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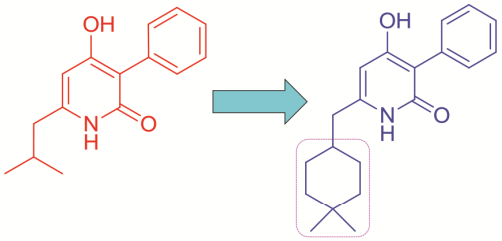
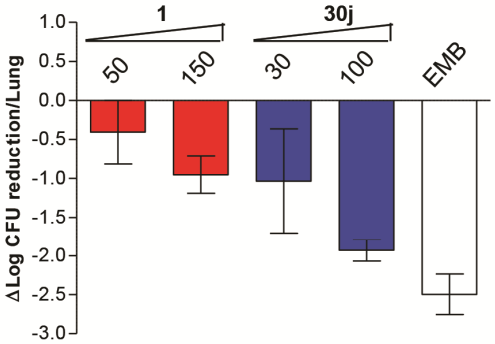
Graphical abstract



	Hit **1**	Lead **30j**
Mtb MIC_{50} (µM)	1.54	0.05
HepG2 CC_{50} (µM)	>20	>20
cLogP	3.04	5.27
AUC (µM.h)	27.18	65.79



Structure Activity Relationships of 4-Hydroxy-2-pyridones: a Novel Class of Antituberculosis Agents

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KEYWORDS. 4-Hydroxy-2-pyridones, Tuberculosis, Antituberculosis agents, Structure activity relations, phenotypic screen.

Abstract. Pyridone **1** was identified from a high-throughput cell-based phenotypic screen against *Mycobacterium tuberculosis* (Mtb) including multi-drug resistant tuberculosis (MDR-TB) as a novel anti-TB agent and subsequently optimized series using cell-based Mtb assay. Preliminary structure activity relationship on the isobutyl group with higher cycloalkyl groups at 6-position of pyridone ring has enabled us to significant improvement of potency against Mtb. The lead compound **30j**, a dimethylcyclohexyl group on the 6-position of the pyridone, displayed desirable in vitro potency against both drug sensitive and multi-drug resistant TB clinical isolates. In addition, **30j** displayed favorable oral pharmacokinetic properties and demonstrated in vivo efficacy in mouse model. These results emphasize the importance of 4-hydroxy-2-pyridones as a new chemotype and further optimization of properties to treat MDR-TB.

Introduction

Tuberculosis (TB) caused by the bacterium *Mycobacterium tuberculosis* (Mtb), is one of the most deadly bacterial infectious diseases. It has been reported that 2 billion people are latently infected and approximately 1 in 10 latent infections will progress to the active disease. There were over 8.6 million cases of TB in 2012, which resulted in nearly 1.3 million deaths.[1] The most widely used chemotherapy for uncomplicated TB is a combination of isoniazid, rifampicin, pyrazinamide, and ethambutol. This complex regimen still requires treatment for at least 6 months, often compromising TB patient compliance. Emergence of various forms of drug-resistant strains of Mtb and co-infection with HIV make the TB more difficult to treat.[2]

Nearly 400, 000 new cases of multi drug resistant TB (MDR-TB) are reported every year, which are resistant to isoniazid and rifampicin. MDR-TB further acquired additional resistance to fluoroquinolones and at least one of the injectable drugs leading to the emergence of

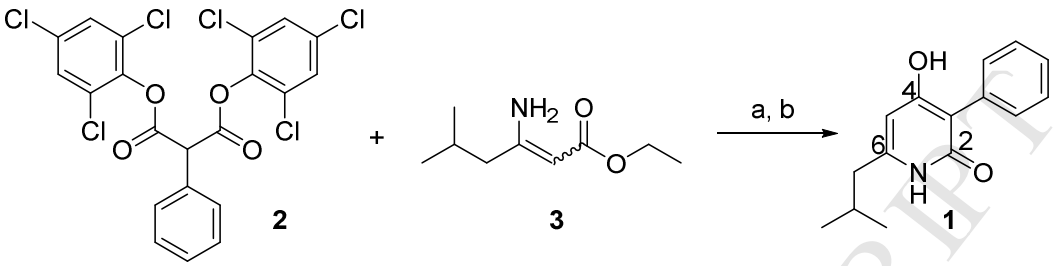
extensively-drug resistant TB (XDR-TB).[1] Second-line drugs used in the treatment of MDR and XDR-TB are less effective and not well tolerated. Although a number of novel chemical agents such as, PA-824, SQ109, oxazolidinones, BTZ043, Q203, NITD304 [3, 4] *etc.*, are fueling the TB pipeline in the last decade, only bedaquiline (TMC207)[5] and delamanid (OPC67683)[6] registered as TB drugs. Thus, there is an urgent unmet medical need to combat drug-resistant forms of Mtb with new chemical entities.

Cell-based high-throughput screen of nearly 2.2 million compound collection using Mtb H37Ra resulted in ~ 8000 hits. Pyridone **1** presented as an attractive hit series based on the in vitro pharmacokinetic properties and moderate potency against Mtb (MIC_{50} = 1.54 µM). Pyridone **1** has a low molecular weight (243 Da), good aqueous solubility (550 µM at pH 6.8), moderate cLogP (3.04), good microsomal stability (CL_{int} rat/human/mouse = 3.4/20/22 µL/min/mg) and did not inhibit major cytochrome P450 isoforms CYP 3A4, 2D6 and 2C9 (IC_{50} >20 µM). In the present study, we describe the structure activity relationship (SAR) and structure property relationship (SPR) of 4-hydroxy-2-pyridones to improve their potency against Mtb.

CHEMISTRY

Pyridone **1** was prepared as shown in Scheme 1 via a condensation of bis(2,4,6-trichlorophenyl) 2-phenylmalonate **2** and ethyl 3-amino-5-methylhex-2-enoate **3**[8] followed by hydrolysis and decarboxylation with NaOH as reported earlier.[9] The intermediate **2** was prepared from commercial available 2-phenylmalonic acid and oxalyl chloride as reported in literature.[10]

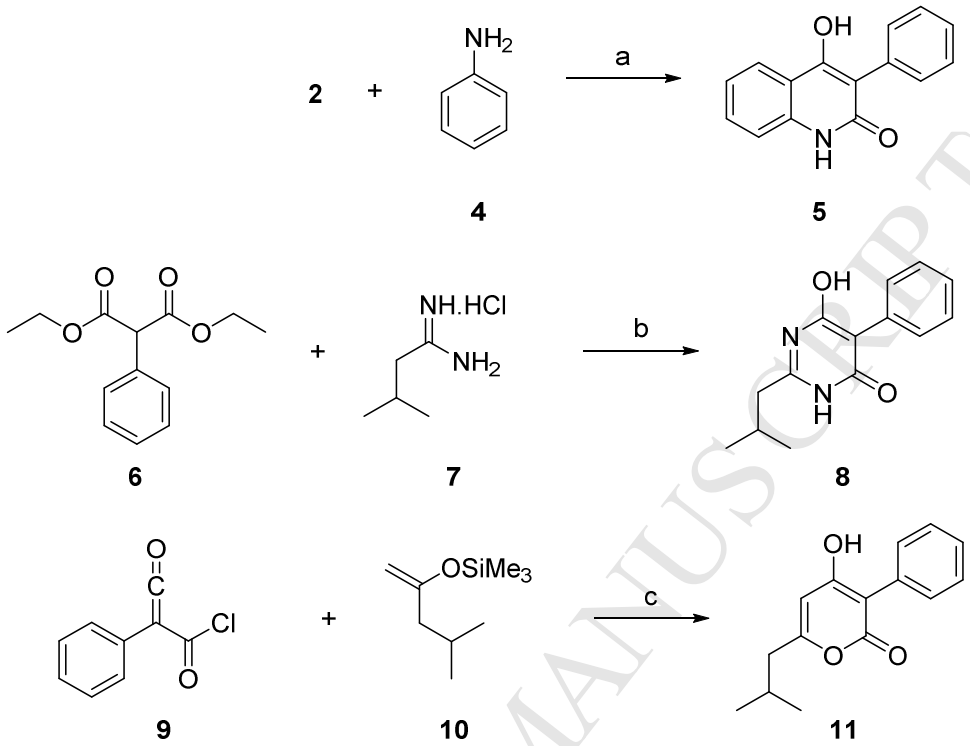
Scheme 1. Synthesis of 4-hydroxy-2-pyridone **1**[a]



[a]Reagents and conditions: (a) neat or Dowtherm, 230 °C, 30 min (b) 2 M NaOH solution, 130 °C, 24 h, 14% (two steps).

Quinolinone **5** was obtained via condensation of bis(2,4,6-trichlorophenyl) 2-phenylmalonate **2** with aniline **4** under microwave conditions (Scheme 2). Direct condensation of diethyl 2-phenylmalonate **6** with 3-methylbutanimidamide hydrochloride **7**[11] in presence of NaOMe yielded pyrimidinone **8**. Treatment of commercial available 2-phenylmalonic acid with oxalyl chloride afforded 3-oxo-2-phenylacryloyl chloride **9**, which upon condensation with silyl enol ether **10** gave pyranone **11**. Both 3-oxo-2-phenylacryloyl chloride **9** and silyl enol ether **10** were prepared as described previously.[12]

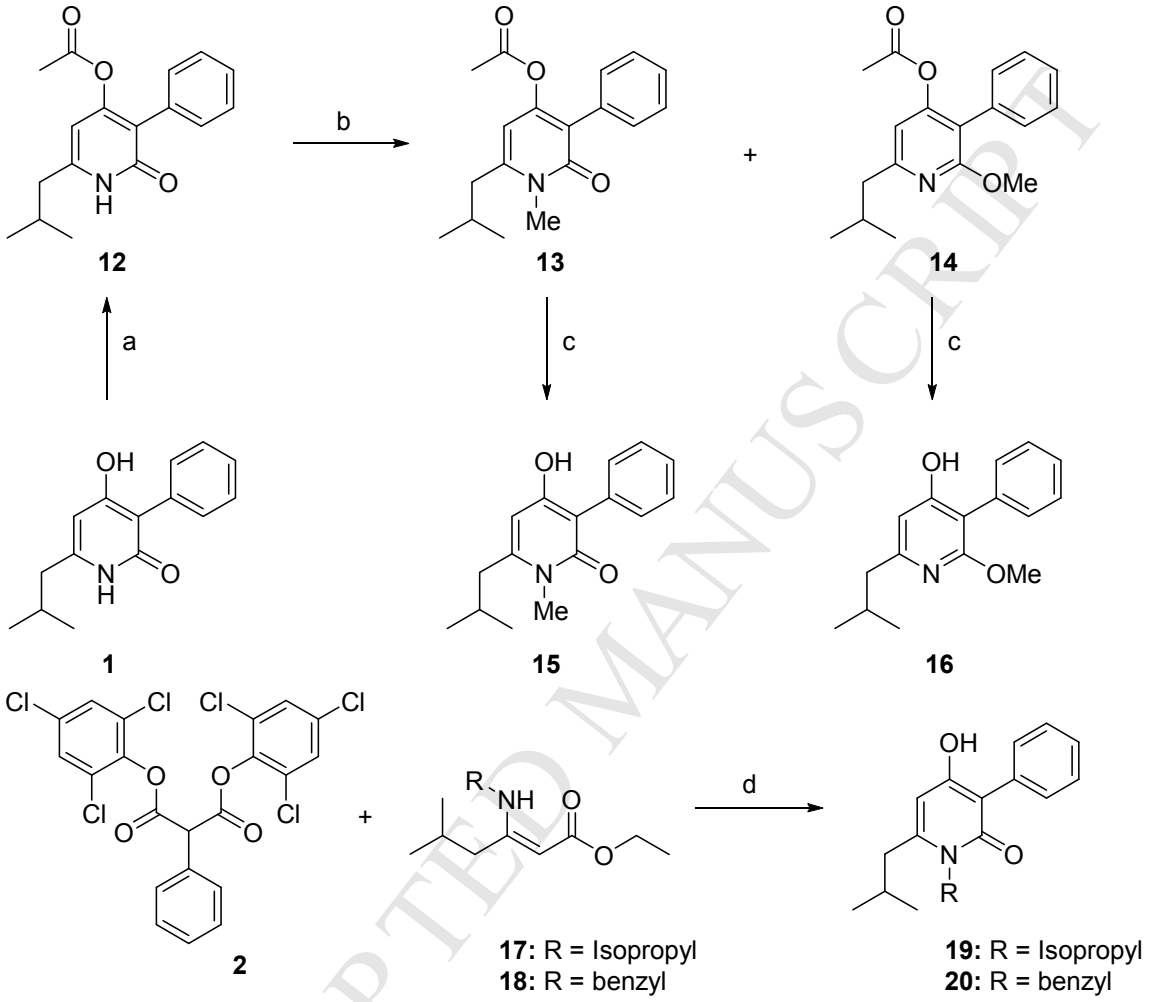
Scheme 2. Synthesis of pyridone ring modifications[a]



[a]Reagents and conditions: (a) Dowtherm, MW, 210 °C, 15 min, 40% (b) NaOMe, 2-methoxyethanol, reflux, 72 h, 6% (c) Xylene, reflux, 10 h, 17%.

The *N*-methyl substituted pyridone **15** was prepared in three steps from the corresponding unsubstituted pyridone **1** (Scheme 3). The phenolic OH of **1** was converted to acetate **12** using acetyl chloride. Methylation of acetate **12** with K_2CO_3 and MeI provided the key intermediates **13** and **14** which were converted to **15** and **16** with NaOMe/MeOH. Both *N*-isopropyl and *N*-benzyl substituted pyridones **19** and **20** were obtained in a single step. Condensation of bis(2,4,6-trichlorophenyl) 2-phenylmalonate **2** with **17** or **18** under neat conditions gave corresponding *N*-substituted pyridones **19** or **20** respectively.

Scheme 3. Synthesis of *N*-substituted 4-hydroxy-2-pyridone analogues[a]

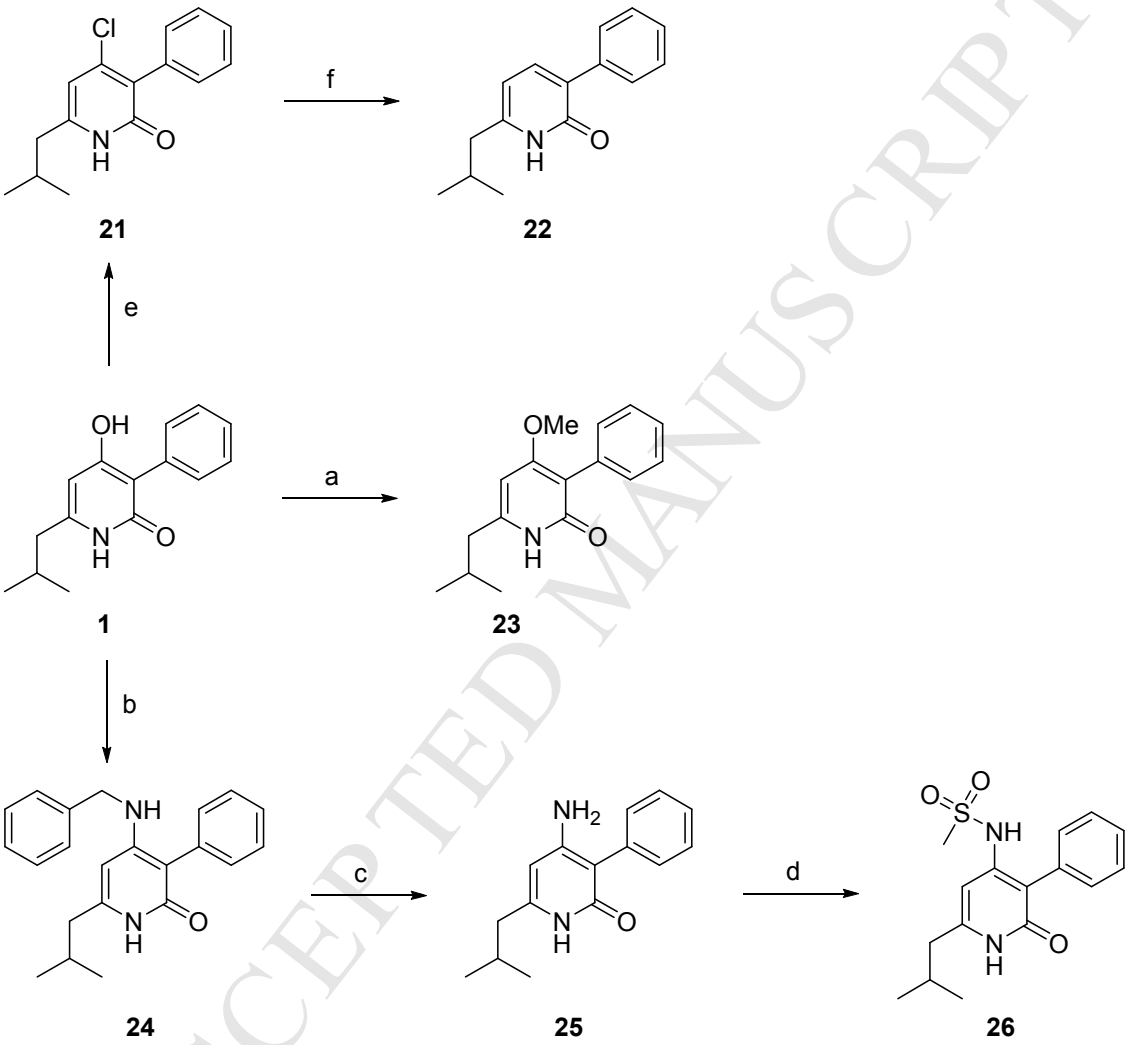


[a]Reagents and conditions: (a) acetyl chloride, pyridine, 1,4-dioxane, rt, 2 h, 63% (b) MeI, K_2CO_3, MW, 100 °C, 30 min, 66% (c) NaOMe, MeOH, rt, 30 min, 46% (**15**) and 38% (**16**) (d) neat, 200 °C, 1 h, 38% (**19**) and 37% (**20**).

Dehydroxy analog **22** was obtained from **1** by conversion of hydroxy to chloro **21** followed by dehalogenation with 10% Pd-C. Methoxy compound **23** was obtained directly from **1** by treatment with diazomethane under basic conditions (Scheme 4). The 4-amino analog **25** was prepared via a two-step sequence starting from **1**. Heating of **1** with benzylamine under microwave conditions provided **24**, which underwent reductive debenzylation using 10% Pd-C in

presence of hydrogen to afford the 4-amino analog **25**. The corresponding sulfonamide analog **26** was obtained by treating **25** with methanesulfonyl chloride and pyridine.

Scheme 4. Synthesis of C4-substituted analogues[a]

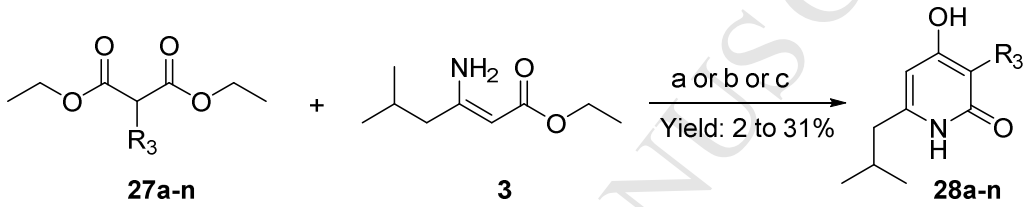


[a]Reagents and conditions: (a) TMS-CH=N_2, DIPEA, 9:1 MeCN/MeOH, rt, 4 h, 33% (b) benzylamine, MW, 220 °C, 10 h, 27% (c) H-Cube, 10% Pd/C, EtOH, 65 °C, 4 min (1 mL/min), 60% (d) $MeSO_2Cl$, pyridine, CH_2Cl_2, rt, 30 min, 26% (e) $SOCl_2$, PCl_5, 1,2-dichloroethane, reflux, 24 h, 23% (f) H_2, 10% Pd/C, EtOH, rt, 1 h, 52%.

The synthetic procedures for the preparation of 3-substituted pyridones **28a-n** are depicted in Scheme 5. The substituted malonic esters **27a-n** were either obtained from commercial sources

or synthesized from diethyl malonate following the literature procedure.[10] The corresponding pyridones **28a-n** were obtained via a condensation-decarboxylation sequence either under microwave conditions or by conventional heating. Decarboxylation of the intermediate carboxylates generally proceeded well under basic conditions with the exception of pyridyl substituted pyridones **28l-n** which required acidic conditions.

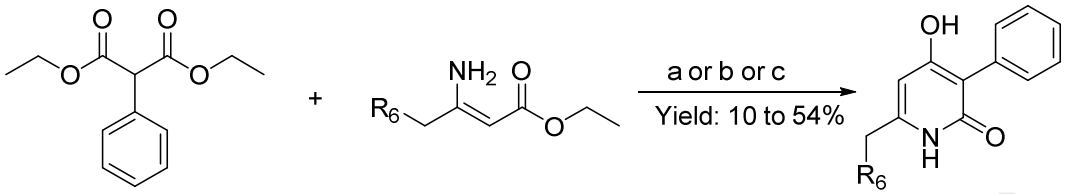
Scheme 5. Synthesis of 3-substituted pyridones from aminocrotonate and substituted malonic esters[a]



[a]Reagents and conditions: (a) (**27a-b, 27d-k**): (i) neat, 220 °C, 45 min (ii) 2 M NaOH solution, MW (160 °C), 1 h (b) (**27c**): (i) neat or Dowtherm, 250 °C, 30 min (ii) 2 M NaOH solution, 130 °C, 24 h (c) (**27l-n**): (i) neat or Dowtherm, 250 °C, 2 h (ii) 2 M HCl solution, 130 °C, 16 h.

Ethyl 3-amino-4-substituted but-2-enoates **29a, 29c-l** were prepared over a 4-step sequence utilizing Meldrum's acid as a key building block as reported in a previous procedure.[8] Intermediate **29b** was prepared according to the literature.[13] The synthesis of 6-substituted pyridones **30a-l** (Scheme 6) was accomplished by utilizing the same condensation-decarboxylation sequence as described earlier in the synthesis of 3-substituted pyridones.

Scheme 6. Synthesis of 6-substituted pyridones from substituted aminocrotonate and diethylphenylmalonate [a]



2 **29a-l** **30a-l**

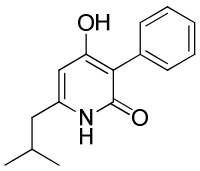
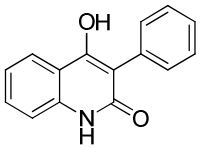
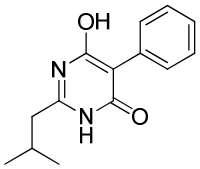
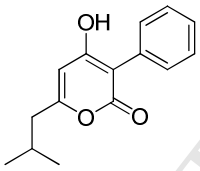
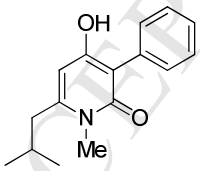
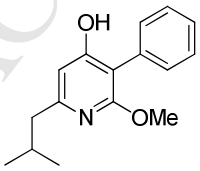
[a]Reagents and conditions: (a) (**30i, 30a, 30c-f, l)**: (i) neat, 220 °C, 45 min (ii) 2 M NaOH solution, MW (160 °C), 1 h (b) (**30b, 30g, 30h, 30j**): (i) neat or Dowtherm, 250 °C, 30 min (ii) 2 M NaOH solution, 130 °C, 24 h (c) (**30k**): (i) neat or Dowtherm, 250 °C, 2 h (ii) 2 M HCl solution, 130 °C, 16 h.

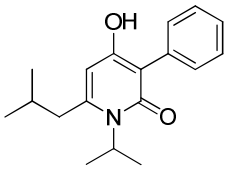
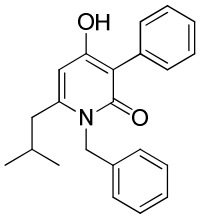
Results and discussion

Considering the promising microbiological profile and favorable in vitro PK properties of 4-hydroxy-2-pyridone, the medicinal chemistry strategy aimed at improving the antitubercular activity. The SAR investigations focused on the (1) role of pyridone core (2) importance of planar 3-phenyl group (3) significance of phenolic OH and (4) importance of lipophilic isobutyl group. The new pyridone analogs were screened in vitro against the *M. tuberculosis* H37Rv in order to obtain the MIC values using turbidometric assay and cytotoxicity against HepG2 cells was measured for selected compounds.

Pyridone Core Modification. Our initial efforts focused on compounds **1** to understand the importance of the pyridone core for the anti-TB activity. Changing of pyridone to quinolinone **5,** introducing nitrogen at the 5-position of the pyridone **8** or a NH replacement with oxygen **11** reduced potency (Table 1). Aromatization of the pyridone to 2-methoxy pyridine **16**, also resulted in loss of anti-TB activity. Methyl substituted analog **15** at the NH position resulted in decreased potency against Mtb, whilst, bulky N-substituted isopropyl **19** and benzyl **20** groups were inactive (Table 1).

Table 1. Anit-TB activity of pyridone ring modifications and *N*-substituted 4-hydroxy-2-pyridones

Compound	Structure	Mtb MIC_{50} (μM)
1		1.54
5		13.2
8		>20
11		13.8
15		8.1
16		>20

19		>20
20		>20

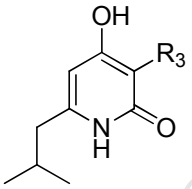
Importance of phenolic OH. Modification of the phenolic OH to NH_2 **25** or NHBn **24** led to the loss of anti-TB activity which could be due to significant decrease in pKa from acidic OH (pKa: 6.58) to basic NH_2 (pKa: 4.7) or NHBn (pKa: 3.32). Modified Moka[14] version 2.5.4 was used to calculate pKa values. Other replacements of the phenolic OH with H **22** or OMe **23** or the bioisostere $NHSO_2Me$ **26** also resulted in significant loss of potency (Table 2). The above results indicated that the pyridone core, phenolic OH and NH are important for anti-TB activity.

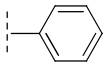
Table 2: SAR on phenolic OH

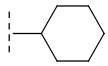
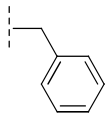
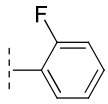
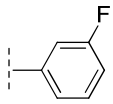
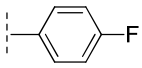
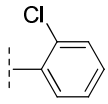
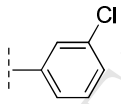
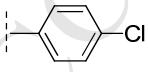
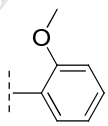
Compound	R_4	Mtb MIC_{50} (µM)
1	OH	1.54
22	H	>20
23	OMe	> 20
24	NHBn	> 20
25	NH_2	>20
26	$NHSO_2Me$	>20

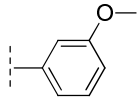
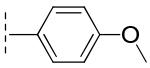
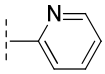
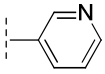
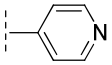
Modifications on the 3-Position of Pyridones. Replacement of the 3-position phenyl group with cyclohexyl **28a** or benzyl **28b** reduced potency, possibly indicating that the planarity of the phenyl group could be essential for anti-TB activity (Table 3). A scan of *ortho* **28c**, *meta* **28d** or *para* **28e** fluoro substitutions on the 3-position of the pyridone retained similar range of anti-TB activity compared to unsubstituted pyridone **1**(Table 3). Substitution on the 3- phenyl with *ortho* **28f** or *meta* **28g** chloro substitution maintained anti-TB activity similar to **1**. By contrast, the *para*-chloro substituted compound **28h** was inactive (Mtb MIC > 20 µM). The fact that chloro and fluoro substitutions at the *ortho* and *meta* positions were tolerated, but not the bulkier chloro substitution on the *para* position suggests that narrow space for substituents. Introducing an electron-donating group OMe on the *ortho* **28i**, *meta* **28j** or *para* **28k** positions of the phenyl group or replacing with 2/3/4 pyridyl **28l-n** also led to complete loss of anti-TB activity. Overall, the phenyl group was found to be optimal for Mtb potency on the 3-position of 4-hydroxy-2-pyridones.

Table 3: SAR of 3-substituted 4-hydroxy-2-pyridones



Compounds	R_3	Mtb MIC_{50} (µM)
1		1.54

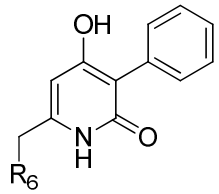
28a		>20
28b		>20
28c		6.31
28d		2.69
28e		6.75
28f		3.32
28g		2.67
28h		>20
28i		>20

28j		>20
28k		>20
28l		>20
28m		>20
28n		>20

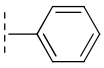
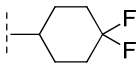
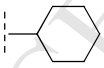
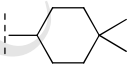
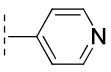
Modifications to the 6-Position Isopropyl Group. We further explored scope and possibilities at the pyridone 6-position to improve anti-TB activity. Pyridones with hydrogen **30a** and dimethyl amine **30b** substitution at the 6-position were inactive compared to the isopropyl group in pyridone **1**. Modifications with isobutyl **30c** and phenyl **30d** substitutions showed anti-TB activity similar to **1**. Other groups like cyclopropyl **30e**, cyclobutyl **30f**, cyclopentyl **30g** and difluorocyclohexyl **30h** on the 6-position retained anti-TB activity (Table 4). Further, cyclohexyl **30i** and dimethylcyclohexyl **30j** substitutions at the 6-position of the pyridone significantly improved anti-TB activity by 1 and ~2 log orders compared to **1**, respectively . These cycloalkyl substitutions also showed increased lipophilicity, with cLogP increasing from 1.58 to 5.27. Furthermore, a plot of cLogP against Mtb MIC for 6-substituted 4-hydroxy-2-pyridones indicated a positive correlation of cLogP with anti-TB activity with an r^2 of 0.91 (Figure 1). The loss of activity of **30a** (cLogP 1.58) and **30b** (cLogP 0.92) could be due to the decreased

lipophilicity of the methyl and dimethyl amino groups (Figure 1). Previously Dannhardt and his coworkers[9] reported activity of **30b** against Mtb H37Ra as 80-160 µM. However, in that study no in vitro and in vivo pharmacokinetic parameters were described. In order to improve solubility, 4-pyridyl **30k** and 4-pyranyl **30l** groups were explored on the pyridone 6-position. Although **30k** and **30l** showed significant improved solubility significantly, they were inactive . Unlike the pyridone core and the 3-position phenyl modifications, substitutions at the 6-position of the pyridone significantly increased anti-TB activity. The lead compound **30j** is the most potent 4-hydroxy-2-pyridone analog with Mtb potency of 0.05 µM. The in vitro potency of **30j** is superior to isoniazid (MIC_{50} = 0.33 µM), PA824 (MIC_{50} = 0.4 µM) and comparable to bedaquiline (MIC_{50} = 0.05 µM). The lead compound **30j** and the other 4-hydroxy-2-pyridone analogs also showed potent activity against MDR-TB clinical isolates[7], suggesting a potential role of this series of compounds in treating MDR-TB infections.

Table 4: SAR of 6-substituted 4-hydroxy-2-pyridones and their physicochemical properties



Compounds	R_6	Mtb MIC_{50} (µM)	cLogP	Solubility µM pH 6.8
Isoniazid	--	0.33	-0.67	
PA-824	--	0.4	2.79	--
Bedaquiline	--	0.05	7.2	--
1		1.54	3.04	550

30a		> 20	1.58	>1000
30b		> 20	0.92	671
30c		1.51	3.57	128
30d		1.40	3.15	40
30e		4.53	2.56	891
30f		1.19	3.12	280
30g		0.92	3.68	144
30h		1.32	3.62	104
30i		0.16	4.24	24
30j		0.05	5.27	25
30k		6.80	1.66	>1000

30l		10.1	1.84	>1000

A difference of in vitro potency and PK properties was observed between the hit **1** and lead compound **30j**. Hit **1** had moderate potency with good Lipinski's rule-of-five compliance, whilst, the lead compound **30j** had increased potency and cLogP (5.27). A similar positive correlation between lipophilicity and anti-TB activity was also observed in our earlier series indole-2-carboxamides[15] and a few other scaffolds.[16] The lead compound **30j** exhibited low solubility (25 µM) and moderate in vitro microsomal clearance (Cl_{int}- mouse/human = 45/59 µL/min/mg).

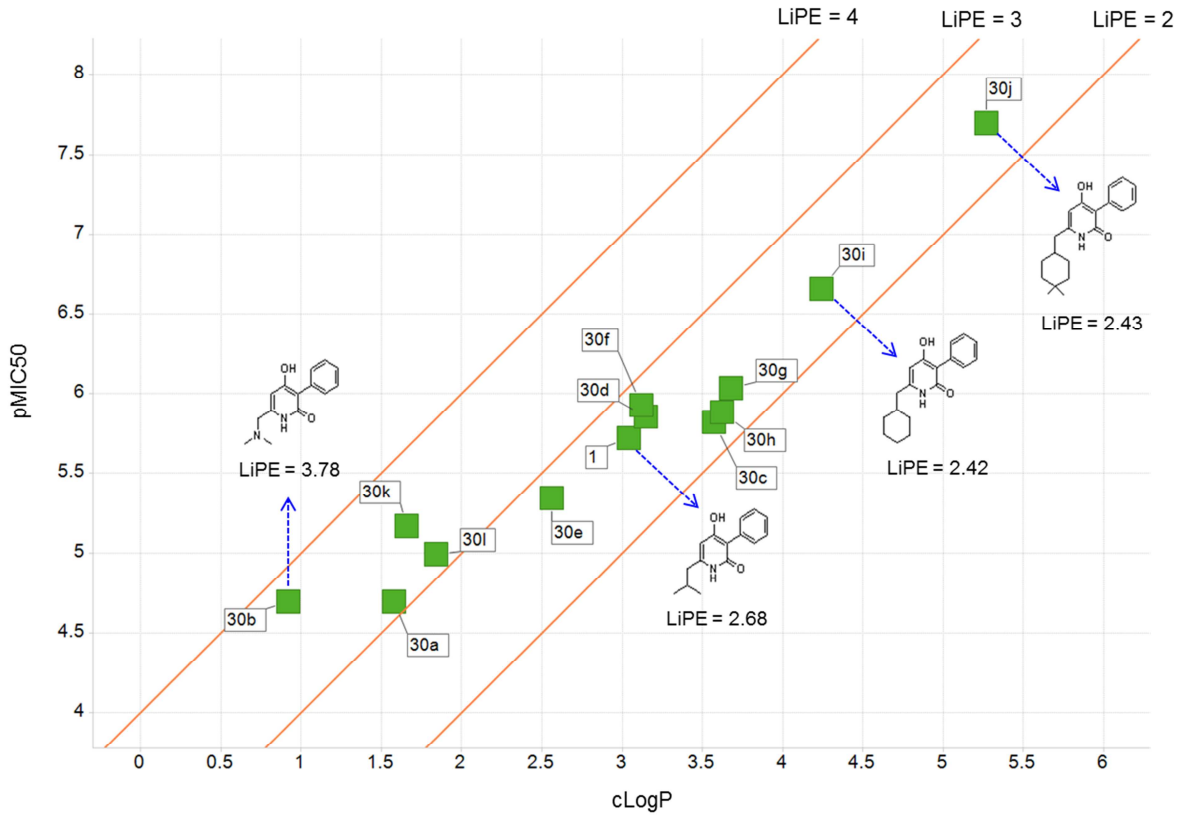


Figure 1. Correlation plot of Mtb MIC_{50} with cLogP for 6-substituted 4-hydroxy-2-pyridones.

Mechanism of Action of Pyridones: Detailed mechanism of action studies unambiguously demonstrated InhA, Mtb enoyl-ACP-reductase, as the molecular target of 4-hydroxy-2-

pyridones.[7] InhA is the clinically validated target of isoniazid and is essential for mycolic acid biosynthesis.[17] Recently, we have reported that **1** and **30j** bind to InhA only in presence of reduced cofactor (NADH) and inhibits the enzyme activity with an IC_{50} of 9.6 µM and 0.57 µM respectively (Table 5). A co-crystal structure of **30j** complexed with InhA and NADH (PDB: 4R9S) revealed that (i) the pyridone core has a pi-pi stacking interaction with dihydropyridine ring of NADH ii) the phenolic hydroxy group makes a hydrogen bond interaction with the hydroxy group of Tyr-158 and the 2'-OH of the ribose sugar of NADH iii) the 6-position dimethylcyclohexyl group occupies a hydrophobic enoyl-ACP substrate binding pocket iv) the nitrogen of the pyridone core interacts with sulphur of methionine (Figure 2).[7]

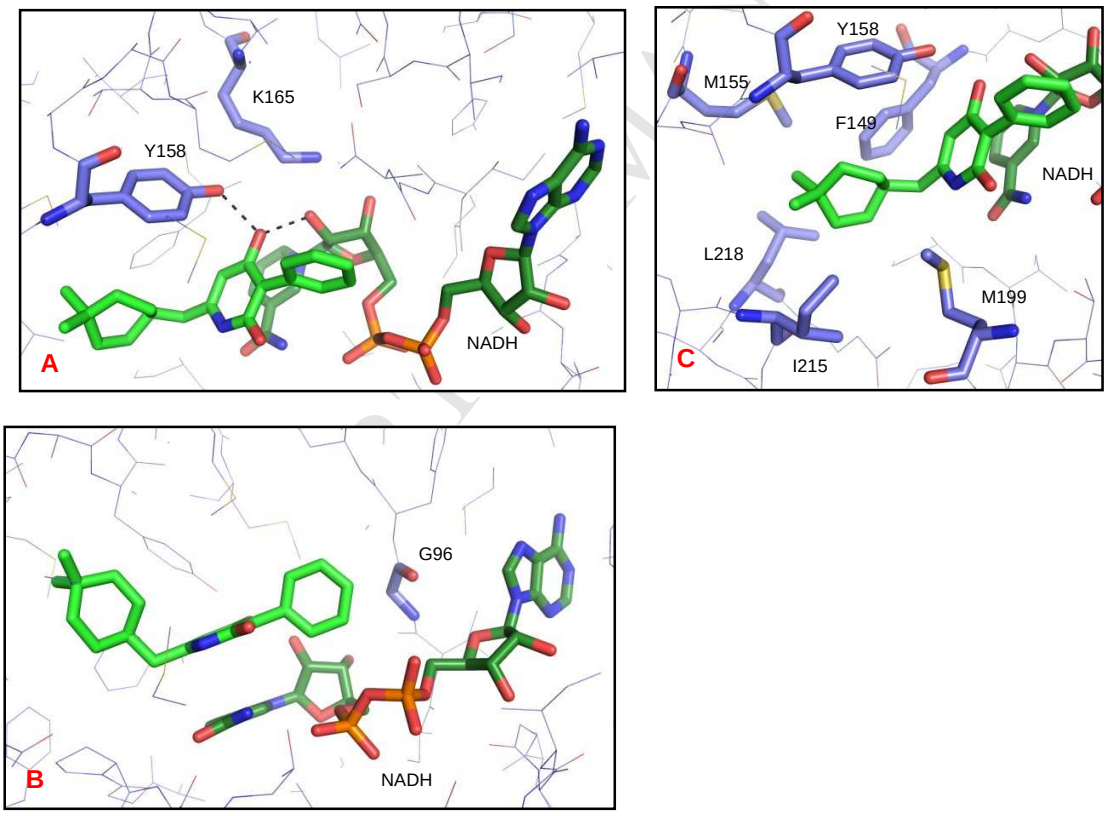


Figure 2. Co-crystal structure of pyridone **30j** with InhA-NADH complex. **A**: Phenolic OH hydrogen bonding with Tyr-158 and 2'-OH of the ribose sugar in NADH. **B**: A narrow binding pocket for 3-phenyl *para* position. **C**: Dimethylcyclohexyl interacting with lipophilic amino acids.

A retrospective analysis using X-ray structure of the **30j**–InhA-NADH complex (Figure 2) was employed to rationalize the SAR of 4-hydroxy-2-pyridones. The hydrogen bond interaction observed in the **30j** co-crystal structure potentially explains the importance of the phenolic OH (Figure 2A). The space available for substituents at 4-position is limited due to the protein environment and bulky substitutions like OMe **23**, NHBn **24** and SO_2NHMe **26** showed a detrimental effect on the anti-TB activity as shown in Table 2.

The 3-phenyl ring lies at an angle to the pyridone and occupies a small pocket with limited room to accommodate *ortho*- and *para*-substituents. In particular, substitution on the *para*-position appears particularly not favored could be due to the steric clash with the Gly-96 backbone carbonyl oxygen (Figure 2B). There appears to be some space within the binding pocket to accommodate a small *meta*-substituent, which is consistent with the activity of *meta*-Cl analogue **28g**. Although space at the ortho position is limited, the *ortho*-Cl analogue (**28f**) also retained similar anti-TB activity compared to unsubstituted phenyl group. By contrast, *ortho*, *meta* and *para* OMe substituted analogues (**28i-k**) and pyridyl analogues **(28l-n)** were devoid of anti-TB activity. This is most likely due to both unfavorable electronic as well as steric reasons.

The SAR on the 6-postion of the pyridone is consistent with X-ray structure of the **30j**–InhA-NADH complex. The hydrophobic 4,4-dimethylcyclohexyl moiety in the compound **30j** is positioned in the hydrophobic cavity formed by the amino acids Phe-149, Met-155, Tyr-158, Met-199, Ile-215, Leu-218 (Figure 2C). The cumulative effect of these interactions increased the potency of the compound **30j** (Mtb MIC_{50} = 0.05 µM). Further it explains lower potency of other less lipophilic substitutions (**30a and 30b**) on the 6-position of pyridone. SAR of the pyridone analogs described in this manuscript was optimized by cell-based activity but not with InhA enzyme inhibition. The cell permeability and drug efflux mechanisms are important factors in

cell-based activity. Especially, permeation of compounds through mycobacterial cell wall is more challenging due to the presence of waxy outer layer.[18] It is worth noting that earlier efforts to identify direct InhA inhibitors to overcome KatG mediated resistance have yielded many inhibitors that block the lipid-binding site similar to 4-hydroxy-2-pyridone analogues.[19-22]

***In vivo* PK and mouse efficacy.** None of the 4-hydroxy-2-pyridone analogues described in this study showed cytotoxicity against mammalian HepG2 cells (CC_{50} > 20µM). Further, in vivo PK of **1** and **30j** were evaluated in mice by intravenous and oral routes. Both compounds exhibited low total systemic clearance (13 to 18 ml/min/kg) and low volume of distribution (0.5 L/kg) with an elimination half-life of ~1.2 hours (Table 5). Compound **1** and **30j** achieved reasonable oral exposure with an oral bioavailability of 55-66%. Despite the low volume of distribution, the plasma concentrations of both **1** and **30j** exceeded their Mtb MICs, thus we subjected these candidates for in vivo acute mouse efficacy studies.

Despite the moderate in vitro potency, compound **1** demonstrated a statistically significant 0.95 log lung CFU reduction at 150 mg/kg compared to the untreated control (Figure 3A). Compound **30j** showed a dose dependent 1 and 1.92 log lung CFU reductions at 30 and 100 mg/kg respectively (Figure 3B). Similarly, compound **30j** showed 2.5 and 2.8 log CFU reduction in spleen with 30 and 100 mg/kg respectively (Figure 3C).[7] The in vivo efficacy of **30j** at 100 mg/kg dose was comparable to the first-line TB drug ethambutol (100 mg/kg). These results showed that the 4-hydroxy 2-pyridone class has the potential to be active against Mtb not only in vitro but also under in vivo conditions.[16]

Table 5: Pharmacological and biological properties of **1** and **30j**

	Compound **1**		Compound **30j**	
PK properties	IVPK	POPK	IVPK	POPK
Dose (mg/kg)	5	50	5	25
V_{ss} (liters/kg)	0.46	-	0.54	-

CL (ml/min/kg)	18	-	13.6	-
Elim $T_{1/2}$ (hours)	1.21	-	1.13	-
C_{max} (μM)	-	13.15	-	13.73
AUC (μM hour)	-	27.18	-	65.79
T_{max} (hours)	-	0.5	-	6
F (%)	-	55	-	66
Biological properties				
InhA IC_{50} (μM)	9.6		0.57	
Mtb MIC_{50} (μM)	1.54		0.05	
HepG2 CC_{50} (μM)	>20		>20	
MDRTB MIC range (μM)	2.5-3.13		0.04-0.16	

IVPK, Intravenous pharmacokinetics; POPK, per oral pharmacokinetics; -, not applicable; C_{max}, maximal plasma concentration; AUC, area under the curve (t = 0 to 24 h); F: absolute oral bioavailability; V_{ss}, volume of distribution at steady state; CL, clearance from plasma; $T_{1/2}$, terminal elimination half-life (non-compartmental estimate); InhA EC_{50}, InhA enzyme inhibition.[7]

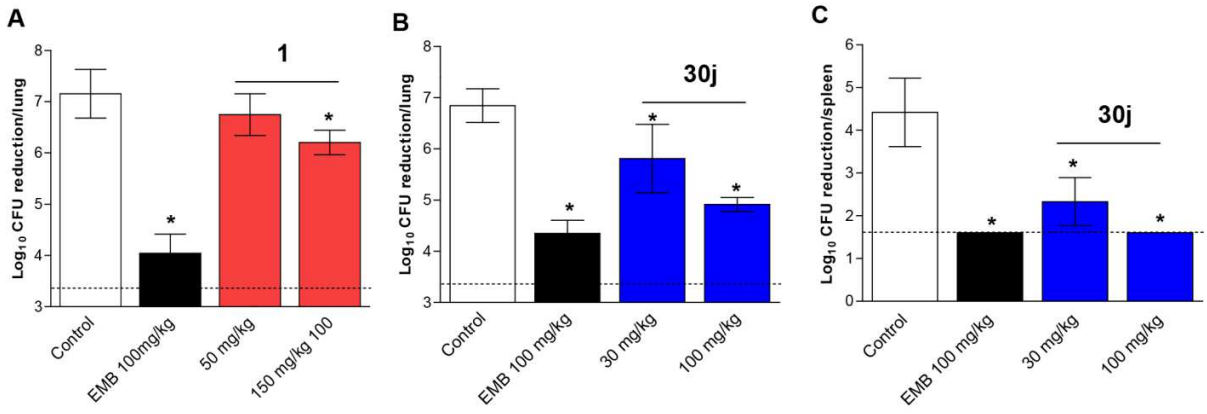


Figure 3. In vivo antimycobacterial activity of 4-hydroxy-2pyridones. Mean log CFU reductions per lung in mice treated with **1** (A), **30j** (B) and mean log CFU reductions per spleen in mice treated with **30j** (C). Ethambutol (EMB) was used as positive control in all the studies. Statistical evaluation was done using one way ANOVA and the data was analyzed using Tukey's multiple comparison test. Statistical significance (*) was accepted with P values <0.05.

SUMMARY

A 4-hydroxy-2-pyridone **1** was selected as one of the promising hits from a phenotypic high throughput screen of 2.2 million library compounds against Mtb in a cell based assay. The preliminary SAR on 6-position of 4-hydroxy-2-pyridones with more lipophilic cycloalkyl groups

significantly improved anti-TB activity by two log orders of magnitude. The improved in vitro activity of lead compound **30j** was translated to a better in vivo efficacy against Mtb in mouse model. At the time of the hit to lead stage, the molecular target of 4-hydroxy-2-pyridones was not identified; hence, the SAR was primarily driven by cell-based assay against Mtb. Later, the mechanism of action studies indicated that 4-hydroxy-2-pyridones are direct inhibitors of InhA. However, lead compound **30j** was not further optimized based on the co-crystal structure information and InhA enzyme assay. The results described here suggest that **30j** is an attractive lead compound having better in vitro potency and in vivo efficacy[7] for further optimization based on structure guided drug design to improve its drug-like properties. Furthermore, the binding mode of 4-hydroxy-2-pyridones could open up new opportunities for medicinal chemists to design new scaffolds active against this important Mtb target.

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% trifluoroacetic acid):CH_3CN (0.025% trifluoroacetic acid) to 10:90 H_2O (0.025% trifluoroacetic acid):CH_3CN (0.025% trifluoroacetic 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% trifluoroacetic acid):CH_3CN (0.025% trifluoroacetic acid) to 20:80 H_2O (0.025% trifluoroacetic acid):CH_3CN (0.025% trifluoroacetic acid) for 6 minutes run time with 0.3 mL/min flow rate [method 1, method 2 and method 4 were used for compounds **13**, **14**, **15**, **16**, **23**, **24**, **25**, **26**, **28a**, **28b**, **28d**, **28e**, **28f**, **28g**, **28h**, **28i**, **28j**, **28k**, **30a**, **30c**, **30d**, **30e**, **30f** and **30l** and method 3 and method 5 were used for compounds **1**, **5**, **8**, **11**, **19**, **20**, **21**, **22**, **28c**, **28l**, **28m**, **28n**, **30b**, **30g**, **30h** and **30k**].

Minimum Inhibitory Concentration (MIC) Determination. The *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 3-fold serial diluted in duplicates in 384-well clear plates. A volume of 50 µL of early log-phase culture of Mtb diluted to an OD_{600} of 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}, the concentration required to inhibit growth by 50%, was

determined by plotting nonlinear curves using GraphPad Prism 5 software (Rao et al., 2013). Isoniazid was used as the internal control in all of our assays, which had an MIC_{50} of 0.33 μM.

Solubility. Solubility was measured using a high-throughput equilibrium solubility (HT-Eq sol) assay using a novel miniaturized shake-flask approach and streamlined HPLC analysis. Briefly, a DMSO sample stock solution from a source plate was transferred to an MPV (miniprep filer vials, Whatman) chamber and subjected to centrifugal evaporation to remove DMSO. Aqueous buffer was added to the MPV chamber, and the MPV plunger was inserted until the membrane on the bottom of the plunger touched the surface of the solution in the chamber. The assembled MPV were incubated on an orbital shaker for 24 h and the plunger was then pushed to the bottom of the chamber followed by shaking for another 30 min. The filtrate was analyzed using HPLC.[23]

Mouse, Rat, and Human in Vitro Metabolic Stability. The metabolic stability of drug candidates was determined in mouse, rat, and human liver microsomes using a compound depletion approach and was 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.[24, 25]

In vivo PK. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Novartis Institute for Tropical Diseases. Mice were allowed to acclimatize before the 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.). Compound **1** and **30j** were formulated using 20% 1,2-propandiol, 10%

solutol HS15, and top up with pH 7.0 buffer for i.v. For oral administration, compound **1** was formulated in 0.5% Tween 80 and 0.5% Methyl cellulose and **30j** was formulated in MEPC (10% w/w Capmul MCM, 25% w/w Cremophor-RH40, 15% w/w Ethanol and 50% w/w 50 mM Phosphate buffer pH 6.8). Blood samples were collected at 0.08, 0.25, 0.75, 1.5, 3, 8, and 24 h following oral dosing. The sampling schedule was identical following i.v. dosing except that the first time point was 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 °C, and plasma was harvested and stored at − 20 °C until analysis.

Extraction and LCMS Analysis. Plasma samples were extracted with acetonitrile/methanol/acetic acid (90:9.8:0.2) containing warfarin as an internal standard using a 8.8:1 extractant-to-plasma ratio. Analyte quantitation was performed by LC/MS/MS. Liquid chromatography was performed using an Agilent 1200 HPLC system coupled with a API4000 triple quadrupole mass spectrometer (Applied Biosystems) using an Agilent Zorbax Phenyl (3.5 μm, 4.6 × 75 mm) column at 45 °C. 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 a flow rate of 1.0 mL/min and run time of 5 min. 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), by noncompartmental 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 with minor modifications.[15] Briefly, on day 0, animals were infected by an intranasal route with 10^3 Mtb H37Rv bacilli. For initial in vivo efficacy evaluation with compound **1**, one week post infection animals were treated for 3 weeks (7 days a week) and for a lead candidate **30j** animals were treated for 4 weeks (7 days a week). In both experiments, ethambutol was used as positive control. After treatment, lungs were aseptically removed and homogenized, and the bacterial load was estimated by serial-dilution plating on 7H11 agar plates. Compound **1** and **30j** were formulated similar to in vivo pharmacology studies. All procedures involving mice were reviewed and approved by the institutional animal care and use committee of the Novartis Institute for Tropical Diseases.

*4-Hydroxy-3-phenylquinolin-2(1H)-one (**5**).* A mixture of *bis*(2,4,6-trichlorophenyl)-2-phenylmalonate **2** (250 mg, 0.47 mmol) and aniline **4** (22 mg, 0.23 mmol) in dowtherm (5 mL) was heated under microwave at 210 °C for 15 min. The reaction mixture was diluted with diethyl ether (20 mL) and the precipitated solid was collected by filtration. The solid was washed with diethyl ether (5 mL) and dried to afford 4-hydroxy-3-phenylquinolin-2(1H)-one **5** (45 mg, 40% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 10.09 (s, 1H), 7.93 (d, J = 8 Hz, 1H), 7.52-7.30 (m, 7H), 7.20-7.15 (m, 1H). ESI MS: *m/z* 238 $[M+H]^+$. HPLC purity: 99.9%.

*6-Hydroxy-2-isobutyl-5-phenylpyrimidin-4(3H)-one (**8**).* To a suspension of 3-methylbutanimidamide hydrochloride **7** (500 mg, 3.68 mmol) and diethyl phenylmalonate **6**

(1.73 g, 7.35 mmol) in 2-methoxyethanol (10 mL) was added NaOMe (0.79 g, 14.7 mmol) and stirred at reflux temperature for 72 h. The reaction mixture was cooled to 0 °C and the precipitated solid was filtered. The solid was taken up in water and acidified with 1 M HCl to pH ~6 and extracted with 10% methanol in chloroform (2 x 20 mL). The combined organic layer was evaporated under reduced pressure and washed with diethyl ether (5 mL) to afford 6-hydroxy-2-isobutyl-5-phenylpyrimidin-4(3H)-one **8** (60 mg, 6% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (br s, 2H), 7.56-7.54 (m, 2H), 7.28-7.24 (m, 2H), 7.15-7.12 (m, 1H), 2.39 (d, *J* = 7.4 Hz, 2H), 2.15-2.09 (m, 1H), 0.93 (d, *J* = 6.5 Hz, 6H). ESI MS: *m/z* 245 $[M+H]^+$. HPLC purity: 97.6%.

*4-Hydroxy-6-isobutyl-3-phenyl-2H-pyran-2-one (**11**).* To a cold solution of trimethyl(4-methylpent-1-en-2-yloxy)silane **10** (714 mg, 4.15 mmol) in xylene (5 mL), was added a solution of 3-oxo-2-phenylacryloyl chloride **9** (500 mg, 2.77 mmol) in xylene (5 mL) dropwise at rt. The resulting mixture was refluxed for 10 h before it was cooled to 0 °C. The precipitated solid was filtered and washed with pentane. The crude solid was re-precipitated in methanol to afford 4-hydroxy-6-isobutyl-3-phenyl-2H-pyran-2-one **11** (120 mg, 17% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53-7.40 (m, 5H), 6.26 (br s, 1H), 5.98 (s, 1H), 2.37 (d, *J* = 7.1 Hz, 2H), 2.2-2.1 (m, 1H), 0.99 (d, *J* = 6.6 Hz, 6H). ESI MS: *m/z* 245 $[M+H]^+$. HPLC purity: 99.7%.

*6-Isobutyl-2-oxo-3-phenyl-1,2-dihydropyridin-4-yl acetate (**12**).* To a suspension of **1** (50.8 mg, 0.20 mmol) in 1,4-dioxane (3 mL) and cooled to 0 °C was added acetyl chloride (16 µL, 0.219 mmol) and pyridine (18.5 µL, 0.230 mmol). The mixture was allowed to gradually warm to room temperature and stirred at room temperature for 2 hr. The reaction mixture was concentrated *in vacuo* and the residue was taken up in CH_2Cl_2 (3 mL). The organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated *in vacuo* to give light yellow

residue. The residue was purified by column chromatography (ISCO Combiflash®, 4 g silica gel column, 0-40% EtOAc/cyclohexane) to give pyridone **12** as white solid (37.6 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47-7.33 (m, 5H), 6.21 (s, 1H), 2.50 (d, *J* = 7.28 Hz, 2H), 2.15-2.09 (m, 1H), 2.07 (s, 3H), 1.00 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 286 $[M+H]^+$.

*6-Isobutyl-1-methyl-2-oxo-3-phenyl-1,2-dihydropyridin-4-yl acetate (**13**) & 6-isobutyl-2-methoxy-3-phenylpyridin-4-yl acetate (**14**).* To a solution of **12** (37.6 mg, 0.132 mmol) in dry MeCN (2 mL) was added K_2CO_3 (18.0 mg, 0.132 mmol) and MeI (11 µL, 0.172 mmol). The resultant mixture was heated at 100 °C for 30 minutes in Biotage microwave reactor. Reaction mixture was cooled and diluted with EtOAc (4 mL). The organics were washed sequentially with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give colorless oil. The crude material was purified by column chromatography (ISCO Combiflash®, 4 g silica gel column, 0-50% EtOAc/cyclohexane) to give **13** (26 mg, 66% yield) and pyridine **14** (7 mg, 17% yield). **13:** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16 - 7.32 (m, 5H), 5.83 (s, 1H), 3.45 (s, 3H), 2.42 (d, *J* = 7.20 Hz, 2H), 1.91 (s, 3H), 1.83 - 1.88 (m, 1H), 0.94 (d, *J* = 6.40 Hz, 6H). ESI MS: *m/z* 258 $[M-Ac]^+$. **14**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 - 7.43 (m, 2H), 7.30-7.37 (m, 3H), 6.56 (s, 1H), 3.91 (s, 3H), 2.59 (d, *J* = 7.28 Hz, 2H), 2.12-2.24 (m, 1H), 2.00 (s, 3H), 0.99 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 258 $[M-Ac]^+$.

*4-Hydroxy-6-isobutyl-1-methyl-3-phenylpyridin-2(1H)-one (**15**).* To a solution of **13** (26 mg, 0.08 mmol) in MeOH (2 mL) was added 30% NaOMe in MeOH (0.2 mL, 10%v/v) at rt. The resultant mixture was stirred at rt for 30 minutes before it was concentrated under *vacuo* to give a white residue. The white residue was taken up in EtOAc (3 mL) and washed with 10% citric acid solution, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a white residue. The crude material was dissolved in MeOH and purified on reversed-phase HPLC using solvent

gradient of 20-95% MeCN/0.1% formic acid in H_2O to give desired product **15** as a white solid (10.3 mg, 46% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.35 (d, *J* = 7.20 Hz, 2H), 7.28 (t, *J* = 7.60 Hz, 2H), 7.17 (t, *J* = 7.20 Hz, 1H), 5.89 (s, 1H), 3.36 (s, 3H), 2.47 (s, 2H), 1.94-1.87 (m, 1H), 0.97 (d, *J* = 6.80 Hz, 6H)). ESI MS: *m/z* 258 $[M+H]^+$. HPLC purity: > 99%.

*6-Isobutyl-2-methoxy-3-phenylpyridin-4-ol (**16**).* To a solution of **14** (7 mg, 0.02 mmol) in MeOH (1 mL) was added 30% NaOMe in MeOH (0.1 mL, 10%v/v) at rt. The resultant reaction mixture was stirred at rt for 30 minutes before it was concentrated under *vacuo* to give white residue. The white residue was taken up in EtOAc (2 mL) and washed with 10% citric acid solution, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a white residue. The crude material was dissolved in MeOH and purified on reversed-phase HPLC using solvent gradient of 40-95% MeCN/0.1% formic acid in H_2O to give desired product **16** as an off-white solid (2.3 mg, 38% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53-7.46 (m, 2H), 7.43-7.35 (m, 3H), 6.46 (s, 1H), 3.89 (s, 3H), 2.55 (d, *J* = 7.03 Hz, 2H), 2.23-2.13 (m, 1H), 0.98 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 258 $[M+H]^+$. HPLC purity: 91.0%.

*4-Chloro-3-phenylpyridin-2(1H)-one (**21**).* A solution of 4-hydroxy-6-isobutyl-3-phenylpyridin-2(1H)-one **1** (1.6 g, 6.57 mmol), PCl_5 (4.79 g, 23.0 mmol) and $SOCl_2$ (1.67 mL, 23.0 mmol) in 1,2-dichloroethane (16 mL) was heated at 90 °C for 24 h. The reaction mixture was cooled to rt, quenched with ice water and extracted with chloroform (2 x 20 mL). The combined organic layer was washed with aqueous sat. $NaHCO_3$, brine, dried over anhydrous Na_2SO_4 solution 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% ethyl acetate in petroleum ether as eluent to afford 4-Chloro-3-phenylpyridin-2(1H)-one **21** (0.40 g, 23% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.8 (br s, 1H), 7.41-7.38 (m,

5H) 6.16 (s, 1H), 2.35 (d, *J* = 7.30 Hz, 2H), 2.05- 1.96 (m, 1H), 0.94 (d, *J* = 6.60 Hz, 6H). ESI MS: 262 $[M+H]^+$. HPLC purity: 98%.

*6-Isobutyl-3-phenylpyridin-2(1H)-one (**22**).* A solution of 4-chloro-3-phenylpyridin-2(1H)-one **21** (200 mg, 0.76 mmol) in ethanol (3 mL), was added 20 mg of 10% of Pd-C (10% w/w). The resulting suspension was stirred under hydrogen atmosphere at rt for 1 h. The reaction mixture was filtered through a celite bed and filtrate was concentrated under vacuum. The crude compound was purified by column chromatography over silica gel (100-200 mesh) using a solvent gradient of 25% ethyl acetate in petroleum ether as eluent to afford 6-isobutyl-3-phenylpyridin-2(1H)-one **22** (90 mg, 52%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.75 (br s, 1H), 7.71 (d, *J* = 7.30 Hz, 2H), 7.57 (d, *J* = 7.18 Hz, 1H), 7.40-7.32 (m, 3H), 6.09 (d, *J* = 7.10 Hz, 1H), 2.35 (d, *J* = 7.30 Hz, 2H), 1.98-1.93 (m, 1H), 0.90 (d, *J* = 6.60 Hz, 6H). ESI MS: 228 $[M+H]^+$. HPLC purity: 99.6%.

*6-Isobutyl-4-methoxy-3-phenylpyridin-2(1H)-one (**23**).* To a stirred solution of **1** (42.3 mg, 0.17 mmol) in 1:9 MeOH/MeCN (2 mL) at rt was added DIPEA (42.0 µL, 0.24 mmol) followed by trimethylsilyldiazomethane (2 M solution, 0.120 mL, 0.24 mmol). The resultant mixture was stirred at rt for 4 h before it was concentrated under *vacuo* to give an off-white solid. The crude material was dissolved in DMSO and purified on reversed-phase HPLC using solvent gradient of 30-95% MeCN/0.1% formic acid in H_2O to give desired product **23** as fluffy white solid (14.9 mg, 33% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41 (br s, 1H), 7.34-7.27 (m, 4H), 7.22-7.16 (m, 1H), 6.12 (s, 1H), 3.73 (s, 3H), 2.36 (d, *J* = 7.28 Hz, 2H), 2.05-1.94 (m, 1H), 0.92 (d, *J* = 6.78 Hz, 6H). ESI MS: *m/z* 258 $[M+H]^+$. HPLC purity: 99.2%.

*4-(Benzylamino)-6-isobutyl-3-phenylpyridin-2(1H)-one (**24**).* A solution of **1** (50 mg, 0.20 mmol) in benzylamine (2 mL) was heated in Biotage microwave reactor at 220 °C for 10 h. The reaction mixture was diluted in DMSO and purified on reversed-phase HPLC using solvent gradient of 10-95% MeCN/0.1% formic acid in H_2O to give desired product **24** as white solid (18.6 mg, 27% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.65 (br s, 1H), 7.41-7.37 (m, 2H), 7.32-7.18 (m, 8H), 5.69 (t, *J* = 6.00 Hz, 1H), 5.58 (s, 1H), 4.31 (d, *J* = 6.00 Hz, 2H), 2.13 (d, *J* = 7.20 Hz, 2H), 1.85-1.78 (m, 1H), 0.76 (d, *J* = 6.80 Hz, 6H). ESI MS: *m/z* 333 $[M+H]^+$. HPLC purity: > 99%.

*4-Amino-6-isobutyl-3-phenylpyridin-2(1H)-one (**25**).* A solution of **24** (23.1 mg, 0.060 mmol) in EtOH (4 mL) was subjected to hydrogenation on the H-Cube at 65 °C using 10%Pd/C cartridge at flow rate of 1 mL per minute under full hydrogen mode. The reaction mixture was concentrated under *vacuo* to give white residue. The crude material was dissolved in MeOH and purified on reversed-phase HPLC using solvent gradient of 5-95% MeCN/0.1% formic acid in H_2O to give desired product **25** as white solid (10.0 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.47 (br s, 1H), 7.36-7.33 (m, 2H), 7.26-7.19 (m, 3H), 5.58 (s, 1H), 5.41 (br s, 2H), 2.17 (d, *J* = 7.20 Hz, 2H), 1.94-1.87 (m, 1H), 0.89 (d, *J* = 6.40 Hz, 6H). ESI MS: *m/z* 243 $[M+H]^+$. HPLC purity: > 99%.

*N-(6-Isobutyl-2-oxo-3-phenyl-1,2-dihydropyridin-4-yl)methanesulfonamide (**26**).* To a suspension of **25** (28.3 mg, 0.11 mmol) in dry DCM (2 mL) was added methanesulfonyl chloride (10.8 µL, 0.140 mmol) and pyridine (18.5 µL, 0.23 mmol) at rt. The resultant mixture was stirred at rt for 30 minutes. The mixture was diluted with CH_2Cl_2 (3 mL) and washed sequentially with saturated NH_4Cl solution, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material was dissolved in DMSO and purified on reversed-phase HPLC

using solvent gradient of 20-95% MeCN/0.1% formic acid in H_2O to give desired product **26** as a sticky solid (10 mg, 26% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.51-7.44 (m, 2H), 7.41-7.35 (m, 1H), 7.28-7.23 (m, 2H), 6.50 (s, 1H), 5.83 (br s, 1H), 3.50 (s, 3H), 2.40 (d, *J* = 7.28 Hz, 2H), 2.07-1.96 (m, 1H), 0.92 (d, *J* = 6.78 Hz, 6H). ESI MS: *m/z* 321 $[M+H]^+$. HPLC purity: 92.2%.

4-Hydroxy-6-isobutyl-1-isopropyl-3-phenylpyridin-2(1H)-one (***19***). A mixture of Ethyl 3-(isopropylamino)-5-methylhex-2-enoate **17** (300 mg, 1.40 mmol) and *bis*(2,4,6-trifluoro phenyl)2- phenylmalonate **2** (453 mg, 0.84 mmol) was heated at 200 °C in a sealed tube for 1 h. The reaction mixture was cooled to rt and a 3:1 mixture of petroleum ether and diethyl ether (20 mL) was added. The precipitated solid was collected by filtration. The crude compound was purified by column chromatography over silica gel (100-200 mesh) using a solvent gradient of 20% of ethyl acetate in petroleum ether as eluent to afford 4-hydroxy-6-isobutyl-1-isopropyl-3-phenylpyridin-2(1H)-one **19** as an off-white solid (90 mg, 38% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 7.34-7.25 (m, 4H), 7.18-7.16 (m, 1H), 5.81 (s, 1H), 4.40-4.30 (m, 1H), 2.45 (d, *J* = 7.10 Hz, 2H), 1.82-1.79 (m, 1H), 1.50 (d, *J* = 6.5 Hz, 6H), 0.97 (d, *J* = 6.6 Hz, 6H). ESI MS: *m/z* 286 $[M+H]^+$. HPLC purity: 95.2%.

The following 4-Hydroxy-2-pyridones **20** was prepared using the above method from *bis*(2,4,6-Trifluoro phenyl)2- phenylmalonate **2** and Ethyl 3-(benzylamino)-5-methylhex-2-enoate **18**.

1-Benzyl-4-hydroxy-6-isobutyl-3-phenylpyridin-2(1H)-one (***20***). Yield: 37%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 7.40-7.18 (m, 8H), 7.18-7.10 (m 2H), 5.97 (s, 2H), 5. 24 (br s,

1H), 2.37 (d, *J* = 7.0 Hz, 2H), 1.81-1.78 (m, 1H), 0.88 (d, *J* = 6.0 Hz, 6H). ESI MS: *m/z* 334 $[M+H]^+$. HPLC purity: 95%.

*6-(Cyclohexylmethyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30i**).* A mixture of ethyl 3-amino-4-cyclohexylbut-2-enoate **29i** (100 mg, 0.47 mmol) and diethyl 2-phenylmalonate **6** (101 µL, 0.47 mmol) was heated neat at 220 °C for 45 minutes. Consumption of starting materials and the formation of carboxylate intermediate was monitored by LC-MS. The crude residue was then dissolved in 2 M NaOH solution (2 mL, 4 mmol) and the resultant mixture was heated in a Biotage microwave reactor at 160 °C for 1 hour. The reaction mixture was cooled and acidified to ~pH 6 with 6 M HCl solution. The precipitated solids were collected and dried under *vacuo*. The crude material was dissolved in DMSO (3 mL) and purified by reversed-phase HPLC (30-95% MeCN in 0.1% formic acid/H_2O, Atlantis prep dC18 OBD prep column, 10 µm, 19 x 250 mm) to give **30i** as a white solid (20.5 mg, 15% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.00 (br s, 1H), 10.19 (s, 1H), 7.39 (d, *J* = 7.03 Hz, 2H), 7.27 (t, *J* = 7.53 Hz, 2H), 7.19-7.12 (m, 1H), 5.76 (s, 1H), 2.26 (d, *J* = 6.78 Hz, 2H), 1.73-1.52 (m, 6H), 1.27-1.09 (m, 3H), 0.85-0.99 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 162.7, 146.7, 134.1, 130.7, 126.9, 125.5, 108.3, 98.1, 36.8, 32.2, 25.8, 25.5. HPLC purity: > 99%. ESI MS: *m/z* 284 $[M+H]^+$. HRMS calculated for $C_{18}H_{22}NO_2$ $[M+H]^+$, 284.1645; found, 284.1647.

The following substituted pyridones were prepared according to the above method using corresponding 2-substituted malonates and ethyl 3-amino-4-substituted but-2-enoate

*3-Cyclohexyl-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28a**).* Yield: 2.4%. ^{1}H NMR (400 MHz, DMSO-d_6) : δ 10.71 (br s, 1H), 9.80 (br s, 1H), 5.59 (s, 1H), 2.83 (t, *J* = 12.0 Hz, 1H), 2.15 (d, *J* = 7.20 Hz, 2H), 2.07-1.98 (m, 2H), 1.89-1.81 (m, 1H), 1.69 (d, *J* = 10.4 Hz, 2H), 1.62 (d, *J* = 7.60 Hz, 1H), 1.34-1.31 (m, 2H), 1.26-1.12 (m, 3H), 0.84 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 250 $[M+H]^+$. HPLC purity: 92.4%.

*3-Benzyl-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28b**).* Yield: 13.9%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.91 (br s, 1H), 10.10 (br s, 1H), 7.25-7.14 (m, 4H), 7.12-7.05 (m, 1H), 5.68 (s, 1H), 3.60 (s, 2H), 2.21 (d, *J* = 7.03 Hz, 2H), 1.93-1.80 (m, 1H), 0.85 (d, *J* = 6.40 Hz, 6H). ESI MS: *m/z* 258 $[M+H]^+$. HPLC purity: 93.9%.

*3-(3-Fluorophenyl)-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28d**).* Yield: 31.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (br s, 1H), 7.33-7.28 (m, 2H), 7.24 (d, *J* = 8.53 Hz, 1H), 7.02-6.94 (m, 1H), 5.79 (s, 1H), 2.26 (d, *J* = 7.53 Hz, 2H), 1.92 (td, *J* = 6.93, 13.49 Hz, 1H), 0.89 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 262 $[M+H]^+$. HPLC purity: 99.6%.

*3-(4-Fluorophenyl)-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28e**).* Yield: 28.6%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (br s, 1H), 10.32 (br s, 1H), 7.49-7.38 (m, 2H), 7.17-7.04 (m, 2H), 5.78 (s, 1H), 2.25 (d, *J* = 7.28 Hz, 2H), 1.98-1.86 (m, 1H), 0.89 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 262 $[M+H]^+$. HPLC purity: 99.2%.

*3-(2-Chlorophenyl)-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28f**).* Yield: 7.3%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.06 (br s, 1H), 10.25 (br s, 1H), 7.46-7.39 (m, 1H), 7.31-7.24 (m, 2H), 7.22-7.18 (m, 1H), 5.76 (s, 1H), 2.27 (d, *J* = 7.53 Hz, 2H), 1.98-1.84 (m, 1H), 0.90 (d, *J* = 6.50 Hz, 6H). ESI MS: *m/z* 278 $[M+H]^+$. HPLC purity: 96.4%.

*3-(3-Chlorophenyl)-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28g**).* Yield: 18.1%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.13 (br s, 1H), 10.59-10.36 (m, 1H), 7.47 (t, *J* = 1.76 Hz, 1H), 7.43-7.38 (m, 1H), 7.31 (t, *J* = 7.91 Hz, 1H), 7.24-7.19 (m, 1H), 5.79 (s, 1H), 2.26 (d, *J* = 7.28 Hz, 2H), 1.98-1.86 (m, 1H), 0.89 (d, *J* = 6.78 Hz, 6H). ESI MS: *m/z* 278 $[M+H]^+$. HPLC purity: 99.0%.

*3-(4-Chlorophenyl)-4-hydroxy-6-isobutylpyridin-2(1H)-one (**28h**).* Yield: 13.3%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.12 (br s, 1H), 10.39 (br s, 1H), 7.48-7.43 (m, 2H), 7.35-7.30 (m, 2H), 5.79 (s, 1H), 2.26 (d, *J* = 7.53 Hz, 2H), 1.96-1.86 (m, 1H), 0.89 (d, *J* = 6.78 Hz, 6H). ESI MS: *m/z* 278 $[M+H]^+$. HPLC purity: 99.3%.

*4-Hydroxy-6-isobutyl-3-(2-methoxyphenyl)pyridin-2(1H)-one (**28i**).* Yield: 17.9%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 (br s, 1H), 7.25-7.18 (m, 1H), 7.01 (dd, *J* = 1.63, 7.40 Hz, 1H), 6.96 (d, *J* = 8.28 Hz, 1H), 6.90-6.85 (m, 1H), 5.71 (s, 1H), 3.66 (s, 3H), 2.25 (d, *J* = 8.03 Hz, 2H), 1.96-1.88 (m, 1H), 0.90 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 274 $[M+H]^+$. HPLC purity: 96.2%.

*4-Hydroxy-6-isobutyl-3-(3-methoxyphenyl)pyridin-2(1H)-one (**28j**).* Yield: 19.7%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.96 (br s, 1H), 10.23 (br s, 1H), 7.21-7.15 (m, 1H), 7.01-6.94 (m, 2H), 6.75 (dd, *J* = 1.63, 8.16 Hz, 1H), 5.76 (s, 1H), 3.72 (s, 3H), 2.25 (d, *J* = 7.28 Hz, 2H), 1.99-1.87 (m, 1H), 0.89 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 274 $[M+H]^+$. HPLC purity: 99.2%.

*4-Hydroxy-6-isobutyl-3-(4-methoxyphenyl)pyridin-2(1H)-one (**28K**).* Yield: 21.6%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.97 (br s, 1H), 10.10 (br s, 1H), 7.33 (d, *J* = 8.78 Hz, 2H), 6.85 (d, *J*

= 8.78 Hz, 2H), 5.76 (s, 1H), 3.75 (s, 3H), 2.24 (d, *J* = 7.28 Hz, 2H), 1.99-1.85 (m, 1H), 0.89 (d, *J* = 6.78 Hz, 6H). ESI MS: *m/z* 274 $[M+H]^+$. HPLC purity: 98.2%.

*4-Hydroxy-6-methyl-3-phenylpyridin-2(1H)-one (**30a**).* Yield: 27.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (br. s, 1H), 10.06 (br. s., 1H), 7.37 (d, *J* = 7.28 Hz, 2H), 7.26 - 7.29 (m, 2H), 7.14 - 7.18 (m, 1H), 5.79 (s, 1H), 2.11 (s, 3H). ESI MS: *m/z* 202 $[M+H]^+$. HPLC purity: 99.7%.

*4-Hydroxy-6-isopentyl-3-phenylpyridin-2(1H)-one (**30c**).* Yield: 26.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.09 (br s, 1H), 10.16 (br s, 1H), 7.38 (d, *J* = 7.03 Hz, 2H), 7.28 (t, *J* = 7.53 Hz, 2H), 7.16 (t, *J* = 8.00 Hz, 1H), 5.81 (s, 1H), 2.39 (t, *J* = 8.00 Hz, 2H), 1.55 (td, *J* = 6.56, 13.24 Hz, 1H), 1.50-1.41 (m, 2H), 0.90 (d, *J* = 6.53 Hz, 6H). ESI MS: *m/z* 258 $[M+H]^+$. HPLC purity: 99.0%.

*6-Benzyl-4-hydroxy-3-phenylpyridin-2(1H)-one (**30d**).* Yield: 20.6%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.27 (br s, 1H), 10.18 (br s, 1H), 7.40-7.31 (m, 6H), 7.27 (t, *J* = 7.65 Hz, 3H), 7.19-7.13 (m, 1H), 5.70 (s, 1H), 3.75 (s, 2H). ESI MS: *m/z* 278 $[M+H]^+$. HPLC purity: 98.9%.

*6-(Cyclopropylmethyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30e**).* Yield: 9.8%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (br s, 1H), 7.42-7.35 (m, 2H), 7.32-7.23 (m, 2H), 7.20-7.13 (m, 1H), 5.94 (s, 1H), 2.30 (d, *J* = 7.03 Hz, 2H), 1.06-0.94 (m, 1H), 0.55-0.45 (m, 2H), 0.25-0.18 (m, 2H). ESI MS: *m/z* 242 $[M+H]^+$. HPLC purity: 99.3%.

*6-(Cyclobutylmethyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30f**).* Yield: 30.7%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.04 (br s, 1H), 10.16 (br s, 1H), 7.40-7.35 (m, 2H), 7.27 (t, *J* = 7.65 Hz, 2H), 7.19-7.13 (m, 1H), 5.77 (s, 1H), 2.62-2.48 (m, 3H), 2.08-1.99 (m, 2H), 1.88-1.79 (m, 2H), 1.74-1.63 (m, 2H). ESI MS: *m/z* 256 $[M+H]^+$. HPLC purity: 99.8%.

*4-Hydroxy-3-phenyl-6-((tetrahydro-2H-pyran-4-yl)methyl)pyridin-2(1H)-one (**30l**).* Yield: 40.2%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (br s, 1H), 10.18 (br s, 1H), 7.40-7.35 (m, 2H), 7.31-7.24 (m, 2H), 7.20-7.13 (m, 1H), 5.80 (s, 1H), 3.83 (dd, *J* = 3.01, 11.54 Hz, 2H), 3.30-3.22 (m, 2H), 2.33 (d, *J* = 7.28 Hz, 2H), 1.82 (br. s., 1H), 1.52 (d, *J* = 12.30 Hz, 2H), 1.28-1.15 (m, 2H). ESI MS: *m/z* 286 $[M+H]^+$. HPLC purity: 98.5%.

4-Hydroxy-6-isobutyl-3-phenylpyridin-2(1H)-one (***1***). A mixture of bis(2,4,6-trichlorophenyl) 2-phenylmalonate **2** (12.7 g, 23.56 mmol) and Ethyl 3-amino-5-methylhex-2-enoate **3** (5.79 g, 33.8 mmol) in dowtherm oil (30 mL) was heated to 230 °C for 30 min. The resulting reaction mixture was cooled to rt and 100 mL petroleum ether was added to reaction mixture. The residue was filtered, washed with pentane and dried to afford 1.5 g of crude intermediate ethyl 4-Hydroxy-2-isobutyl-6-oxo-5-phenyl-1,6-dihydropyridine-3-carboxylate which was taken up in 2 M NaOH solution (16 mL) and refluxed for 24 h. The reaction mixture was acidified to ~pH 6 with 1 M HCl and extracted with ethyl acetate (2 x 25 mL). The combined organic layer was washed with water, 5% aqueous $NaHCO_3$ solution, brine, dried over anhydrous Na_2SO_4 and concentrated to afford 4-hydroxy-6-isobutyl-3-phenylpyridin-2(1H)-one **1** as an off-white solid (0.80 g, 14% yield over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.18 (br s, 1H), 10.2 (s,

1H), 7.39-7.26 (m, 4H), 7.18-7.15 (m, 1H), 5.80 (s, 1H), 2.30 (d, *J* = 7.10 Hz, 2H), 1.95-1.93 (m, 1H), 0.90-0.88 (d, *J* = 6.42 Hz, 6H). ESI MS: *m/z* 244 $[M+H]^+$. HPLC purity: 99.9%.

The following 4-Hydroxy-2-pyridones were prepared using the above procedure from corresponding 2-aryl malonates and 4-substituted ethyl 3-aminobut-2-enoate.

3-(2-Fluorophenyl)-4-hydroxy-6-isobutylpyridin-2(1H)-one (***28c***). Yield: 13% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (s, 1H), 10.30 (br s, 1H), 7.30-7.22 (m, 2H), 7.14-7.09 (m, 2H), 5.78 (s, 1H), 2.27 (d, *J* = 7.5 Hz, 2H), 1.96-1.89 (m, 1H), 0.90 (d, *J* = 6.6 Hz, 6H). ESI MS: *m/z* 262 $[M+H]^+$. HPLC purity: 97.3%.

*6-((Dimethylamino)methyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30b**).* Yield: 16% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.86 (br s, 1H), 10.25 (s, 1H), 7.39-7.30 (m, 2H), 7.28-7.19 (m, 2H), 7.17 (t, *J* = 6.8 Hz, 1H), 6.0 (s, 1H), 3.21 (s, 2H), 2.21 (s, 6H). ESI MS: *m/z* 245 $[M+H]^+$. HPLC purity: 99.5%.

*6-(Cyclopentylmethyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30g**).* Yield: 17% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (s, 1H), 10.18 (br s, 1H), 7.38 (d, *J* = 7.50 Hz, 2H), 7.27 (t, *J* = 7.50 Hz, 2H), 7.17 (t, *J* = 7.0 Hz, 1H), 5.86 (s, 1H), 2.40 (s, 2H), 2.16-2.10 (m, 1H), 1.70-1.50 (m, 6H), 1.23-1.19 (m, 2H). ESI MS: *m/z* 270 $[M+H]^+$. HPLC purity: 95.9%.

*6-((4,4-Difluorocyclohexyl)methyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30h**).* Yield: 39% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (s, 1H), 10.18 (s, 1H), 7.37 (m, 2H), 7.28 (m, 2H), 7.17 (m, 1H), 5.80 (s, 1H), 2.36 (d, *J* = 6.6 Hz, 2H), 2.10-1.80 (br. s., 2H), 1.85-1.65 (m, 5H), 1.22 (m, 2H). ESI MS: *m/z* 320 $[M+H]^+$. HPLC purity: 98.7%.

*6-((4,4-Dimethylcyclohexyl)methyl)-4-hydroxy-3-phenylpyridin-2(1H)-one (**30j**).* Yield: 40% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (s, 1H), 10.20 (s, 1H), 7.38-7.26 (m, 4H), 7.18-7.14 (m, 1H), 5.78 (s, 1H), 2.30 (d, *J* = 6.1 Hz, 2H), 1.46-1.34 (m, 5H), 1.25 (br s, 4H) 0.87 (d, *J* = 5.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 162.8, 146.8, 134.1, 130.7, 126.9, 125.5, 108.3, 98.2, 38.3, 36.7, 32.3, 29.6, 27.9, 24.3. ESI MS: *m/z* 312 $[M+H]^+$. HRMS calculated for $C_{20}H_{26}NO_2$ [M+H], 312.1958; found, 312.1956. HPLC purity: > 99%.

*4-Hydroxy-6-isobutyl-3-(pyridin-2-yl)pyridin-2(1H)-one (**28l**).* To a solution of diethyl 2-(pyridin-2-yl)malonate **27l** (400 mg, 1.68 mmol) in dowtherm oil (10 mL) was added ethyl 3-amino-5-methylhex-2-enoate **3** (231 mg, 1.34 mmol) and heated at 250 °C for 2 h. The reaction mixture was diluted with 30 mL petroleum ether, the precipitated solid was filtered and dried under vacuum to afford 200 mg of intermediate ethyl 4-hydroxy-2-isobutyl-6-oxo-5-(pyridin-2-yl)-1,6-dihydropyridine-3-carboxylate. The crude intermediate (200 mg, 0.632 mmol) was taken up in 2 M HCl (2 mL) and refluxed at 130 °C for 16 h in a sealed tube. The reaction mixture was diluted with cold water and basified to pH 8 using saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (2 x 15 mL). The combined organic layer was washed with brine (2 x 30 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude compound was

purified by triturating with n-pentane and diethyl ether to afford 4-hydroxy-6-isobutyl-3-(pyridin-2-yl)pyridin-2(1H)-one **28l** as an off-white solid (85.0 mg, 25% yield over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 18.28 (s, 1H), 11.08 (s, 1H), 9.19 (d, *J* = 8.6 Hz, 1H), 8.49 (d, *J* = 4.8 Hz, 1H), 8.00-7.96 (m, 1H), 7.34-7.31 (m, 1H), 5.75 (s, 1H), 2.28 (d, *J* = 7.0 Hz, 2H), 1.98-1.95 (m, 1H), 0.89 (d, *J* = 6.4 Hz, 6H). ESI MS: *m/z* 245 $[M+H]^+$. HPLC purity: 98.5%.

The following 4-Hydroxy-2-pyridones were prepared using above procedure from corresponding 2-aryl malonates and ethyl 3-amino-5-methylhex-2-enoate **3**.

*4-Hydroxy-6-isobutyl-3-(pyridin-3-yl)pyridin-2(1H)-one (**28m**).* Yield: 13% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.20 (s, 1H), 10.51 (s, 1H), 8.60 (s, 1H), 8.0 (br s, 1H), 7.81 (d, *J* = 7.5 Hz, 1H), 7.32 (t, *J* = 5.95 Hz, 1H), 5.82 (s, 1H), 2.28 (d, *J* = 7.1 Hz, 2H), 1.94-1.91 (m, 1H), 0.90 (d, *J* = 6.6 Hz, 6H). ESI MS: *m/z* 245 $[M+H]^+$. HPLC purity: 97.5%.

*4-Hydroxy-6-isobutyl-3-(pyridin-4-yl)pyridin-2(1H)-one (**28n**).* Yield: 10% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.23 (s, 1H), 10.7 (br s, 1H), 8.45 (d, *J* = 4.8 Hz, 2H), 7.51 (br s, 2H), 5.81 (s, 1H), 2.27 (d, *J* = 7.0 Hz, 2H), 1.95-1.88 (m, 1H), 0.90 (d, *J* = 6.2 Hz, 6H). ESI MS: *m/z* 245 $[M+H]^+$. HPLC purity: 95.1%.

*4-Hydroxy-3-phenyl-6-(pyridin-4-ylmethyl)pyridin-2(1H)-one (**30k**).* Yield: 54% for two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.39 (s, 1H), 10.3 (br s, 1H), 8.54 (d, *J* = 4.8 Hz, 2H), 7.36-7.26 (m, 6H), 7.18 (m, 1H), 5.75 (s, 1H), 3.8 (s, 2H). ESI MS: *m/z* 279 $[M+H]^+$. HPLC purity: 94.1%.

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ABBREVIATIONS

Mtb: Mycobacterium tuberculosis; TB: Tuberculosis; MDR: multi-drug resistant; XDR: extensively drug resistant; TDR: totally drug resistant; NaOH: Sodium hydroxide; NaOMe: Sodium methoxide; K_2CO_3: Potassium carbonate; MeOH: Mehtanol; EtOH: Ethanol; EtOAc: Ethyl acetate; $SOCl_2$: Thionyl chloride; DIPEA: *N*,*N*-diisopropylethylamine; DMF: dimethylformamide; MeCN: Acetonitrile; HCl: Hydrochloric acid; MW: Microwave; DCM: Dichloromethane; CH_2Cl_2: Dichloromethane; Na_2SO_4: Sodium sulphate; rt: room temperature; MIC: minimum inhibitory concentration; SAR: structure–activity relationships

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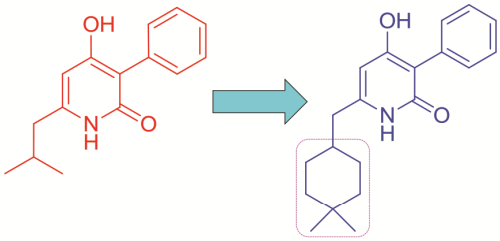
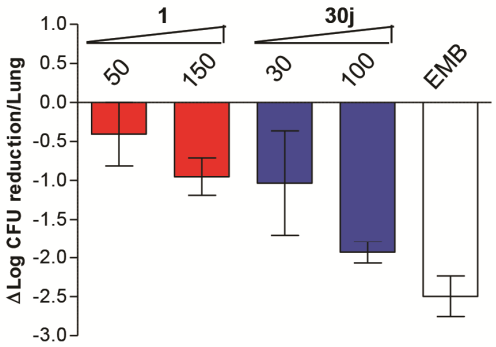


	Hit **1**	Lead **30j**
Mtb MIC_{50} (µM)	1.54	0.05
HepG2 CC_{50} (µM)	>20	>20
cLogP	3.04	5.27
AUC (µM.h)	27.18	65.79



Highlights for review

- A high-throughput cell-based phenotypic hit pyridone **1** displayed active against *Mycobacterium tuberculosis* (Mtb) including multi-drug resistant tuberculosis (MDR-TB)
- SAR expansion on 6-position of pyridone **1** hit significantly improved potency and lead compound **30j** displayed 50 nM activity against Mtb
- Lead compound **30j** showed favorable oral pharmacokinetic properties and demonstrated in vivo efficacy in mouse model
- Mechanism of action studies indicated that 4-hydroxy-2-pyridones are direct inhibitors of InhA
- Lead compounds **30j** is attractive lead compound for further optimization as direct inhibitors of InhA