. In addition, compound **16** showed acceptable PK properties, further study is currently underway to improve its stability in liver microsome and hepatocyte. These compounds adds to the growing list of imidazopyridine carboxamide derivatives that have been reported to possess anti-TB properties in literature(17, 18, 20, 22-24, 27-30).

Experimental details

1. Chemistry method

General information. 7-chloroimidazo[1,2-*a*]pyridine-2-carboxylic acid was purchased from Combi-blocks. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer (300 and 75 MHz), with TMS as an internal standard. Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quadruplet, m = multiplet and br = broad. HRMS experiments were performed on micOTOF-Q (Bruker) instrument. TLC was performed with Merck 60 F254 silica gel plates. Flash chromatography was performed using YamazenFlash® Rf system with Yamazen pre-packed columns or alternatively using Sigma-Aldrich silica gel (230–400 mesh). Purities of final compounds were established by analytical HPLC, which was carried out using the Agilent 1100 HPLC system with a Synergi 4 μm Hydro-RP 80A column, on a variable wavelength detector G1314A. Method: flow rate = 1.0 mL/min; gradient elution over 20 minutes, from 30% MeOH-H_2O to 100% MeOH with 0.05% TFA, and 100% MeOH with 0.05% TFA for an additional 5 minutes. The purity of all tested compounds was >95% (unless otherwise stated) as determined by the method described above.

General procedure for the synthesis of **2-19**

To a solution of the appropriate carboxylic acid (1 equiv) in anhydrous dichloromethane (CH_2Cl_2) (4 mL/mmol) at room temperature were added anhydrous hydroxybenzotriazole (HOBt, 1 equiv) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride **(**EDC.HCl, 1 equiv) under an argon atmosphere. After stirring for 10 min, the appropriate substituted amine (1 equiv) and triethylamine (1.5 equiv) were added, and the reaction mixture was stirred at room temperature until disappearance of the starting material (usually 12 to 16 h). After this time water (2 mL) was added, and the mixture was extracted with EtOAc (3×10 mL), the organic layers were separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (dichloromethane–methanol) gradient, 0-10% methanol in dichloromethane over 20-30 minutes (unless specified differently) to obtain the imidazopyridinamides in yields ranging from 33 to 77%.

Experimental details

2,6-dimethyl-*N*-(thiophen-2-ylmethyl)imidazo[1,2-*a*]pyridine-3-carboxamide (2). Yield 65% (off-white solid). HPLC: t_R 8.0 min, purity 98.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.21 (s, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.23 (d, J = 3.0 Hz, 1H), 7.18 (d, J = 9.0 Hz, 1H), 7.05 (d, J = 3.0 Hz, 1H), 6.98-6.95 (t, J = 3.0, 6.0 Hz, 1H), 6.19

(s, 1H), 4.86 (d, J = 6.0 Hz, 2H), 2.65 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.4, 145.5, 145.1, 141.1, 130.1, 127.0, 126.0, 135.3, 123.0, 115.7, 114.9, 38.3, 18.4, 16.8. HRMS (ESI) calcd for $C_{15}H_{16}N_3OS$ ($[M+H]^+$) 286.1009; found: 286.0986.

***N*-cyclohexyl-2, 6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (3).** Yield 70 % (white solid). HPLC: t_R 9.0 min, purity 97.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 9.0 Hz, 1H), 5.67 (d, J = 6.0 Hz, 1H), 4.08-3.93 (m, 1H), 2.61 (s, 3H), 2.33 (s, 3H), 2.11-1.99 (m, 2H), 1.81-1.67 (m, 2H), 1.55-1.16 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.9, 144.9, 144.5, 129.7, 125.9, 122.7, 115.6, 115.4, 48.1, 33.4, 25.6, 24.8, 18.3, 16.6. HRMS (ESI) calcd for $C_{16}H_{22}N_3O$ ($[M+H]^+$) 272.1757; found: 272.1744.

***N*-cycloheptyl-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (4).** Yield 74 % (white powder). HPLC: t_R 10.3 min, purity 97.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 9.0 Hz, 1H), 5.74 (d, J = 6.0 Hz, 1H), 4.29-4.10 (m, 1H), 2.66 (s, 3H), 2.33 (s, 3H), 2.15-1.98 (m, 2H), 1.76-1.44 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 144.9, 144.5, 129.7, 125.9, 122.7, 115.7, 115.4, 50.4, 35.4, 28.0, 24.2, 18.3, 16.7. HRMS (ESI) calcd for $C_{17}H_{24}N_3O$ ($[M+H]^+$) 286.1914; found: 286.1911.

***N*-cyclooctyl-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (5).** Yield 61 % (white powder). HPLC: t_R 12.5 min, purity 98.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.46 (d, J = 9.0 Hz, 1H), 7.16 (d, J = 9.0 Hz, 1H), 5.82 (d, J = 9.0 Hz, 1H), 4.28-4.20 (m, 1H), 2.66 (s, 3H), 2.32 (s, 3H), 1.95 (m, 2H), 2.02-1.60 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.5, 146.0, 144.8, 144.1, 129.9, 125.9, 122.9, 115.5, 49.4, 32.5, 27.2, 25.5, 23.7, 18.3, 16.5. HRMS (ESI) calcd for $C_{18}H_{26}N_3O$ ($[M+H]^+$) 300.2070; found: 300.2072.

6-chloro-*N*-cyclooctyl-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (6). Yield 50 % (white powder). HPLC: t_R 15.3 min, purity 97.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.49 (s, 1H), 7.49 (d, J = 9.0 Hz, 1H), 7.27 (d, J = 3.0 Hz, 1H), 5.78 (d, J = 6.0 Hz, 1H), 4.32-4.15 (m, 1H), 2.68 (s, 3H), 2.03-1.99 (m, 2H), 1.71-1.61 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 145.3, 144.1, 128.0, 126.1, 121.4, 116.6, 116.2, 49.5, 32.5, 27.2, 25.5, 23.7, 16.6. HRMS (ESI) calcd for $C_{17}H_{23}ClN_3O$ ($[M+H]^+$) 320.1524; found: 320.1531.

7-chloro-*N*-cyclooctyl-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (7). Yield 51 % (white powder). HPLC: t_R 13.9 min, purity 97.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.17 (d, J = 6.0 Hz, 1H), 7.41 (s, 1H), 6.76 (d, J = 6.0 Hz, 1H), 5.85 (d, J = 6.0 Hz, 1H), 4.19-4.13 (m, 1H), 2.57 (s, 3H), 1.91-1.86 (m, 2H), 1.65-1.48 (m, 12H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.0, 145.5, 145.4, 133.2, 128.1, 115.9, 115.2, 114.3, 49.4, 32.4, 27.1, 25.4, 23.6, 16.4. HRMS (ESI) calcd for $C_{17}H_{23}ClN_3O$ ($[M+H]^+$) 320.1524; found: 320.1537.

7-chloro-*N*-cyclooctyl-2-phenylimidazo[1,2-*a*]pyridine-3-carboxamide (8). Yield 56 % (white powder). HPLC: t_R 19.7 min, purity 97.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.67-7.64 (m, 2H), 7.61 (d, J = 9.0 Hz, 1H), 7.51-7.49 (m, 3H), 7.34 (d, J = 9.0 Hz, 1H), 5.82 (d, J = 9.0 Hz, 1H), 4.12-4.07 (m, 1H), 1.77-1.71 (m, 2H), 1.51-1.27 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 148.3, 144.4, 133.5, 129.7, 129.6, 129.0, 128.5, 126.4, 121.9, 117.3, 115.4, 49.2, 31.4, 27.2, 25.3, 23.3. HRMS (ESI) calcd for $C_{22}H_{25}ClN_3O$ ([M+H]$^+$) 382.1681; found: 382.1701.

***N*-(cyclohexylmethyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (9).** Yield 58 % (off-white solid). HPLC: t_R 10.8 min, purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (s, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 9.0 Hz, 1H), 5.86 (s, 1H), 3.35-3.31 (t, J = 6.0, 6.0 Hz, 2H), 2.67 (s, 3H), 2.33 (s, 3H), 1.81-1.60 (m, 6H), 1.32-1.14 (m, 3H), 1.06-0.96 (m, 2H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.8, 145.0, 144.6, 129.8, 126.0, 122.8, 115.6, 115.3, 45.6, 38.1, 31.0, 26.4, 25.8, 18.3, 16.7. HRMS (ESI) calcd for $C_{17}H_{24}N_3O$ ([M+H]$^+$) 286.1914; found: 286.1895.

***N*-(cycloheptylmethyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (10).** Yield 47 % (off-white powder). HPLC: t_R 12.4 min, purity 98.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.17 (d, J = 9.0 Hz, 1H), 5.85 (s, 1H), 3.37-3.33 (t, J = 6.0, 6.0 Hz, 2H), 2.69 (s, 3H), 2.34 (s, 3H), 1.91-1.71 (m, 4H), 1.72-1.68 (m, 2H), 1.58-1.45 (m, 5H), 1.34-1.24 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.8, 145.0, 144.6, 129.8, 126.0, 122.8, 115.7, 115.4, 45.9, 39.8, 32.3, 28.3, 26.4, 18.4, 16.7. HRMS (ESI) calcd for $C_{18}H_{26}N_3O$ ([M+H]$^+$) 300.2070; found: 300.2058.

***N*-(cyclooctylmethyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (11).** Yield 72 % (off-white powder). HPLC: t_R 11.9 min, purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.40 (d, J = 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 5.91 (s, 1H), 3.30-3.26 (t, J = 6.0, 6.0 Hz, 2H), 2.63 (s, 3H), 2.29 (s, 3H), 1.79-1.27 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.7, 144.9, 144.6, 129.7, 125.9, 122.7, 115.6, 115.4, 46.1, 37.9, 30.4, 26.9, 26.2, 25.4, 18.3, 16.6. HRMS (ESI) calcd for $C_{19}H_{28}N_3O$ ([M+H]$^+$) 314.2227; found: 314.2213.

***N*-(adamantan-1-ylmethyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (12).** Yield 73 % (tan solid). HPLC: t_R 12.5 min, purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.17 (s, 1H), 7.43 (d, J = 9.0 Hz, 1H), 7.14 (d, J = 9.0 Hz, 1H), 5.87-5.82 (t, J = 6.0, 9.0 Hz, 1H), 3.19 (d, J = 6.0 Hz, 2H), 2.70 (s, 3H), 2.31 (s, 3H), 2.05-1.96 (m, 3H), 1.73-1.56 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.9, 145.0, 144.5, 129.7, 126.0, 122.8, 115.6, 115.4, 50.7, 40.4, 36.9, 33.9, 28.2, 18.3, 16.9. HRMS (ESI) calcd for $C_{21}H_{28}N_3O$ ([M+H]$^+$) 338.2227; found: 338.2212.

***N*-(2-cyclohexylethyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (13).** Yield 51 % (off-white powder). HPLC: t_R 12.6 min, purity 98.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.40 (d, J = 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 5.82-5.79 (t, J = 3.0, 6.0 Hz, 1H), 3.50-3.43 (q, J = 6.0, 6.0, 9.0 Hz, 2H), 2.65 (s, 3H), 2.32 (s, 3H), 1.74-1.60 (m, 5H), 1.53-1.46 (q, J = 6.0, 21.0, 12.0 Hz, 2H), 1.33 (m, 1H), 1.22-1.15 (m, 3H), 0.98-0.90 (t, J = 12.0, 12.0 Hz, 2H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 144.9, 144.6, 129.7, 125.9, 122.7, 115.6, 115.3, 37.3, 35.6, 33.2, 26.4, 26.2, 18.3, 16.6. HRMS (ESI) calcd for $C_{18}H_{26}N_3O$ ($[M+H]^+$) 300.2070; found: 300.2073.

***N*-(2-cycloheptylethyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (14).** Yield 33 % (tan solid). HPLC: t_R 14.2 min, purity 96.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.37 (d, J = 9.0 Hz, 1H), 7.10 (s, J = 9.0 Hz, 1H), 5.89-5.85 (t, J = 6.0, 6.0 Hz, 1H), 3.46-3.40 (q, J = 6.0, 6.0 Hz, 2H), 2.59 (s, 3H), 2.26 (s, 3H), 1.71-1.32 (m, 13H), 1.22-1.13 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.6, 144.8, 144.6, 129.6, 125.8, 122.7, 115.5, 115.3, 37.9, 37.7, 37.0, 34.4, 28.4, 26.3, 18.3, 16.5. HRMS (ESI) calcd for $C_{19}H_{28}N_3O$ ($[M+H]^+$) 314.2227; found: 314.2220.

***N*-(3-cyclohexylpropyl)-2,6-dimethylimidazo[1,2-*a*]pyridine-3-carboxamide (15).** Yield 77 % (tan solid). HPLC: t_R 14.0 min, purity 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.17 (s, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 9.0 Hz, 1H), 5.81-5.77 (t, J = 6.0, 6.0 Hz, 1H), 3.49-3.42 (q, J = 6.0, 9.0, 6.0 Hz, 2H), 2.66 (s, 3H), 2.33 (s, 3H), 1.74-1.62 (m, 7H), 1.31-1.12 (m, 6H), 0.93-0.83 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.7, 144.9, 144.7, 129.8, 125.9, 122.8, 115.7, 115.4, 39.8, 37.4, 34.8, 33.3, 27.3, 26.6, 26.3, 18.3, 16.7. HRMS (ESI) calcd for $C_{19}H_{28}N_3O$ ($[M+H]^+$) 314.2227; found: 314.2224.

6-chloro-*N*-(3-cyclohexylpropyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (16). Yield 55 % (off-white powder). HPLC: t_R 17.7 min, purity 97.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (s, 1H), 7.52 (d, J = 12.0 Hz, 1H), 7.30 (d, J = 12.0 Hz, 1H), 5.84-5.83 (t, J = 3.0, 3.0 Hz, 1H), 3.51-3.44 (q, J = 6.0, 6.0, 6.0 Hz, 2H), 2.69 (s, 3H), 1.70-1.68 (m, 7H), 1.28-1.18 (m, 6H), 0.95-0.88 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1, 145.3, 144.1, 128.2, 126.1, 121.5, 116.6, 116.1, 39.9, 37.3, 34.7, 33.3, 27.2, 26.6, 26.3, 16.5. HRMS (ESI) calcd for $C_{18}H_{25}ClN_3O$ ($[M+H]^+$) 334.1681; found: 334.1672.

7-chloro-*N*-(3-cyclohexylpropyl)-2-phenylimidazo[1,2-*a*]pyridine-3-carboxamide (17). Yield 70 % (tan powder). HPLC: t_R 20.5 min, purity 93.8% ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.65 (s, 1H), 7.68-7.61 (m, 3H), 7.52-7.51 (m, 3H), 7.37-7.33 (m, 1H), 5.81 (s, 1H), 3.31-3,24 (q, J = 6.0, 9.0, 6.0 Hz, 2H), 1.69-1.61 (m, 7H), 1.43-1.33 (m, 2H), 1.22-1.10 (m, 4H), 1.05-0.97 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 148.4, 144.5, 133.5,

128.7, 129.1, 128.7, 126.8, 126.4, 122.0, 117.4, 115.2, 39.6, 37.3, 34.5, 33.2, 26.6, 26.5, 26.3. HRMS (ESI) calcd for $C_{23}H_{27}ClN_3O$ ([M+H]$^+$) 396.1837; found: 396.1837.

7-chloro-*N*-cyclooctylimidazo[1,2-a]pyridine-2-carboxamide (18). Yield 46 % (white powder). HPLC: tR 17.5 min, purity 96.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.11 (s, 1H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.28 (d, *J* = 6.0 Hz, 1H), 7.19 (d, *J* = 9.0 Hz, 1H), 4.20-4.17 (m, 1H), 1.97-1.90 (m, 2H), 1.71-1.57 (m, 12H); ^{13}C NMR (75 MHz, CDCl3) δ 161.0, 142.8, 141.4, 127.4, 124.2, 121.5, 118.4, 114.4, 49.2, 32.2, 27.3, 25.4, 23.7. HRMS (ESI) calcd for $C_{16}H_{21}ClN_3O$ ([M+H]+) 306.1368; found: 306.1358.

7-chloro-*N*-(3-cyclohexylpropyl)imidazo[1,2-*a*]pyridine-2-carboxamide (19). Yield 61 % (white powder). HPLC: t_R 18.7 min, purity 97.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.11 (s, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.35-7.34 (t, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 3.47 (q, *J* = 6.0, 6.0, 6.0 Hz, 2H), 1.68-1.61 (m, 7H), 1.29-1.16 (m, 6H), 0.93-0.86 (m, 2H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.1, 142.8, 141.2, 127.5, 124,2, 121.7, 118.4, 114.4, 39.6, 37.4, 34.6, 33.3, 27.1, 26.7, 26.3. HRMS (ESI) calcd for $C_{17}H_{23}ClN_3O$ ([M+H]$^+$) 320.1524; found: 320.1532.

2. Biological method

Minimum Inhibitory Concentration (MIC) determination. MIC was determined by using MABA assay. *Mtb* H37Rv and five clinical isolates were cultured to mid-log phase (OD600 = 0.5) in 7H9 broth at 37ºC. The culture was diluted to OD600 of 0.001 using 7H9 without Tween 80 targeting 10^5 colony forming units (CFU) per mL. Two-fold serial dilution was made for compounds from 64 to 0.0039 µg/mL using 100 µL of 7H9 without Tween 80 on 96-well plates. One hundred µL of the above-mentioned diluted culture was added to each testing wells. Test plates were incubated at 37 ºC for 7 days, then 32.5 µL of alamarBlue reagent was added to all wells. Plates were further incubated for 16-18 h at 37^o and read at Ex544/Em590 using a BMG Optima fluorescence plate reader. Data were analyzed and the lowest concentration that inhibited 90% of growth was defined as MIC.

Vero Cytotoxicity (IC_{50}) determination. Cytotoxicity of selected compounds, expressed as IC_{50}, was determined using Vero cells and MABA assay. Briefly, Vero cell linage (ATCC CCL-81) was grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). Flat-bottomed 96-well plate was seeded with 1×10^4 cells per well. The plate was incubated at 37^{o}C with 5% CO_2 for 16 h. For compound preparation, 2-fold serial dilution was made using a deep-well block using DMEM containing 5%

FBS with a volume of 200 μL. Culture media was replaced with 160 μL of the compound-containing media, with 100% DMSO as positive (100% kill) control and media-only as blank (100% viability) control. The plate was incubated for 72 h and then washed twice with PBS before adding 100 μL of DMEM with 5% FBS medium freshly mixed with 10% alamarBlue. The plate was incubated for 2 h and then immediately read with a fluorescence microplate reader at 544Ex/590Em. The minimum concentration that killed at least 50% of the cells was identified as IC_{50}.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding this work

Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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