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Discovery and preclinical profile of sudapyridine (WX-081), a novel anti-tuberculosis agent

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ABSTRACT

Multidrug resistant tuberculosis (MDR-TB) remains a major human health challenge. Bedaquiline was approved in 2012 by the US FDA, and listed by WHO as a treatment for multidrug-resistant tuberculosis (MDR-TB) in 2018. However, the side effects of bedaquiline including the risk of unexplained mortality, QTc prolongation and hepatotoxicity limit its wide clinical use. Based on bedaquiline, we describe herein discovery and development of a novel diarylpyridine series, which led to identification of WX-081 (sudapyridine, **21l**). It displayed excellent anti-mycobacterial activity against M. tuberculosis H37Rv *in vitro* and *in vivo* and low cytotoxicity; additionally WX-081 had excellent pharmacokinetic parameters in animals, better lung exposure and lower QTc prolongation potential compared to bedaquiline. WX-081 is currently under clinical phase II development (NCT04608955).

Tuberculosis (TB) is a contagious bacterial infection, caused by a bacillus *Mycobacterium tuberculosis*. TB is one of the top ten causes of death, especially in young adults worldwide.[1,2] Even though improvement has been made, TB is still a substantial health threat due to emergence of multidrug resistant tuberculosis (MDR-TB).[3] The proportion of the MDR-TB population has remained about 3–4 % for initial diagnosis and about 18–21 % for treatment experienced population. The current lengthy and complex treatment regimens are simply inadequate to defeat MDR-TB infections, triggering revitalized search for better TB agents.[3].

Since approval of rifampin in 1962, the US FDA had not approved new TB drug with novel mechanism until 2012. SIRTURO® (bedaquiline, Figure 1) with novel mechanism of action (MOA) was approved for pulmonary MDR-TB in 2012. The approval was based on its>20 % cure rate improvement vs background treatment options in MDR-TB patients. Bedaquiline (BDQ) is a diarylquinoline anti-TB antibiotic which features a novel MOA of inhibiting the proton pump of the mycobacterial ATP syntheses. This inhibition leads to bacterial energy depletion and eventually kills mycobacteria.[4,5] Bedaquiline was listed by WHO as preferred prescription for rifampin-resistant tuberculosis (RR-TB) and multidrug-resistant tuberculosis (MDR-TB) in 2018.[6] However, its use has been partially limited by toxicity, which include the risk of unexplained mortality, QTc prolongation, phospholipidosis and hepatotoxicity.[7–11] Therefore, a potent and safer anti-tuberculosis agent related to bedaquiline is desirable in clinic. This letter describes our discovery and preclinical profile of WX-081 (sudapyridine, **21l**), a novel diarylpyridine analogue with anti-mycobacterial activity similar to bedaquiline. Furthermore it displayed safety advantages over bedaquiline, and better lung exposure when compared to bedaquiline. Sudapyridine (WX-081) has been taken to clinical developments and currently in phase II clinical trials (NCT04608955).

In our search for a safer and proprietary anti-mycobacterial chemical series, we focused on replacements of quinoline of bedaquiline with phenylpyridines as shown in Fig. 1, 6-phenylpyridine compound **2** and

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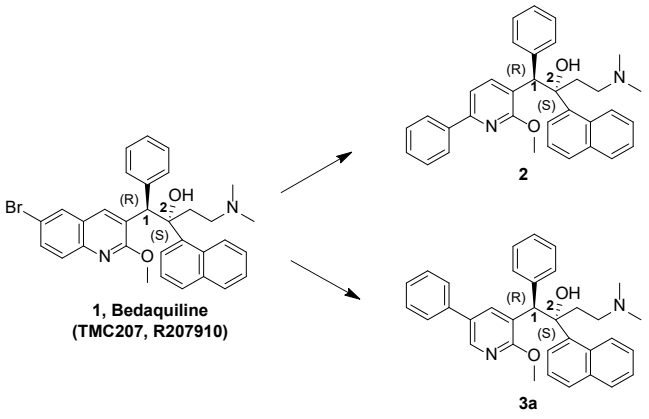
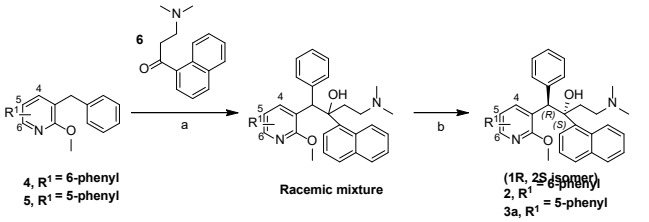


Fig. 1. Chemical structures of bedaquiline and novel designs **2** and **3a**.

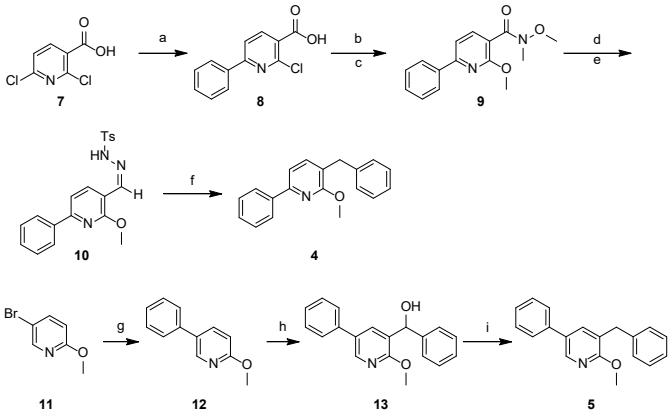
5-phenylpyridine **3a** were our initial designs.[12] The preparation of compounds **2** and **3a** are described in Scheme 1. Both compounds used a coupling of anions of 3-benzylpyridines (**4**, **5**) with naphthalene aminoketone (**6**) in the presence of LDA at −70 °C to produce a mixture of four possible chiral isomers. The four isomers were first separated by column chromatography to get a pair of diastereomeric mixture, and by chiral resolution using supercritical fluid chromatography (SFC, see supplementary material for separation conditions) to separate all four enantiomers. Only one of the four chiral isomers is biologically active, and the active enantiomer identified as (1*R*, 2*S*) optical isomer with absolute chirality identical to bedaquiline (as shown in Figure 1). Compounds **2** and **3a** were thus made and separated starting from precursor 3-benzylpyridines **4** and **5**. The prerequisite 3-benzyl-2-methoxy-6-phenylpyridine **4** for making compound **2** was prepared as shown in Scheme 2. Starting from 2,6-dichloronicotinic acid, reaction with phenylboronic acid under Suzuki coupling conditions yielded 6-phenylnicotinic acid **8**. Substitution of chloride with sodium methoxide followed by coupling with N,O-dimethyl hydroxylamine hydrochloride in the presence of EDCI and HOBT produced Weinreb amide **9**. Reduction of the Weinreb amide with $LiAlH_4$, then treatment of the resultant aldehyde with *p*-toluenesulfonylhydrazine obtained hydrazone **10**. Treatment of compound **10** with phenylboronic acid at basic conditions produced compound **4**. The prerequisite 3-benzyl-2-methoxy-5-phenylpyridine **5** for making compound **3a** was made starting from commercially available 5-bromo-2-methoxypyridine **11**. Reaction with phenylboronic acid in the presence of palladium catalyst generated compound **12**. Methoxy-directed lithiation of compound **12** using TMPLi at −70 °C followed by reaction with benzaldehyde afforded alcohol **13**. Reduction using BF_3-Et_2O and Et_3SiH transformed compound **13** to compound **5**.

Prior to evaluate the anti-mycobacterial activity of compounds against *M. tuberculosis* H37Rv, we used a primary screen panel including *M. smegmatis* ATCC19420, *M. smegmatis* ATCC700084, *M. peregrinum* ATCC700686 and *M. bovis* ATCC 35,737 as surrogates. The minimum inhibitory concentrations (MIC) were determined in the primary panel, and compounds showing MIC<0.5 μg/mL in the primary panel progressed to evaluations in *Mycobacterium tuberculosis* (*M.tb* H37Rv). New compounds were screened in both normaxic MABA and hypoxic LORA conditions using the methods developed by Cho et al.[13] The cytotoxicity was evaluated in both HeLa and Vero cell lines. Compounds **2** and **3a** were profiled in this testing scheme, and their surrogate MICs summarized in Table 1. Compound **2** showed MIC about 1 μg/mL in our primary panel, while compound **3a** showed very potent activity in the panel. Compound **3a** was further assessed in *M. tuberculosis* H37Rv using the MABA and LORA assays, MICs were determined and reported in Table 2. Compound **3a** exhibited respectable *M.tb* H37Rv MICs of 1.1 μg/mL (MABA) and 0.7 μg/mL (LORA). However, comparing potency of compound **3a** to that of bedaquiline, further optimization became necessary.



*Reagents and conditions: a) LDA, THF, -70 °C, 3h; b) chiral SFC resolution, 3% yield for the two steps.

Scheme 1. * A general synthesis of compounds **2** and **3a** *Reagents and conditions: a) LDA, THF, −70 °C, 3 h; b) chiral SFC resolution, 3 % yield for the two steps.



Scheme 2. * Syntheses of prerequisite 3-benzyl-phenylpyridines **4** and **5** for preparation of compound **2** and **3a** *Reagents and conditions: a) Phenylboronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, EtOH/DME/H_2O, 100 °C, 5 h, 60 % yield; b) MeOH, Sodium methoxide, 60 °C, 12 h; c) N,O-dimethyl hydroxylamine hydrochloride, EDCI, HOBT, Et_3N, DMF, 25 °C, 12 h, 94 % yield; d) LAH, THF, 0 ~ 25 °C, 2 h, 49 % yield; e) p-toluenesulfonylhydrazine, MeOH, 25 °C, 2 h; f) Phenylboronic acid, K_2CO_3, dioxane, 80 °C, 2 h; g) $Pd(dppf)Cl_2$, K_2CO_3, dioxane/H_2O, 90 °C, 16 h, 85 % yield; h) benzaldehyde, 2,2,6,6-tetramethylpiperidine (TMP), *n*-BuLi, THF, −20 ~ -70 °C, 12 h, 65 % yield; i) BF_3-Et_2O, Et_3SiH, DCM, 50 °C, 16 h, 95 % yield. Abbreviations: Index: EDCI: 1-ethyl-3(3-dimethylpropylamine carbodiimide, HOBT: 1-Hydroxybenzotriazole.

Our optimization of compound **3a** began from SAR studies of replacements of naphthalene in compound **3a**. To reduce aromaticity, a variety of substituted phenyls as the replacements were explored as shown in compounds **3b-e**. These compounds were prepared as shown in Scheme 3. The syntheses followed the preparation of compound **3a**, except different known aminoketones (**14b-e**) were used in the place of aminoketone **6**.

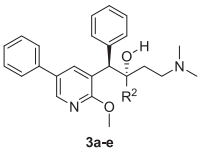
Compounds **3b-e** were evaluated and their *M.tb* H37Rv MICs are reported in Table 2, the surrogate MIC data from *M. smegmatis, M. peregrinum* and *M. bovis* were omitted for clarity. Replacing the naphthyl with phenyl (**3b**) can achieve similar anti-mycobacterial MICs under both MABA and LORA conditions, but cytotoxicity in HeLa cell is apparent. Difluorophenyl analogues (**3c-e**) appeared to show somewhat

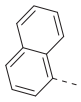
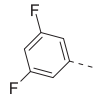
Table 1
* Anti-mycobacterial activity of compounds **2** and **3a**.

Compd.	MIC (μg/mL)			
	M.smegmatis ATCC19420	*M.smegmatis* ATCC700084	*M.peregrinum* ATCC700686	*M.bovis* ATCC35737
2	1.0	1.0	0.5	ND
3a	0.03125	0.0625	0.0625	0.5

*MIC data are average of two tests; ND: not determined.

Table 2
* SAR of compound **3a-e** assayed against *M. tuberculosis* and their cytotoxicity profile.

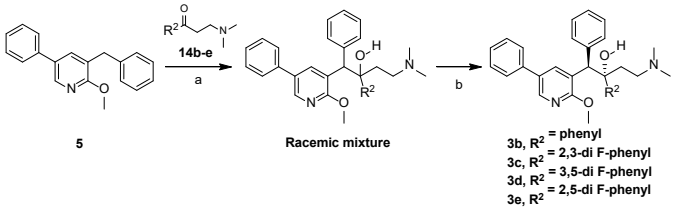


Compd.	R^2	*M. tuberculosis* H37Rv MIC (μg/mL)		CC_{50} (μg/mL)	CC_{50} (μg/mL)
		MABA	LORA	HeLa cell	Vero Cell
3a		1.1 ± 0.2	0.7 ± 0.3	30.4	>50
3b		1.5 ± 0.1	1.2 ± 0.2	11.9	>50
3c		0.6 ± 0.2	0.5 ± 0.1	28.1	>50
3d		0.7 ± 0.3	0.4 ± 0.1	28.8	>50
3e		0.7 ± 0.3	0.7 ± 0.2	ND	>50
Bedaquiline	–	0.03 ± 0.01	<0.2	>64	>50

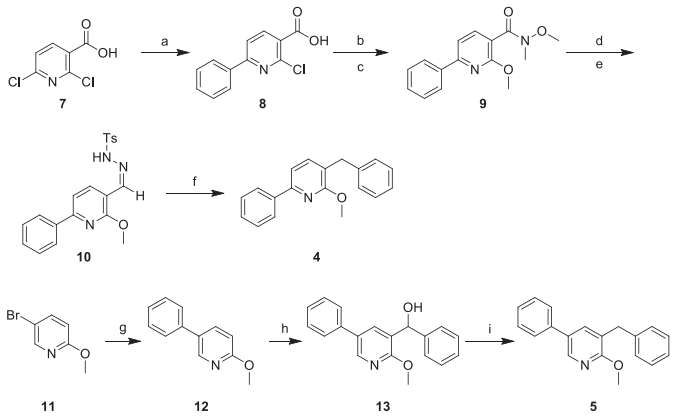
*The MIC assays followed Cho et al protocols (ref.13); MIC data are average of two tests; CC_{50} data are a single test or average of two tests; ND: not determined.

improved activity against *M. tuberculosis* H37Rv, especially compound **3c**, where MICs improved by 2-fold when compared to compound **3a**. However, the material availability for making difluorophenyl analogues is not as widely available as that of naphthyl compound **3a**.

Next, our optimization of compound **3a** focused on SAR studies of 5-phenyl replacements on the pyridine in compound **3a**. A variety of compounds **21a-f** were prepared as shown in Scheme 4. Regioselective bromine-metal exchange reaction of 3,5-dibromo-2-methoxypyridine **15** with *n*-BuLi in Et_2O, followed by reaction with benzaldehyde, and the resultant benzyl alcohol was reduced by using BF_3-Et_2O and Et_3SiH to form compound **16**. Transforming 3-benzyl-5-bromo-2-methoxypyridine **16** to key intermediate **17** was done similarly as described in Scheme 1. From key intermediate **17**, there were two pathways to get to desired compounds **21a-r**. Pathway A: Conversion of the key intermediate **17** to its corresponding boronate ester **19**, which was coupled with isothiazole-4-bromide, and SFC separation to give desired compound

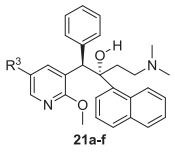


Scheme 3. * A general synthesis of SAR compounds *Reagents and conditions: a) LDA, THF, −70 °C, 2 ~ 3 h; b) chiral SFC resolution, about 3–10 % isolated yield for the two steps.



Scheme 4. *Reagents and conditions: a) *n*-BuLi, Et_2O, −70 °C to rt, 1 h, then benzaldehyde, 56 % yield; b) BF_3-Et_2O, Et_3SiH, DCM, 50 °C, 16 h, 94 % yield; c) di-isopropylamine, *n*-BuLi, THF, −70 °C, 1.5 h, 43 % yield; d) KOAc, Pd(dppf)Cl_2, dioxane, 80 °C, 16 h, 86 % yield; e) Ar-boronic acid, KOAc, Pd(dppf)Cl_2, dioxane/H_2O, 80 °C, 2 ~ 3 h; f) isothiazole-4-bromide, K_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O, 80 °C, 16 h; g) chiral SFC resolution.

Table 3
* SAR of compounds **3a** and **21a-f** assayed against *M. tuberculosis* and their cytotoxicity profile.



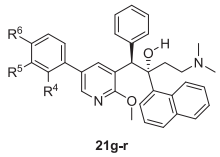
Compd.	R^3	*M. tuberculosis* H37Rv MIC (μg/mL)		CC_{50} (μg/mL)	CC_{50} (μg/mL)
		MABA	LORA	HeLa cell	Vero Cell
3a		1.1 ± 0.2	0.7 ± 0.3	30.4	>50
21a		1.9 ± 0.4	3.9 ± 0.6	28.8	26.3
21b		0.9 ± 0.2	1.4 ± 0.3	>64	24.4
21c		2.1 ± 0.3	0.9 ± 0.1	9.6	>50
21d		0.7 ± 0.2	1.5 ± 0.3	57.7	32.3
21e		1.7 ± 0.2	1.8 ± 0.2	>64	27.6
21f		1.3 ± 0.1	2.7 ± 0.3	22.5	>50
Bedaquiline	–	0.03 ± 0.01	<0.2	>64	>50

*The MIC assays followed Cho et al protocols (ref.13); MIC data are average of two tests; CC_{50} data are a single test or average of two tests.

21a. Pathway B: The key intermediate **17** reacted with known arylboronic acids under Suzuki conditions to obtain a mixture of isomers, separation by SFC to provide compounds (**21b-r**).

The MIC data for compounds **21a-f** are summarized in Table 3. A variety of heteroaryls (**21a-c**) can replace the phenyl on the pyridine of compound **3a** to provide similar anti-mycobacterial potency. Cycloalkyl

Table 4
* SAR of compounds **3a** and **21g-r** assayed against *M. tuberculosis* and their cytotoxicity profile.



21g-r

Compd.	R^4	R^5	R^6	*M. tuberculosis* H37Rv MIC (μg/mL)		CC_{50} (μg/mL)	CC_{50} (μg/mL)
				MABA	LORA	HeLa cell	Vero Cell
3a	H	H	H	1.1 ± 0.2	0.7 ± 0.3	30.36	>50
21g	F	H	H	1.41 ± 0.15	0.57 ± 0.1	ND	>50
21h	H	F	H	0.48 ± 0.09	1.18 ± 0.11	62.78	>50
21i	H	H	F	0.34 ± 0.07	0.71 ± 0.16	>64	>50
21j	Cl	H	H	1.17 ± 0.22	3.03 ± 0.45	>64	>50
21k	H	Cl	H	2.73 ± 0.24	1.36 ± 0.13	ND	>50
21l	H	H	Cl	0.08 ± 0.04	0.56 ± 0.12	>64	>50
21*m*	H	H	Br	0.22 ± 0.11	0.22 ± 0.08	>64	>50
21*n*	H	H	CN	0.44 ± 0.09	0.77 ± 0.14	>64	>50
21o	H	H	CF_3	0.96 ± 0.22	0.63 ± 0.15	>64	>50
21p	H	H	OMe	0.33 ± 0.08	0.19 ± 0.05	>64	>50
21q	H	H	OCF_3	0.03 ± 0	0.61 ± 0.14	>64	>50
21r	H	F	Cl	0.19 ± 0.05	0.52 ± 0.11	>64	>50
Bedaquiline	–			0.03 ± 0.01	<0.2	>64	>50

*The MIC assays followed Cho et al protocols (ref.13); MIC and CC_{50} data are average of two tests; ND: not determined.

(**21d**), alkyl (**21e**) and tetrahydropyranyl (**21f**) replacements also provide similar potency compared to compound **3a**.

Next, our optimization of compound **3a** focused on substituting the 5-phenylpyridine in compound **3a**. A variety of substituted 5-phenylpyridine compounds **21g-r** were prepared as shown in Scheme 4 through pathway B. The selected analogues and their activities were reported in Table 4. Mono-substitutions of the phenyl with fluorine and chlorine at *ortho-*, *meta-* and *para*-positions were investigated. Among them, *para*-substitutions were found significantly improved potency. This is evident among two groups (*o, m, p*) of fluorine and chlorine substituted compounds (**21g, h, i** and **21j, k, l**), the *p*-fluorophenyl compound (**21i**) and *p*-chlorophenyl (**21l,** WX-081) displayed the best MIC against *M. tuberculosis* H37Rv in each group. Next, a variety of *para*-substitutions, such as Br (**21m**), –CN (**21n**), -CF_3 (**21o**), CH_3O– (**21p**) and CF_3O- (**21q**) substituted compounds prepared, and their activity profile as shown in Table 4. Among them, *p*-trifluoromethoxyphenyl analogue (**21q**) displayed similar potency to WX-081 (**21l**). Disubstituted-phenyl analogue (**21r**) was not as potent as WX-081. Having considered its profile and cost of manufacture, WX-081 was picked as a compound going forward to further profiling as potential clinical candidate.

Anti-mycobacterial profile of WX-081 was evaluated in a panel of contemporary clinical isolates. This panel included both drug-susceptible (DS-TB, n = 10) and multidrug-resistant (MDR-TB, n = 30) *Mycobacterium tuberculosis* strains. WX-081 was tested side-by-side with bedaquiline, rifampin, isoniazid and other known anti-TB agents. Table 5 summarizes drug profiles. WX-081 exhibited potent activity with MICs range from 0.017 to 0.219 μg/mL, MIC_{50} of 0.083 μg/mL, MIC_{90} of 0.152 μg/mL for DS-TB strains; while MIC range from 0.026 to 0.966 μg/mL, MIC_{50} of 0.11 μg/mL, MIC_{90} of 0.46 μg/mL for MDR-TB strains. These results are similar to bedaquiline profile, but superior to all other tested agents in MDR-TB. In addition, minimum bactericidal concentration (MBC) assay indicated that WX-081 is a bactericidal agent, just like bedaquiline. [14].

The spontaneous resistance mutation rates of WX-081 and bedaquiline against *M. smegmatis* ATCC700824 were assessed. The frequency of resistance was 5.17×10^{-9} and 3.45×10^{-9} for WX-081 and bedaquiline respectively in the presence of 16-fold MIC concentration. The resistant clones of *M. smegmatis* ATCC700824 were cross-screened using WX-081 or bedaquiline, and the MIC test results showed that these mutants were WX-081 and bedaquiline cross-resistant, suggesting that both WX-081 and bedaquiline exerted their anti-mycobacterial activity through the same mechanism of action.

With its potent *in vitro* anti-mycobacterial activity, WX-081 was evaluated for *in vitro* ADMET and PK properties in CD-1 mice. WX-081was tested side-by-side with bedaquiline, and the results are shown in Table 6 and 7. Both WX-081 and bedaquiline showed low cell

Table 5
* Anti-mycobacterial profile of WX-081, bedaquiline and other TB agents tested against recent clinical isolates of *M. tuberculosis* strains (n = 40).

Strains	Compounds	MIC (μg/mL)		
		Range	MIC_{50}	MIC_{90}
MDR-TB (n = 30)	WX-081	0.026–0.966	0.111	0.465
	Bedaquiline	0.006–0.468	0.032	0.111
	Isoniazid	0.283->40	4.205	>40
	Rifampin	0.02->40	>40	>40
	Moxifloxacin	0.019–4.633	0.605	2.165
	Kanamycin	0.788->40	1.235	>40
DS-TB (n = 10)	WX-081	0.017–0.219	0.083	0.152
	Bedaquiline	0.019–0.053	0.029	0.043
	Isoniazid	<0.02–0.034	0.024	0.032
	Rifampin	<0.02–0.066	0.035	0.057
	Moxifloxacin	0.03–0.122	0.038	0.071
	Kanamycin	1.146–2.414	1.219	1.907

*MIC_{50} and MIC_{90} indicate minimum inhibitory concentrations required to inhibit growth of 50% and 90% of clinical isolates tested.

Table 6
* ADMET parameters for WX-081 and bedaquiline in mice.

Parameter		Bedaquiline	WX-081
MDCK Permeability (P_{app})	A to B (10^{-6} cm/s)	0.06	0.04
	B to A (10^{-6} cm/s)	0.09	0.11
	Efflux Ratio	1.53	2.54
PPB H/R (% bound)		>99.0/>99.0 %	>99.0/>99.0 %
LMS H/R/M CL (μL/min/mg)		34.1/87.8/183	39.4/45.7/71.3
CYP1A2/2C9/2C19/2D6/3A4 IC_{50}: (μM)		All > 10	All > 10
hERG inhibition IC_{50}: (μM)		>30	>30

*H: human, R: rat, M: mouse; LMS: liver microsomal stability; CL: clearance.

Table 7
Mouse pharmacokinetic parameters for WX-081 and bedaquiline.

Parameter	Bedaquiline	WX-081
Dose (*iv*/*po*) (mg/kg)	1/6.25	1/6.25
CL (*iv*) (mL/min/kg)	7.76	3.59
V_d (*iv*) (L/kg)	6.21	10.4
$T_{1/2}$ (*iv*) (h)	21.3	46.3
C_{max} (*po*) (ng/mL)	608	503
$AUC_{0\text{-last}}$(*po*) (ng.h/mL)	6038	10,155
F%	47.1	40.7

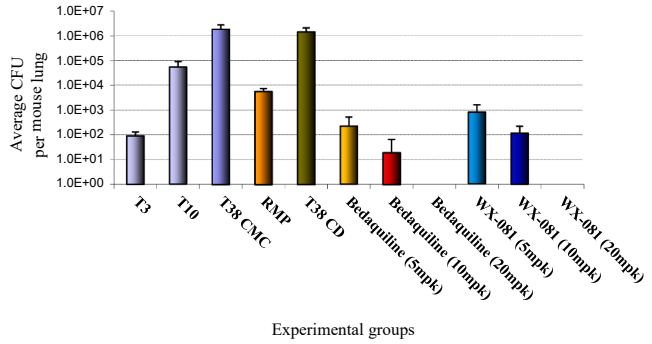


Fig. 2. Mouse bacterial load (average CFU per mouse lung) after treatment with WX-081, bedaquiline and rifampin (RMP) at day-38 vs vehicle controls (rifampin was dosed in 0.5% aqueous carboxymethylcellulose and others in 20% aqueous cyclodextrin) in an acute TB infection model (see supplementary material for detailed protocol).

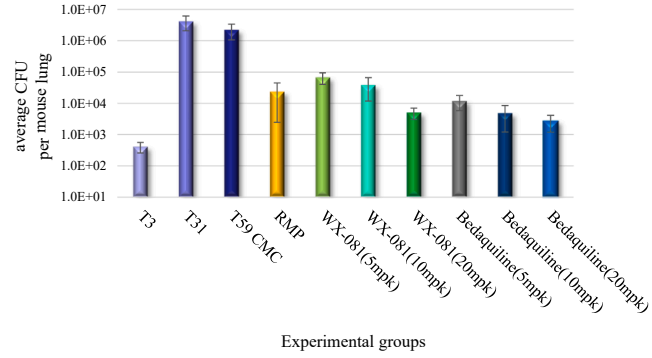


Fig. 3. Mouse bacterial load (average CFU per mouse lung) after treatments with WX-081, bedaquiline and rifampin (RMP) at day-59 vs vehicle controls (0.5% aqueous carboxymethylcellulose was used as vehicle) in a chronic TB infection model (see supplementary material for detailed protocol).

Table 8
Rat and Dog pharmacokinetic parameters for WX-081 and bedaquiline (rat only).

Parameter	Bedaquiline	WX-081	
Species	Rat	Rat	Dog
Dose (*iv*/*po*) (mg/kg)	1/5.0	1/5.0	0.5/2.0
CL (*iv*) (mL/min/kg)	8.67	8.25	2.04
V_d (*iv*) (L/kg)	10.3	9.2	6.04
$T_{1/2}$ (*iv*) (h)	37.6	25.6	56.2
C_{max} (*po*) (ng/mL)	354	328	390
$AUC_{0\text{-last}}$(*po*) (ng.h/mL)	2614	4075	8500
F%	27.7	36.7	58.9

permeability, high plasma protein binding (>99 % bound in all species), no inhibition of CYP enzymes at 10 μM screen concentration, moderate clearance in liver microsomes (LMS). And both compounds exhibited no inhibitory activity to hERG channel with IC_{50} > 30 μM. The mouse PK studies showed that both displayed low *in vivo* clearance, moderate V_d and good oral bioavailability (Table 7), but WX-081 had longer $t_{1/2}$ and higher exposure (AUC) compared to bedaquiline in mice.

With good mouse PK, WX-081 and bedaquiline were evaluated for *in vivo* anti-tuberculosis efficacy in both acute and chronic TB infection models (see supplementary material for detailed protocol). The difference was number of days TB infection was allowed to take root before the treatment was initiated.[15] In the acute TB infection model, treatments were commenced 10 days post aerosol infection. WX-081, bedaquiline, rifampin (as a positive control) and vehicles were given orally. Both WX-081 and bedaquiline were given three dosages of 5, 10 and 20 mg/kg to evaluate their dose response, while rifampin was given 15 mg/kg. The drugs were administered daily for five consecutive days per week for a total of four weeks. The results are shown in Figure 2. Both WX-081 and bedaquiline treatments yielded remarkable bacterial load reduction as measured by colony forming units (CFUs) in mouse lung in a dose dependent fashion with clearance of bacteria in the mouse lungs at 20 mg/kg. At 5 mg/kg dose, both compounds reduced CFU by more than three logs when compared to day-38 vehicle controls (T38 CMC or T38 CD), and better reduction than rifampin at 15 mg/kg. In the chronic TB infection model, TB bacilli were allowed to grow for 31 days to establish a chronic infection (Figure 3). The treatment groups and doses were similar to that of the acute infection model. In the chronic model, the infection was more difficult to treat than in the acute model. Nevertheless both WX-081 and bedaquiline showed potent efficacy: at low dose of 5 mg/kg, both compounds yielded more than one log CFU reduction, while at high dose of 20 mg/kg, both compounds yielded more than two and a half log CFU reduction when compared to the T59 CMC control.

Multispecies pharmacokinetic properties of WX-081 determined in SD rats and Beagle dogs and PK parameters are summarized in Table 8. WX-081 exhibited low clearance, long half-life, excellent exposure (AUC) and high C_{max} after oral dose, and good oral bioavailability across species tested.

Tissue distribution study was carried out in both CD-1 mice and SD rats, and the drug concentrations in the target organs measured (Table 9). At T = 96 h after PO dosing, the lung concentration of WX-081 was severalfold over that of bedaquiline at the same dose (6.25 mg/kg in mice and 5.0 mg/kg in rats) in both rodents. The lung/plasma ratio of WX-081 was slightly higher than that of bedaquiline as well. The higher concentration in the lung is desirable for a drug to treat tuberculosis.

Bedaquiline reported to have QTc interval prolongation in clinic, and this was due to its major metabolite BDQ-M2, which also had extremely long $t_{1/2}$ in human.[16] WX-081 produced similar major metabolite WX-081-M3 *in vitro* and *in vivo* in our studies (Figure 4).

The hERG inhibition was determined for both metabolites BDQ-M2 and WX-081-M3 and the results are summarized in Table 10. Both metabolites inhibited hERG channel, but their IC_{50} numbers are only moderate (IC_{50}: 1.73 μM and 1.89 μM for BDQ-M2 and WX-081-M3 respectively), which does not account for bedaquiline QTc

Table 9
Lung and plasma concentration of bedaquiline and WX-081 in mice and rats after oral dose at T = 96 h time point.

Parameter	Bedaquiline		WX-081	
Species	Mouse	Rat	Mouse	Rat
PO doses (mg/kg)	6.25	5.0	6.25	5.0
Lung Concentration (ng/g)	135	58.8	857	337
Plasma Concentration (ng/mL)	4.56	1.52	25.4	4.74
L/P Ratio*	29.6	38.7	33.7	71.1

*L/P Ratio = Lung concentration (ng/g) / Plasma concentration (ng/mL).

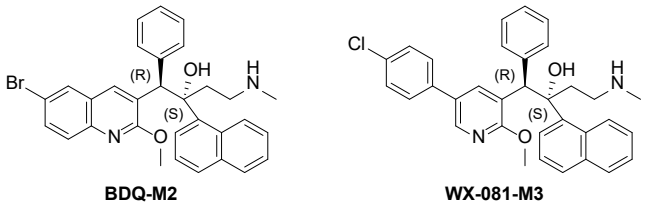


Fig.4. Chemical structures of BDQ-M2 and WX-081-M3.

Table 10
Inhibitory effect of WX-081-M3 and BDQ-M2 on different ion channels.

Parameters	WX-081-M3	BDQ-M2
hERG IC_{50} (μM)	1.89	1.73
hCav1.2 IC_{50} (μM)	> 3	0.75
Nav1.5 IC_{50} (μM)	> 10	> 10
Rabbit Purkinje fiber assay V_{max} change conc. (μM)	> 3	0.3

prolongation observed in clinic. We further assessed the effects of WX-081-M3 and BDQ-M2 on ion channels, such as Nav1.5, hCav1.2 channel and action potential parameters of Purkinje fibers from New Zealand white rabbits *in vitro*, the results are presented on Table 10. Both WX-081-M3 and BDQ-M2 did not inhibit Nav1.5 sodium channel (IC_{50}: >10 μM). In contrast, the inhibitory effect of BDQ-M2 on hCav1.2 calcium channel was stronger than that of WX-081-M3 (IC_{50}: 0.75 μM and > 3 μM for BDQ-M2 and WX-081-M3 respectively). In a rabbit Purkinje fibers assay, WX-081-M3 had no effect on action potential parameters at 3 μM, while BDQ-M2 exhibited rather potent effect on the change of V_{max} (maximal upstroke velocity) at as low as 0.3 μM when compared to control (see Table 4 and 5 in supplementary material for details). Overall, the WX-081-M3 was less inhibitory to ion channels and V_{max} (maximal upstroke velocity) than BDQ-M2, suggesting a possible improvement in QTc interval prolongation for WX-081 in clinic.

In an early exploratory safety evaluation, we evaluated safety of WX-081 head-to-head with bedaquiline in SD rats and Beagle dogs. In a 14-day pilot toxicology study, daily oral administration of WX-081 (30, 100, and 200 mg/kg) and bedaquiline (200 mg/kg) was investigated in rats (eight males, eight females per dose level). WX-081 showed better tolerance than bedaquiline. In WX-081 treated high dose group (200 mg/kg), there were 2 death in 16 treated animals on day 14, with the drug exposure (AUC) of 248 μM·h. In contrast, in bedaquiline treated group (200 mg/kg), there were 6 death in 16 treated animals on day 9–12, the drug exposure (AUC) was 109 μM·h with early animal death occurred in less exposure. Additionally, in a 14-day GLP toxicology study in Beagle dogs, daily dose of WX-081 and bedaquiline (200 mg/kg for both) was studied in parallel, all animals tolerated the treatment. Electrocardiograms (ECG) were measured daily. There was no qualitative ECG changes for WX-081 treated dogs, but QTc interval prolongation by over 10 % was observed on bedaquiline treated dogs, indicating again WX-081 is potentially safer than bedaquiline in clinic.

In our search for a safer antituberculosis agent related to bedaquiline, the bromoquinoline of bedaquiline was replaced with 5-phenylpyridine to form a novel, proprietary diarylpyridine chemical series. Extensive SAR studies were carried out, and WX-081 (sudapyridine) was discovered as our clinical candidate. WX-081 exhibited high antimycobacterial activity against both drug-susceptible and drug-resistant clinical isolates of *M. tuberculosis*, comparable to bedaquiline. In the side-by-side studies *in vivo,* both WX-081 and bedaquiline displayed excellent *in vivo* efficacy in both acute and chronic TB infection models. WX-081 also possessed favorable PK profiles in mice, rats and dogs with good oral bioavailability. Tissue distribution study revealed WX-081 had higher lung concentration than bedaquiline. In the head-to-head safety studies *in vivo*, WX-081 demonstrated better safety and tolerability in rats, and a lower risk of QTc interval prolongation in dogs when compared to bedaquiline. The observed differences in safety between WX-081 and bedaquiline may be due to their difference in chemical structures as their physicochemical properties are similar. The anti-tuberculosis profile, together with excellent safety profile in our GLP toxicology studies, warranted WX-081 moving forward. WX-081 entered clinical development in 2018, and currently in phase II clinical development for multidrug-resistant tuberculosis (NCT04608955).[17]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2022.128824.

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