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Pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones: A Novel Antimycobacterial Class Targeting Mycobacterial Respiration

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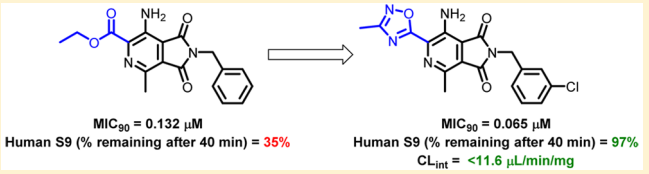
Supporting Information

ABSTRACT: High-throughput screening of a library of small polar molecules against *Mycobacterium tuberculosis* led to the identification of a phthalimide-containing ester hit compound (**1**), which was optimized for metabolic stability by replacing the ester moiety with a methyl oxadiazole bioisostere. A route utilizing polymer-supported reagents was designed and executed to explore structure−activity relationships with respect to the *N*-benzyl substituent, leading to compounds with nanomolar activity. The frontrunner compound (**5h**) from these studies was well tolerated in mice. A *M. tuberculosis* cytochrome *bd* oxidase deletion mutant (Δ*cyd*KO) was hypersusceptible to compounds from this series, and a strain carrying a single point mutation in *qcrB*, the gene encoding a subunit of the menaquinol cytochrome *c* oxidoreductase, was resistant to compounds in this series. In combination, these observations indicate that this novel class of antimycobacterial compounds inhibits the cytochrome *bc1* complex, a validated drug target in *M. tuberculosis*.



INTRODUCTION

Mycobacterium tuberculosis (*Mtb*) is the causative agent of the infectious disease tuberculosis (TB). Although most TB deaths are preventable, the mortality rate owing to *Mtb* infections remains unacceptably high.[1] Furthermore, the six-month treatment duration using the 40-year-old first-line drugs, isoniazid (INH) and rifampicin (RIF), often results in poor patient adherence, which can lead to cases of drug-resistant TB.[2] The WHO estimates that, in 2013, about half a million people developed multidrug-resistant TB (MDR-TB), which is defined as resistance to at least INH and RIF.[1,3] Therefore, new drugs which can shorten the duration of treatment are urgently needed. Some progress has been made with the recent approval of two new drugs for the treatment of MDR-TB patients: the diarylquinoline, Bedaquiline,[4] and the nitroimidazole, Delamanid.[5]

A high-throughput phenotypic screening effort at the Novartis Institute for Tropical Diseases (NITD) identified compounds, which lie in the conventional oral drug space (Mw 300−500 Da and clogP 1−4.5), but multiple lead optimization efforts to improve antimycobacterial potency led to advanced compounds with high lipophilicity and increased molecular weight.[6] Generally lipophilic molecules pose a challenge to formulation as well as other aspects of clinical development. Interestingly, many of the classical TB specific drugs like

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isoniazid, pyrazinamide, ethionamide, *p*-aminosalicylic acid, and cycloserine occupy a unique chemical space in terms of molecular weight (<250 Da) and lipophilicity (clogP < 2.5) (Figure 1). To probe this distinctive chemical space, NITD

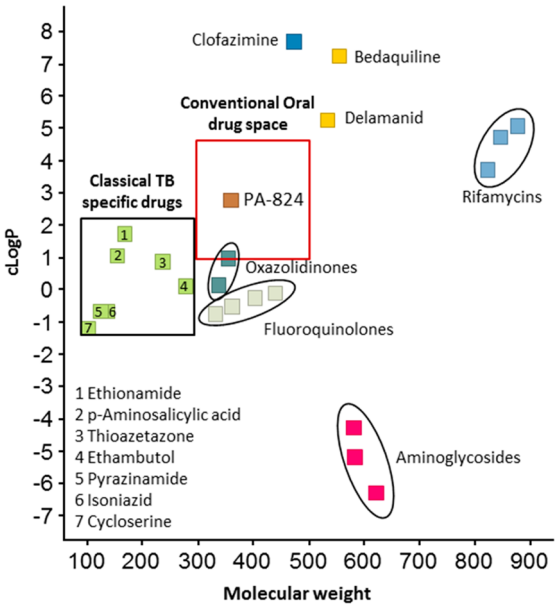
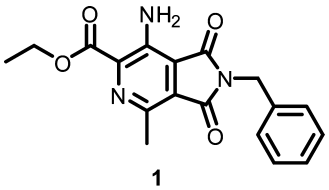


Figure 1. Plots of clogP vs molecular weight. Most TB drugs lie outside of conventional oral drug space as many are small and polar.

assembled a library of ~6000 leadlike[7] compounds with Mw 150−350 Da, clogP −1 to 3.5, and with no obvious reactive groups, and carried out a proof-of-concept phenotypic screen against *M. tuberculosis*. Compound **1** (Figure 2) with a Mw 339 and clogP 3.3 was selected as a representative example for subsequent hit validation through resynthesis, retesting, and ADME profiling.



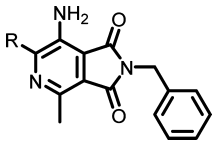
1

MIC_{99} = <0.156 μM
cLogP = 3.34
TPSA = 102.6
MW = 339.35

Figure 2. HTS hit compound **1** from screening a focused library. cLogP and TPSA calculated by ChemDraw Professional 15.

Although compound **1** had good in vitro potency against *Mtb*, the presence of the ester functionality raised concerns regarding metabolic instability owing to hydrolysis of the ester group, both in vitro in liver microsomes and in vivo. Ester group hydrolysis was confirmed by showing that less than 20% of the compound remained after incubation with human, rat, and mouse liver microsomes after 30 min (Table 1). The carboxylic acid was identified as the main metabolite in mouse liver microsomes (MLM) and rat liver microsomes (RLM). In the presence of human S9 fraction, only 35% remained after 40 min. These observations led to the conclusion that compound **1** was not expected to perform well in vivo. Therefore, the

Table 1. SAR for Ester Bioisosteres and in Vitro ADMET Data



compd	R	MIC_{90}[a] (μM)	Solubility[b] (μM)	HLM/RLM/MLM[c] % remaining	VERO IC_{50}[d] (μM)
1		<0.156	<5	14/19/18	>100
2a		>160	<5	98/100/99	n.d.
2b		3.13	177	64/71/79	>100[e]
2c		0.15	7.4	n.d.	n.d.
2d		<0.156	<5	3.4/3.3/8.8	n.d.
2e		1.25	202	61/9.6/36	n.d.
2f		>160	<5	n.d	n.d.
2g		>160	41	n.d.	n.d.
2h		>160	n.d.	80/46/58	n.d.
2i		>160	172	72/51/47	>100
2j		>160	<5	63/50/57	>100
2k		0.625	<5	57/100/98	>100
2l		>160	185	99/92/95	n.d.
2m		20	<5	100/83/20	>100
RIF[f]		0.009	n.d.	n.d.	n.d.

[a]14-day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]FaSSIF kinetic solubility assay at pH 6.5. [c]Percentage remaining at 30 min assessed in human, rat and mouse liver microsomes. [d]Cytotoxicity against kidney epithelial cells extracted from an African green monkey (VERO). [e]Cytotoxicity against Chinese hamster ovarian cells (CHO). [f]Rifampicin positive control. n.d., not determined.

initial medicinal chemistry strategy was to improve the metabolic stability of compound **1** while maintaining good in vitro potency by identifying bioisosteric replacements for the labile ester functionality. Once a suitable ester group replacement was identified, we envisaged that initial structure-activity relationship (SAR) studies would focus on iterative changes to the benzyl substituent and exploration of the phthalimide core. Here, we present results of the SAR analyses along with our efforts toward elucidation of the target of this antimycobacterial series through mechanism of action studies.

RESULTS AND DISCUSSION

Chemistry. Hit compound **1** was prepared by acid-catalyzed condensation of ethyl acetamidocyanoacetate and benzylmaleimide (Scheme 1).[8] Similarly, reaction of *N*-(dicyanomethyl)-acetamide with benzylmaleimide gave nitrile **2a**. Hydrolysis of the ethyl ester **1** under acid conditions gave acid **2b**. Primary, secondary, and tertiary amides were prepared from **2b** by utilizing amide coupling reagents EDC, HOBt, and the appropriate amine in dichloromethane. These conditions and appropriate alcohols were also used for the synthesis of esters (**2c**−**e**), which were explored to determine the influence of the

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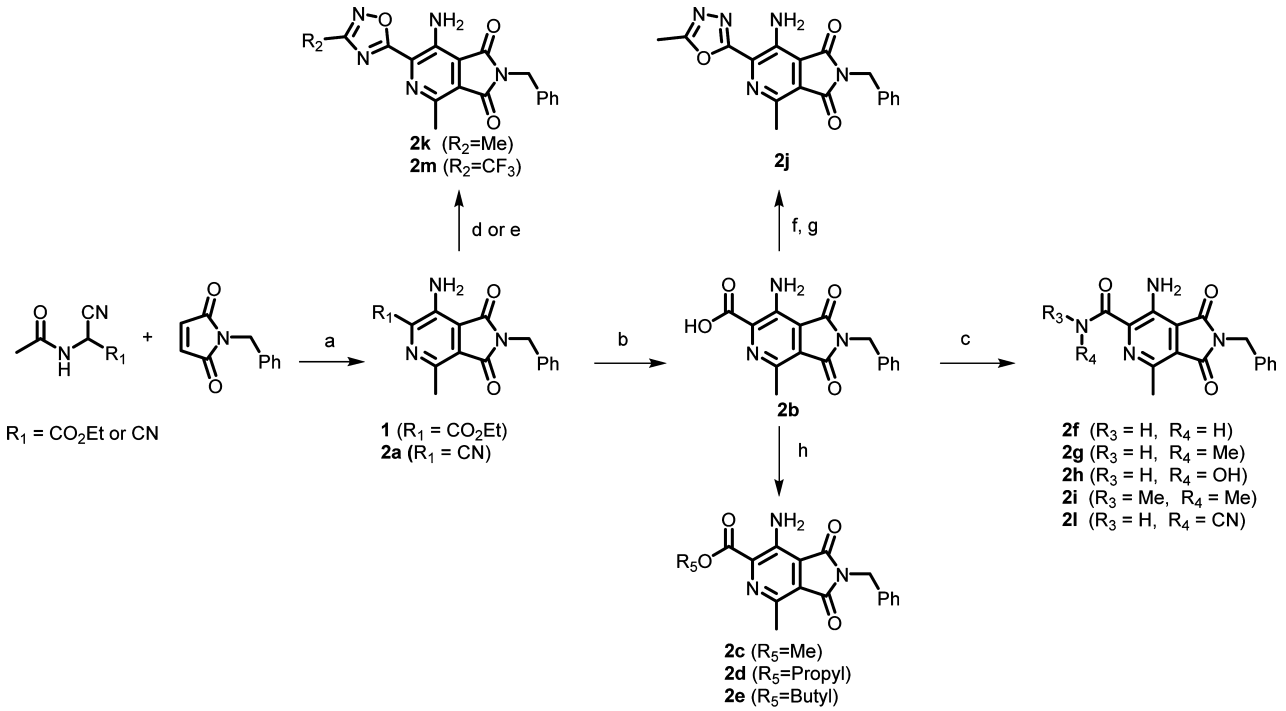
Scheme 1. Synthesis of Ester Bioisosteres[a]



[a]Reagents and conditions: (a) cat. TFA, DCE, reflux, 3 d; (b) **1**, aq HCl, dioxane, reflux; (c) EDC, HOBt, amine, DCM, rt; (d) **1**, $CH_3C(=NOH)NH_2$, NaH, dioxane, 60 °C; (e) (1) **1**, $CF_3C(=NOH)NH_2$, NaH, 65 °C, dioxane, (2) PS-BEMP, dioxane, 75 °C; (f) $CH_3CONHNH_2$, T_3P, NEt_3, EtOAc, reflux; (g) TsCl, NEt_3, $CHCl_3$, 65 °C; (h) EDC, HOBt, ROH, DCM.

size of the ester group on *Mtb* activity. The 1,2,4-oxadiazoles **2k** and **2m** were prepared by reacting **1** with the appropriate amidoxime in sodium hydride in dioxane at 60 °C. 1,3,4-Oxadiazole **2j** was prepared in two steps from **2b** by coupling acetyl hydrazide in the presence of propylphosphonic anhydride (T3P) and then cyclization with tosyl chloride (TsCl) in refluxing chloroform.

In Vitro Antimycobacterial Activity. Compounds prepared in this manner were tested against the fully virulent *Mtb* H37RvMa[9] in GAST/Fe medium using the standard broth micro dilution method[10] and minimum inhibitory concentration (MIC) values determined at 14 days (Table 1). Kinetic solubility of the test compounds was determined in fasted state simulated intestinal fluid (FaSSIF) medium, and metabolic stability of selected compounds in human, rat, and mouse liver microsomes was assessed by incubating for 30 min and measuring the percentage of compound remaining.[11] As shown in Table 1, the methyl (**2c**), ethyl (**1**), propyl (**2d**), and cyclopropyl (**2e**) esters all had good in vitro activity against *Mtb* H37RvMa. The primary (**2f**), secondary (**2g**), tertiary (**2i**), and hydroxamic (**2h**) amides were not active at the highest concentration tested (MIC_{90} > 160 μM). The carboxylic acid **2b** maintained some *Mtb* activity, albeit at least 10-fold lower than the original hit compound **1**. As expected, **2b** had improved microsomal metabolic stability in microsomes relative to **1** and exhibited significantly improved aqueous solubility, as did the inactive amide analogues. The improved metabolic stability of **2b** suggests that ester hydrolysis is the main route of metabolism. The ester **1** was also hydrolyzed into **2b** in mouse plasma, in *Mtb* supernatant (data not shown), and in mouse liver microsomes even in the absence of NADPH, indicating contribution by non-CYP450 enzymes. However, no hydrolysis occurred in a control containing buffer only, indicating that the reaction is enzyme-mediated and not a result of chemical instability. Replacement of the ester functionality with a nitrile also abolished *Mtb* activity but did improve metabolic stability. Next, we turned our attention to five-membered heterocycles, in particular oxadiazoles, because these are well-known bioisosteres for ester groups.[12] The 1,3,4-oxadiazole compound **2j** was shown to be inactive, whereas the 1,2,4-oxadiazole (**2k**) had significant *Mtb* activity and improved metabolic stability over ester **1**. Metabolite identification in the presence of mouse liver microsomes and in mice revealed that the metabolic pathway for compound **2k** was through oxidative ring opening of the oxadiazole ring, most likely mediated by oxidation of the methyl group leading to metabolites **2b**, **2f**, and **2l** (Supporting Information Figure S1). This prompted us to investigate blocking oxidation on the methyl group by replacement with a trifluoromethyl group (**2m**). However, this modification resulted in loss of *Mtb* activity. All compounds tested in this series were not toxic to VERO cells up to 100 μM (IC_{50} > 100 μM).

With the 1,2,4-oxadiazole identified as a more metabolically stable ester bioisostere, we next explored changes to the *N*-benzyl substituent in order to improve potency and aqueous solubility. For rapid SAR exploration, a parallel synthesis route was designed that would enable preparation of various analogues with minimum purification (Scheme 2). Cyclization of maleimide and ethyl acetamidocyanoacetate gave *N*-phthalimide, compound **3**, which could be transformed into the versatile precursor **4** by reacting *N*′-hydroxyacetimidamide with **3** under basic conditions. Alkylation of **4** with a variety of benzyl bromides and chlorides proceeded smoothly in the presence of polymer-supported 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine on polystyrene (PS-BEMP), in dioxane, at 75 °C. After the reaction was

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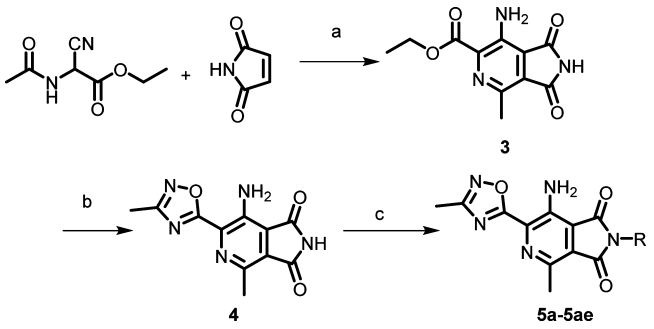
Scheme 2. Synthesis of Methyl Oxadiazole Analogues[a]



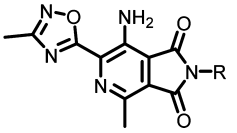
[a]Reagents and conditions: (a) cat. TFA, dichloroethane reflux, 3 d; (b) $CH_3C(=NOH)NH_2$, NaH, dioxane, 60 °C; (c) (1) RBr or RCl, PS-BEMP, dioxane, 24 h, 75 °C, (2) PS-PhSH, 75 °C.

complete, thiophenol on polystyrene (PS-PhSH) was added to scavenge any remaining benzyl bromide or chloride. Removal of the resins by filtration gave the final products, which were often >95% pure. Remaining impurities consisted of unreacted benzyl bromide or **4** which could be easily removed by flash chromatography.

We initially explored the effect of adding small halogens to the benzyl ring. Benzyl analogues with *para* and *meta* halogens (F, Cl, Br, and CF_3) were favored over *ortho* substituted analogues (Table 2). Methoxy and trifluoromethoxy groups also showed good to excellent anti-*Mtb* activity. α-Methylbenzyl analogue **5l** also maintained good anti-*Mtb* activity with some improvement in solubility probably resulting from disrupting the planarity of the molecule. Replacement of the benzyl group with a 4-pyridylmethyl (**5s**) was investigated to improve solubility but resulted in a loss of activity with an increase in solubility. A series of compounds with larger changes in the *para* position was prepared to explore potential new interactions and/or to improve the solubility of this series in aqueous media. Analogues with nitrile (**5u**), carboxamide (**5v**), thiotrifluoromethyl (**5w**), hydroxymethyl (**5x**), and *N*,*N*-dimethylamino (**5y**) functionalities all showed a significant loss in activity. Methylsulfone analogue **5t** showed good *Mtb* potency but no improvement in solubility over **2k**. Heterocycles in the para position, e.g., pyridyl, pyrazole, and pyrrolidinone, were mainly detrimental to activity. However, the *p*-fluorophenyl ether derivative **5ab** maintained good activity and exhibited some improvement in solubility relative to the benzyl analogue **2k**. The increase in lipophilicity of this compound compared to other analogues might have contributed to the improved activity. Finally, saturated cyclic heterocycles such as the piperidinylmethyl analogue **5ad** and tetrahydropyranmethyl **5ae** resulted in loss of activity while improving solubility.

We also investigated some changes to the pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione scaffold of **1** (Scheme 3). These changes, which included removing the benzylic methylene, compound **6**,[8] and the methyl group on the pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione core, compound **7**, both led to a significant loss in activity (Table 3). Reduction of the carbonyls on the phthalimide moiety was also explored. Reduction using $NaBH_4$ gave a mixture of monohydroxyl regio-isomers, which were separated by preparative HPLC. The 1-hydroxy compound **8a** resulted in a 4-fold drop in activity, while the 3-hydroxy isomer **8b** retained the potency of the original hit compound **1**. Furthermore, both of these compounds showed a

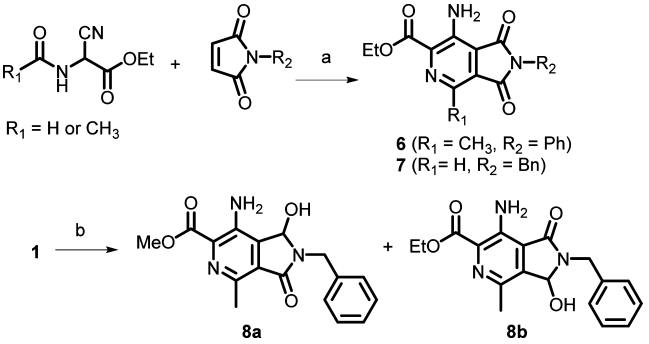
Table 2. SAR and Solubility Data for Changes Made to the Phthalimide Group



compd	R	MIC_{90} GAST/Fe[a] (μM)	Solubility[b] (μM)	compd	R	MIC_{90} GAST/Fe[a] (μM)	Solubility[b] (μM)
5a		0.31	<5	**5q**		<0.156	<5
5b		0.62	<5	**5r**		2.5	<5
5c		20	<5	**5s**		20	26.4
5d		<0.156	<5	**5t**		0.31	<5
5e		0.62	<5	**5u**		>160	<5
5f		<0.156	<5	**5v**		>160	<5
5g		<0.156	<5	**5w**		1.2	<5
5h		<0.156	72	**5x**		0.62	<5
5i		<0.156	<5	**5y**		20	7.4
5j		<0.156	<5	**5z**		>160	<5
5k		0.39	6.3	**5aa**		160	<5
5l		1.25	10.9	**5ab**		<0.156	23.8
5m		0.30	<5	**5ac**		40	<5
5n		1.25	<5	**5ad**		>160	160
5o		>160	<5	**5ae**		20	5.7
5p		0.31	<5	RIF[c]		0.009	n.d.

[a]14-day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]FaSSIF kinetic solubility assay at pH 6.5. [c]Rifampicin positive control. n.d., not determined.

Scheme 3. Synthesis of Analogues with Changes to the Core[a]



[a]Reagents and conditions: (a) cat. TFA, DCE, reflux, 3 d; (b) $NaBH_4$, DCM, MeOH, 0 °C.

significant improvement in aqueous solubility and microsomal stability and were not toxic to VERO cells.

Further in Vitro Biological Profiling of Selected Compounds. Having identified numerous compounds with

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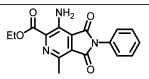
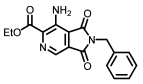
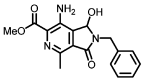
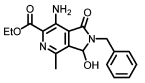
Table 3. SAR of Changes Made to the Heterocyclic Scaffold and in Vitro ADMET Data

compd	R	MIC_{90} GAST/Fe[a] (μM)	Solubility[b] (μM)	HLM/RLM/MLM[c] % remaining	VERO IC_{50}[d] (μM)
6		>160	5.0	n.d.	n.d.
7		20	n.d.	n.d.	n.d.
8a		2.5	200	55/70/69	>100
8b		0.144	41.5	99/81/89	>100
RIF[e]		0.009	n.d.	n.d.	n.d.

[a]14-day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]FaSSIF kinetic solubility assay at pH 6.5. [c]Percentage remaining at 30 min assessed in human, rat and mouse liver microsomes. [d]Cytotoxicity against kidney epithelial cells extracted from an African green monkey (VERO). [e]Rifampicin positive control. n.d., not determined.

submicromolar MIC_{90} values against *Mtb* H37RvMa, and having made improvements to in vitro metabolic stability in some cases (e.g., compound **5h**), we profiled these compounds in additional microbiological assays in vitro. Specifically, compounds **1**, **5h**, and **5k** were tested against a recombinant *Mtb* H37RvMa strain expressing green fluorescent protein (*Mtb*::gfp)[13] (Table 4).[14] Compounds **1**, **5h**, and **5k** were tested in GAST/Fe medium using this *Mtb*::gfp strain, and the dose–response curve confirmed the potent MIC_{90} values. In contrast, the same compounds were completely inactive when MIC_{90} values were determined following 14-days incubation in standard Middlebrook 7H9 medium supplemented with ADC (bovine albumin fraction V), an observation reminiscent of known inhibitors of the mycobacterial cytochrome *bc1* complex possibly suggesting pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones target the respiratory pathway.[15]

To determine the potential utility of this scaffold in a clinical setting, compounds **1**, **5h**, and **5j** were tested for activity against four drug-susceptible clinical *Mtb* isolates, selected from a culture collection in the Department of Molecular Biology and Human Genetics, Stellenbosch University, South Africa (Table 4). The identity of the isolates was determined by IS6110 restriction fragment length polymorphism (RFLP) and their phylogenetic lineages assigned by spoligotyping.[16] The spoligotype patterns indicated that the strains SAWC 3906, SAWC 2371, SAWC 3385, and SAWC 3933 belong to the atypical Beijing, Haarlem, Cas 1/Delhi, and X families, respectively. Pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones retained activity against the full clinical panel (Table 4) in 7H9 medium. This result established the activity of this series against clinical *Mtb* strains from diverse phylogenetic origins and, moreover, suggested that the apparent inactivity against the laboratory strain, H37RvMa, in 7H9 medium was likely a consequence of extended adaption to in vitro medium over decades of research use.[9]

In Vitro and in Vivo Pharmacokinetic Studies. In vitro clearance indicated that there was insignificant turnover for compounds **5h** and **5k** in human, rat, and mouse microsomes (Table 5). However, compound **5h** was more stable across all three species, more stable in the presence of human S9 fraction, and had moderate stability in human plasma. The measured log *D* was similar for these analogues and within an acceptable space for oral drugs, while compound **5h** showed high permeability in a PAMPA assay and was highly protein bound. On the basis of these results, compound **5h** was selected for progression into mouse PK studies.

With the excellent in vitro microsomal stability and moderate solubility observed for **5h**, a mouse PK study was carried out at 5 mg/kg iv and 20 mg/kg po (Table 6). When dosed iv compound **5h** showed a high clearance and volume of distribution and had poor plasma exposure (AUC 8 μM·min and C_{max} = 0.033 μM respectively at 20 mg/kg) when dosed orally. As the compound showed high in vivo clearance leading to poor blood exposures, it was coadministered with aminobenzotriazole (ABT), which is a known nonspecific cytochrome P450 inhibitor, and is used frequently to reduce the CYP-mediated metabolism of compounds.[17] Dosing iv with ABT indicated that the clearance improved to moderate (170.78–94.45 mL/min/kg). Dosing orally with ABT showed an increased exposure, resulting in a C_{max} 2-fold above the MIC_{90} with a 10-fold higher AUC compared to non-ABT exposure. The disconnect between in vitro and in vivo clearance suggests that clearance is through a non CYP-mediated process, most probably through hydrolysis of the oxadiazole by amidases and esterases present in the plasma. This is consistent with the moderate half-life found in human plasma ($t_{1/2}$ = 60 min) and the minor reduction in clearance when codosing the compound with ABT in mice.

Target Identification Studies. To identify the molecular target of pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones and gain insight into the mechanism of action, we attempted to raise spontaneous resistant mutants (SRM) in H37RvMa. We were unable to obtain SRMs of compounds from this series even when plating on solid media containing a range of concentrations from 2-fold to 200-fold the MIC_{90} value determined in the liquid GAST/Fe assay. During routine hit triaging, we observed that the compounds were hyperactive against a cytochrome *bd* oxidase deletion mutant (Δ*cyd*KO) (Table 7).[15] In addition, a derivative Δ*cyd*KO deletion mutant

Table 4. MIC_{90} Values (μM) of Selected Compounds against *M. tuberculosis* Strains

	reference strain			SAWC clinical isolates (7H9)			
compd	H37RvMa (GAST/Fe)	*Mtb*::gfp (GAST/Fe)[a]	*Mtb*::gfp (7H9)[a]	2371 Haarlem	3385 Cas1/Delhi	3906 Atyp-Beijing	3933 LCC
1	<0.156	0.132	>20	<0.08	<0.08	<0.08	n.d.
5h	<0.156	0.065	>20	0.6	0.6	0.15	0.15
5j	<0.156	n.d.	n.d.	0.3	0.075	0.075	0.15
5k	0.39	0.052	>20	n.d.	n.d.	n.d.	n.d.
RIF[b]	0.009	0.0032	0.0016	n.d.	n.d.	n.d.	n.d.

[a]GFP readout. [b]Rifampicin positive control, SAWC, South Africa Western Cape, n.d. not determined.

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Table 5. Properties of Two Leading Compounds 5h and 5k

compd	VERO IC_{50} (μM)[a]	HLM/RLM/MLM CL_{int} (mL/min/mg protein)[b]	human S9 (% remaining at 40 min)	plasma stability $t_{1/2}$ (min)	log *D* pH 7.4	PAMPA pH 6.5 log P_{app} (cm/s)	human PPB (% bound)
5h	>100	<11.6/<11.6/<11.6	97	62	3.34	−5.0	96.7
5k	>100	15.4/32.4/65.7	93	>360	3.64	−6.1	n.d

[a]Cytotoxicity against kidney epithelial cells extracted from an African green monkey (VERO). [b]HLM = human liver microsomes, RLM = rat liver microsomes, MLM = mouse liver microsomes. PPB = plasma protein binding, n.d., not determined.

Table 6. Pharmacokinetic Parameters for Compound 5h in C57/BL6 Mice Following Intravenous and Oral Administration with and without ABT[a]

	compd **5h** without ABT		compd **5h** with ABT	
parameters	IV	oral	IV	oral
dose (mg/kg)	5	20	5	20
apparent $t_{1/2}$ (h)	7.01	2.19	7.31	3.39
CL_{total} (mL/min/kg)	170.78	–	94.45	–
V_d (L/kg)	104.24	–	53.10	–
C_{max} (μM)	0.622	0.033	1.526	0.167
T_{max} (h)	0.17	1	0.17	0.5
$AUC_{0-\infty}$ (min·μM)	76	8	159	80
oral bioavailability (%)	–	2	–	26

[a]Values are the mean from three animals. Dash indicates that the value was not measured or was not relevant.

Table 7. Activity of Selected Compounds against Mutant *Mtb* Strains

compd	MIC_{90} (μM) with H37RvMa[a]	MIC_{90} (μM) with Δ*cyd*KO[a]	MIC_{90} (μM) with Ala317Thr point mutant[a]
1	0.078	0.019	>10
2e	1.25	0.039	>10
5h	<0.156	<0.019	>10
5j	<0.156	0.039	>10
5k	0.0637	<0.019	>10
8b	0.144	0.019	>10

[a]14-day Alamar Blue readout in GAST/Fe medium.

carrying an Ala317Thr point mutation in *qcrB*[15] was resistant to compounds from this series (Table 7). Together with the differential activity in GAST/Fe versus 7H9 medium against H37RvMa (Table 4), the observed hypersensitivity of the Δ*cyd*KO deletion mutant to these compounds and the cross-resistance of the Δ*cyd*KO/QcrBA317T mutant strongly indicated that this series of pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones targets the QcrB subunit of the respiratory *bc1* complex in *Mtb*. Recently, multiple chemically diverse scaffolds have been described that target QcrB, the cytochrome *b* subunit in the cytochrome *bc1* complex, and these include the imidazo-[1,2-*α*]pyridines series from which the exciting new clinical candidate Q203 was derived.[18]

CONCLUSIONS

Screening of a library of compounds in novel chemical space led to the identification of a potent hit compound, the pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione **1**. Hit optimization of **1** gave compound **5h** by replacing the ester moiety with a methyl oxadiazole bioisostere. This compound had good in vitro metabolic stability and retained the excellent potency of the hit compound. Pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones targets mycobacterial respiratory cytochrome *bc1*, a highly promising promiscuous target in *Mtb*.[19] Mouse PK studies with **5h** indicate high clearance and low plasma exposure (C_{max} = 0.033 μM) in contrast to good metabolic stability in liver microsomes. Co-dosing of the compound with ABT gave an improved clearance resulting in a circulation concentration above the MIC_{90} for 6 h when dosed at 20 mg/kg. Future studies will focus on improving exposure of the compounds in plasma by further modifications based on the 3-hydroxyl analogue **8b**, which has good in vitro *Mtb* activity and improved aqueous solubility.

EXPERIMENTAL SECTION

General Methods. Purification of compounds were carried out by either column chromatography on silica gel 60 (Fluka), particle size 0.063−0.2 mm (70−230 mesh), as the stationary phase, or by Waters preparative HPLC using X-bridge C18 5 μm column (4.6 mm × 150 mm). Mobile phase B: 0.4% acetic acid, 10 mM ammonium acetate in a 9:1 ratio of HPLC grade methanol and type 1 water. Mobile phase A: 0.4% acetic acid in 10 mM ammonium acetate in HPLC grade (type 1) water; flow rate = 15.00 mL/min; detector, photodiode array (PDA). All target compounds and intermediates were characterized by ^{1}H NMR and LC-MS. NMR spectra were recorded on either a Varian Mercury-300 (^{1}H 300.1 MHz, ^{13}C 75.5 MHz) or Bruker-400 (^{1}H 400.2 MHz, ^{13}C 100.6 MHz) instrument using $CDCl_3$, CD_3OD, and DMSO-d_6 as solvents. Liquid chromatograph with mass spectrometer (LC-MS) analysis was performed using an Agilent 1260 Infinity binary pump, Agilent 1260 Infinity diode array detector (DAD), Agilent 1290 Infinity column compartment, Agilent 1260 Infinity standard autosampler, and a Agilent 6120 quadrupole (single) mass spectrometer, equipped with APCI and ESI multimode ionization source. Purities were determined by Agilent LC-MS using a Kinetex Core C18 2.6 μm column (50 mm × 3 mm). Mobile phase B: 0.4% acetic acid, 10 mM ammonium acetate in a 9:1 ratio of HPLC grade methanol, and type 1 water. Mobile phase A: 0.4% acetic acid in 10 mM ammonium acetate in HPLC grade (type 1) water, with flow rate = 0.9 mL/min; detector, diode array (DAD), and all compounds were confirmed to have ≥95% purity.

*Ethyl 7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**1**).* Trifluoroacetic acid (0.06 g, 0.58 mmol) was added to a mixture of 1-benzyl-1*H*-pyrrole-2,5-dione (1.0 g, 5.88 mmol) and ethyl acetamidocyanoacetate (1.31 g, 7.05 mmol) in DCE. The reaction mixture was heated at reflux for 48 h. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (10−20% EtOAc/petroleum ether) to afford **1** (1.0 g, 50%). ^{1}H NMR (400 MHz, DMSO) δ 7.26−7.32 (m, 5H), 6.97 (s, 2H), 4.71 (s, 2H), 4.35 (q, J = 7.11 Hz, 2H), 2.58 (s, 3H), 1.32 (t, J = 7.08 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.28, 167.45, 165.94, 142.53, 141.46, 135.93, 134.51, 128.77, 128.63, 128.03, 125.77, 120.59, 62.16, 41.66, 20.04, 14.23. LC-MS (APCI): m/z 340.2 $[M + H]^+$. HPLC purity 95%.

*7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo-[3,4-c]pyridine-6-carbonitrile (**2a**).* To a suspension of malononitrile (30.0 g, 454 mmol) in water (200 mL) and acetic acid (200 mL) was added sodium nitrite (62.70 g, 908.6 mmol) at 0−5 °C over a period of 1 h. The temperature was slowly raised to rt and stirred until complete consumption of malononitrile. The reaction mixture was then extracted with EtOAc (5 × 80 mL). The combined organic layers were washed with 10% sodium bicarbonate solution (5 × 25 mL) and brine, dried over sodium sulfate, and evaporated. The resulting crude product was purified by flash column chromatography (20−30%

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EtOAc/petroleum ether) to afford hydroxycarbonimidoyl dicyanide (16.0 g, 37%). ^{1}H NMR (400 MHz, DMSO) δ 14.45 (s, 1H), 7.90 (s, 1H), 7.83(s, 1H). To a cooled suspension of hydroxycarbonimidoyl dicyanide (16.0 g, 166 mmol) and acetic anhydride (68.0 g, 666 mmol) in acetic acid (200 mL) was added zinc dust (32.69 g, 499.0 mmol) in small portions. The suspension was stirred at rt for 3 h. The reaction mixture was poured into crushed ice, and the pH was adjusted to 8−9 using a saturated sodium carbonate solution. The product was extracted with EtOAc (3 × 120 mL). The combined organic layers were washed with water (2 × 100 mL) and brine, dried over sodium sulfate, and evaporated. The crude product was purified by flash column chromatography (40−50% EtOAc/petroleum ether) to afford *N*-(dicyanomethyl)acetamide (3.30 g, 16%). ^{1}H NMR (400 MHz, DMSO) δ 7.53 (s, 1H), 2.21 (s, 1H). Trifluoroacetic acid (1.48 g, 12.9 mmol) was added to a mixture of 1-benzyl-1*H*-pyrrole-2,5-dione (6.02 g, 32.1 mmol) and *N*-(dicyanomethyl)acetamide (3.30 g, 26.8 mmol) in DCE. The reaction mixture was heated at reflux for 48 h. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (10−20% EtOAc/petroleum ether) to afford **2a** (0.80 g, 10%). ^{1}H NMR (400 MHz, DMSO) δ 8.15 (s, 1H), 7.75 (s, 1H), 7.2−7.36 (m, 5H), 4.72 (s, 2H), 2.61 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ 168.70, 168.27, 167.88, 140.68, 139.88, 136.93, 136.49 129.03, 127.92, 125.63, 119.92, 19.83. LC-MS (APCI): m/z 310.1 $[M + H_2O]^+$. HPLC purity 98%.

*7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo-[3,4-c]pyridine-6-carboxylic Acid (**2b**).* To a mixture of **1** (0.500 g, 1.47 mmol) in dioxane (6 mL) was added aqueous 1 M hydrochloric acid (12.0 mL, 12.0 mmol), and the reaction was refluxed overnight. The organics were removed under reduced pressure and the mixture cooled to 0 °C to give a yellow precipitate. Filtration gave **2b** (366 mg, 78%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO) δ 7.40−7.21 (m, 5H), 7.03 (s, 2H), 4.72 (s, 2H), 2.60 (s, 3H). LC-MS (ESI): m/z 312.1 $[M + H]^+$. HPLC purity 98%.

*Methyl 7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**2c**).* A solution of **2b** (100 mg, 0.32 mmol), methanol (26 μL, 0.64 mmol), HOBt (50 mg, 0.32 mmol), and EDC (61 mg, 0.32 mmol) in DCM (3 mL) was stirred for 24 h. Water was added to the reaction, followed by EtOAc. The organic layer was separated, washed with brine, and dried over $MgSO_4$. After filtration and concentration, the product was purified by recrystallization from MeOH/DCM to give **2c** (60 mg, 57%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.27 (m, 5H), 6.92 (br s, 2H), 4.80 (s, 2H), 4.00 (s, 3H), 2.76 (s, 3H). LC-MS (ESI): m/z 326.0 $[M + H]^+$. HPLC purity 98%.

*Propyl 7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**2d**).* A solution of **2b** (100 mg, 0.32 mmol), 1-propanol (58 μL, 0.64 mmol), HOBt (50 mg, 0.32 mmol), and EDC (61 mg, 0.32 mmol) in DCM (3 mL) was stirred for 24 h. Water was added to the reaction, followed by EtOAc. The organic layer was separated, washed with brine, and dried over $MgSO_4$. After filtration and concentration, the product was purified by preparative HPLC to afford **2d** (7 mg, 6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.38 (m, 2H), 7.36−7.27 (m, 3H), 6.90 (br s, 2H), 4.79 (s, 2H), 4.36 (t, J = 7.0 Hz, 2H), 2.76 (s, 3H), 1.86 (dt, J = 7.5, 7.1, 2H), 1.02 (t, J = 7.4 Hz, 3H). LC-MS (ESI): m/z 354.1 $[M + H]^+$. HPLC purity 99%.

*Cyclopropyl 7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**2e**).* A solution of **2b** (100 mg, 0.32 mmol), EDC (61 mg, 0.32 mmol), and HOBt (50 mg, 0.32 mmol) was stirred in DCM (3.0 mL) for 15 min. Cyclopropanol (0.041 mL, 0.64 mmol) was added, and the reaction was stirred for 24 h. Water (10 mL) was added to the reaction, followed by EtOAc (10 mL). The organic layer was separated, washed with brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo to give the crude product which was purified by preparative HPLC to give **2e** (21 mg, 19%). ^{1}H NMR (300 MHz, DMSO) δ 7.38−7.23 (m, 5H), 6.98 (br s, 2H), 4.72 (s, 2H), 4.37−4.22 (m, 1H), 2.58 (s, 3H), 0.85−0.79 (m, 4H). LC-MS (ESI): m/z 352.1 $[M + H]^+$. HPLC purity 95%.

*7-Amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo-[3,4-c]pyridine-6-carboxamide (**2f**).* To a solution of **2b** (152 mg, 0.482 mmol) in DMF (2 mL) was added triethylamine (0.134 μL, 0.964 mmol) and HATU (275 mg, 0.723 mmol) and stirred for 10 min. Ammonium chloride (31 mg, 0.58 mmol) was then added and the mixture stirred at 40 °C overnight. Addition of water resulted in an orange precipitate, which was isolated by vacuum filtration and purified by column chromatography (30% EtOAc/hexane) to afford **2f** (88 mg, 59%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO) δ 8.09 (s, 1H), 7.70 (s, 1H), 7.23−7.39 (m, 5H), 4.70 (s, 2H), 2.59 (s, 3H). LC-MS (ESI): m/z 311.0 $[M + H]^+$. HPLC purity 98%.

*7-Amino-2-benzyl-N,4-dimethyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxamide (**2g**).* A solution of **2b** (100 mg, 0.32 mmol), methylamine (0.32 mL of a 2 M solution in THF, 0.64 mmol), HOBt (50 mg, 0.32 mmol), and EDC (61 mg, 0.32 mmol) in DCM (3 mL) was stirred for 24 h. Water was added to the reaction, followed by EtOAc. The organic layer was separated, washed with brine, and dried over $MgSO_4$. After filtration and concentration, the product was purified by preparative HPLC to afford **2g** (8 mg, 7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.45−7.23 (m, 5H), 4.79 (s, 2H), 2.99 (d, J = 5.2 Hz, 3H), 2.67 (s, 3H). LC-MS (ESI): m/z 325.1 $[M + H]^+$. HPLC purity 99%.

*7-Amino-2-benzyl-N-hydroxy-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxamide (**2h**).* **2b** (100 mg, 0.32 mmol) and triethylamine (0.058 mL, 0.42 mmol) were added to anhydrous THF (3.0 mL). The solution was cooled to 0 °C in an ice bath, and ethyl chloroformate (0.040 mL, 0.42 mmol) was added dropwise. The mixture was stirred for 20 min at 0 °C and left stirring for a further 2 h at rt. The mixture was filtered and then added in a dropwise manner to a solution of triethylamine (0.045 mL, 0.32 mmol) and hydroxylamine hydrochloride (22 mg, 0.32 mmol) in methanol (3.0 mL) and then allowed to stir for 2 h at rt. The solvents were removed in vacuo, and the residue was dissolved in EtOAc (10 mL). The organics were washed with 0.5 M HCl (5.0 mL) and water (10 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure, and the crude product was purified by preparative HPLC which afforded **2h** (46 mg, 44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.97 (br s, 1H), 7.47−7.21 (m, 5H), 4.80 (s, 1H), 4.79 (s, 2H). LC-MS (ESI): m/z 327.0 $[M + H]^+$. HPLC purity 95%.

*7-Amino-2-benzyl-N,N,4-trimethyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxamide (**2i**).* A solution of **2b** (100 mg, 0.32 mmol), dimethylamine hydrochloride (52 mg, 0.64 mmol), HOBt (50 mg, 0.32 mmol), and EDC (61 mg, 0.32 mmol) in DCM (3 mL) was stirred for 24 h. Water was added to the reaction, followed by EtOAc. The organic layer was separated, washed with brine, and dried over $MgSO_4$. After filtration and concentration, the product was purified by preparative HPLC to afford **2i** (9 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46−7.41 (m, 2H), 7.38−7.27 (m, 3H), 6.05 (s, 2H), 4.82 (s, 2H), 3.17 (s, 3H), 3.15 (s, 3H), 2.73 (s, 3H). LC-MS (ESI): m/z 339.1 $[M + H]^+$. HPLC purity 99%.

*7-Amino-2-benzyl-4-methyl-6-(5-methyl-1,3,4-oxadiazol-2-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**2j**).* To a mixture of **2b** (100 mg, 0.32 mmol) and acethydrazide (25 mg, 0.32 mmol) in EtOAc (10 mL) was added triethylamine (97 mg, 0.130 mL, 0.96 mmol). To the mixture was added T3P (0.20 mL of a 50% solution in EtOAc, 0.32 mmol) and the reaction refluxed overnight at 80 °C. The reaction was diluted with EtOAc (10 mL) and washed with water (10 mL), followed by saturated $NaHCO_3$ (10 mL). After drying the organics over Na_2SO_4, the solvent was reduced in vacuo to give the crude product which was purified by recrystallization from methanol. The product was taken into the next step without further purification. To a stirred suspension of the hydrazide (100 mg, 0.270 mmol) in DCM (10 mL) was added triethylamine (137 mg, 1.36 mmol) followed by tosyl chloride (156 mg, 0.820 mmol). The mixture was heated to 65 °C for 3 h, followed by removal of the solvent in vacuo. The residue was taken up in EtOAc (10 mL) and washed with brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by preparative HPLC to afford **2j** (7 mg, 7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46−7.40 (m, 2H), 7.38−7.27 (m, 3H), 4.82 (s, 2H), 2.80 (s, 3H), 2.70 (s, 3H). LC-MS (ESI): m/z 350.1 $[M + H]^+$. HPLC purity 99%.

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*7-Amino-2-benzyl-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**2k**).* To a solution of *N*′-hydroxyacetimidamide (52 mg, 0.707 mmol) in THF (10 mL) was added NaH (27 mg, 0.707 mmol, 60% in mineral oil) and the mixture stirred for 15 min at rt and then at 60 °C for a further 30 min. To the mixture was added **1** (0.200 g, 0.589 mmol) and the mixture refluxed overnight. The reaction was cooled to rt and quenched by adding water (10 mL) and the product extracted with EtOAc (2 × 10 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by preparative TLC (30% EtOAc/hexane), followed by recrystallization from a mixture of dichloromethane and hexane to give **2k** (20 mg, 10%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.27 (m, 5H), 4.82 (s, 2H), 2.81 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ 172.17, 169.75, 169.46, 167.45, 142.11, 139.40, 129.42, 126.98, 121.90, 19.83, 11.80. LC-MS (ESI): m/z 350.1 $[M + H]^+$. HPLC purity 98%.

*7-Amino-2-benzyl-N-cyano-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxamide (**2l**).* To a solution of **2b** (0.150 g, 0.482 mmol) in a mixture of DMF (2 mL) and acetonitrile (2 mL), were added EDC (0.110 g, 0.578 mmol), HOBt (0.089 g, 0.58 mmol), and cyanamide (0.020 g, 0.48 mmol). After stirring for 30 min, DiPEA (0.170 mL, 0.964 mmol) was added and the reaction mixture was stirred at rt overnight. The crude reaction mixture was concentrated in vacuo and purified by preparative HPLC to give **2l** (12 mg, 7%). ^{1}H NMR (300 MHz, DMSO) δ 7.37−7.23 (m, 5H), 7.10 (br s, 2H), 4.72 (s, 2H), 2.58 (s, 3H). LC-MS (ESI): m/z 336.1 $[M + H]^+$. HPLC purity 97%.

*7-Amino-2-benzyl-4-methyl-6-(3-(trifluoromethyl)-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**2m**).* To a solution of (*E*)-2,2,2-trifluoro-*N*′-hydroxyacetimidamide (91 mg, 0.707 mmol) in anhydrous dioxane (3 mL) at −10 °C was added sodium hydride (71 mg, 1.77 mmol, 60% in mineral oil). The mixture was stirred for 15 min at rt followed by heating for 30 min at 65 °C. Ethyl 7-amino-2-benzyl-4-methyl-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]-pyridine-6-carboxylate (**1**) (200 mg, 0.589 mmol) was then added in one portion and the mixture stirred overnight at 65 °C. After cooling to rt, the reaction was quenched by adding satd NH_4Cl (1 mL) and stirring for 15 min. The mixture was transferred to a separatory funnel, water (20 mL) added, and the product extracted with EtOAc (2 × 20 mL). After drying over Na_2SO_4, filtration and concentration under reduced pressure the crude product was taken up in dioxane (2 mL) and BEMP on polystyrene (234 mg, 0.514 mmol, labeling 2.2 mol/g) was added. The mixture was heated at 75 °C for 2 h. LC-MS of the reaction indicated that all material had been converted to the desired product. The solution was filtered and the solvent removed in vacuo to yield a yellow solid. Purification by preparative HPLC gave **2m** (10 mg, 17%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 7.44−7.47 (m, 2H), 7.31−7.40 (m, 3H), 4.86 (s, 2H), 2.85 (s, 3H). LC-MS (ESI): m/z 404.1 $[M + H]^+$. HPLC purity 99%.

*Ethyl 7-Amino-4-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**3**).* Trifluoroacetic acid (0.060 g, 0.60 mmol) was added to a mixture of 1*H*-pyrrole-2,5-dione (6.0 g, 6.2 mmol) and ethyl acetamidocyanoacetate (10.6 g, 6.2 mmol) in DCE. The reaction mixture was heated at reflux for 48 h. The solvent was evaporated under reduced pressure, and the crude product was triturated with methanol followed by diethyl ether to afford **3** (5.0 g, 32%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 6.91 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 2.56 (s, 3H), 1.33 (t, J = 7.1 Hz, 3H). LC-MS (ESI): m/z 250.1 $[M + H]^+$. HPLC purity 95%.

*7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo-[3,4-c]pyridine-1,3(2H)-dione (**4**).* To a solution of *N*′-hydroxyacetimidamide (2.85 g, 38.5 mmol) in anhydrous dioxane (160 mL) at −10 °C was added sodium hydride (2.57 g, 64.2 mmol, 60% in mineral oil). The mixture was stirred for 15 min at rt followed by heating for 30 min at 65 °C. The ester **3** (8.00 g, 32.1 mmol) was then added in one portion and the mixture stirred overnight at 65 °C. After cooling to rt, the reaction was quenched by adding satd NH_4Cl (100 mL) and stirring for 15 min. The mixture was transferred to a separatory funnel, water (400 mL) added, and the product was extracted with EtOAc (2 × 400 mL). After drying over $MgSO_4$, filtration, and concentration under reduced pressure, the crude product was purified by flash chromatography (gradient of cyclohexane/EtOAc) to give **4** (2.59 g, 27%). ^{1}H NMR (400 MHz, DMSO) δ 11.50 (s, 1H), 7.21 (s, 2H), 2.62 (s, 3H), 2.50 (s, 3H). LC-MS (ESI): m/z 260.1 $[M + H]^+$. HPLC purity 98%.

General Procedure for the Synthesis of Compounds 5a–5ae. To a solution of **4** (50 mg, 0.19 mmol, 1.1 equiv) in dioxane (4.0 mL) was added BEMP on polystyrene (262 mg, 0.576 mmol, labeling 2.2 mmol/g). The reaction was placed on an Activo-PLS organic synthesizer and shaken for 15 min at rt, and then the appropriate chloride or bromide (0.18 mmol, 1.0 equiv) was added and shaking continued for 24 h at 75 °C. To the mixture was added polymer supported thiophenol (152 mg, 1.1 eq, labeling 1.3 mmol/g) and the mixture stirred for 18 h at 75 °C. After filtration on a VacMaster-20 system, the filtrate was concentrated to give the final product. Compounds that were not sufficiently pure were further purified by flash chromatography (gradient of cyclohexane/EtOAc) or triturated with methanol.

*7-Amino-2-(4-fluorobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5a**).* Yield **5a**: (28 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49−7.37 (m, 2H), 7.08−6.95 (m, 2H), 4.78 (s, 2H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 368.1 $[M + H]^+$. HPLC purity 95%.

*7-Amino-2-(4-chlorobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5b**).* Yield **5b**: (45 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 4.78 (s, 2H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 384.1 $[M + H]^+$. HPLC purity 95%.

*7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(4-(trifluoromethyl)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5c**).* Yield **5c**: (64 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63−7.48 (m, 4H), 4.87 (s, 2H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 418.2 $[M + H]^+$. HPLC purity 95%.

*7-Amino-2-(4-methoxybenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5d**).* Yield **5d**: (47 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 4.75 (s, 2H), 3.78 (s, 3H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 380.1 $[M + H]^+$. HPLC purity 95%.

*7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(4-(trifluoromethoxy)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5e**).* Yield **5e**: (40 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 8.3 Hz, 2H), 4.81 (s, 2H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 434.1 $[M + H]^+$. HPLC purity 95%.

*7-Amino-2-(4-bromobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5f**).* Yield **5f**: (73 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 4.76 (s, 2H), 4.66 (s, 1H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 430.0 $[M + H]^+$. HPLC purity 99%.

*7-Amino-2-(3-fluorobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5g**).* Yield **5g**: (29 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.01 (m, 4H), 4.81 (s, 2H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 368.1 $[M + H]^+$; HPLC purity 95%.

*7-Amino-2-(3-chlorobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5h**).* Yield **5h**: (43 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.35−7.19 (m, 3H), 4.76 (s, 2H), 2.80 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.91, 167.96, 167.24, 167.14, 143.92, 139.15, 137.70, 134.67, 130.88, 130.09, 128.74, 128.36, 126.83, 125.03, 120.21, 41.13, 19.94, 11.74. LC-MS (ESI): m/z 384.0 $[M + H]^+$. HPLC purity 95%.

*7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(3-(trifluoromethyl)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5i**).* Yield **5i**: (26 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.56 (t, J = 6.6 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 4.87 (s, 2H), 2.82 (s, 3H), 2.55 (s, 3H). LC-MS (ESI): m/z 418.1 $[M + H]^+$. HPLC purity 96%.

*7-Amino-2-(3-methoxybenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5j**).* Yield **5j**: (50 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dd, J = 8.6, 7.2 Hz, 2H), 7.01 (d, J = 7.6 Hz, 1H), 6.97 (s, 1H), 6.83 (d, J = 8.3 Hz,

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1H), 4.79 (s, 2H), 3.80 (s, 3H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 380.1 $[M + H]^+$. HPLC purity 95%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(3-(trifluoromethoxy)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5k**). Yield **5k**: (35 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.32 (m, 2H), 7.29 (s, 1H), 7.16 (s, 1H), 4.82 (s, 2H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 434.1 $[M + H]^+$. HPLC purity 95%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(1-phenylethyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5l**). Yield **5l**: (21 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 7.6 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.31−7.26 (m, 1H), 5.54 (q, J = 7.3 Hz, 1H), 2.80 (s, 3H), 2.54 (s, 3H), 1.93 (d, J = 7.3 Hz, 3H). LC-MS (ESI): m/z 364.1 $[M + H]^+$. HPLC purity 99%.

7-Amino-2-(2-fluorobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5m**). Yield **5m**: (57 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.35 (m, 1H), 7.32−7.26 (m, 1H), 7.13−7.06 (m, 2H), 4.90 (s, 2H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 368.3 $[M + H]^+$. HPLC purity 99%.

7-Amino-2-(2-chlorobenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5n**). Yield **5n**: (62 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (dd, J = 14.0, 8.2 Hz, 1H), 7.34−7.21 (m, 3H), 5.00 (s, 2H), 2.85 (s, 3H), 2.57 (s, 3H). LC-MS (ESI): m/z 384.3 $[M + H]^+$. HPLC purity 98%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(2-(trifluoromethyl)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5o**). Yield **5o**: (53 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.22 (d, J = 7.7 Hz, 1H), 5.07 (s, 2H), 2.84 (s, 3H), 2.55 (s, 3H). LC-MS (ESI): m/z 418.3 $[M + H]^+$. HPLC purity 99%.

7-Amino-2-(2-methoxybenzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5p**). Yield **5p**: (46 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.15 (m, 2H), 6.97−6.82 (m, 2H), 4.88 (s, 2H), 3.86 (s, 3H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 380.3 $[M + H]^+$. HPLC purity 99%.

7-Amino-2-(4-(difluoromethoxy)benzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5q**). Yield **5q**: (68 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 6.48 (t, J = 73.7 Hz, 1H), 4.80 (s, 2H), 4.77 (s, 1H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 416.1 $[M + H]^+$. HPLC purity 98%.

7-Amino-2-((5-chloropyridin-2-yl)methyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5r**). Yield **5r**: (52 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J = 2.3 Hz, 1H), 7.66 (dd, J = 8.3, 2.5 Hz, 1H), 7.30 (d, J = 8.3 Hz, 1H), 4.95 (s, 2H), 2.82 (s, 3H), 2.55 (s, 3H). LC-MS (ESI): m/z 385.1 $[M + H]^+$. HPLC purity 98%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(pyridin-4-ylmethyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5s**). Yield **5s**: (34 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 5.3 Hz, 2H), 7.30 (d, J = 5.5 Hz, 2H), 4.82 (s, 2H), 2.82 (s, 3H), 2.55 (s, 3H). LC-MS (ESI): m/z 351.1 $[M + H]^+$. HPLC purity 99%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(4-(methylsulfonyl)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5t**). Yield **5t**: (45 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.1 Hz, 2H), 4.90 (s, 2H), 3.02 (s, 3H), 2.82 (s, 3H), 2.55 (s, 3H). LC-MS (ESI): m/z 428.1 $[M + H]^+$. HPLC purity 95%.

4-((7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1,3-dioxo-1,3-dihydro-2H-pyrrolo[3,4-c]pyridin-2-yl)methyl)benzonitrile (**5u**). Yield **5u**: (60 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 4.85 (s, 2H), 2.82 (s, 3H), 2.55 (s, 3H). LC-MS (ESI): m/z 375.1 $[M + H]^+$. HPLC purity 99%.

4-((7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1,3-dioxo-1,3-dihydro-2H-pyrrolo[3,4-c]pyridin-2-yl)methyl)benzamide (**5v**). Yield **5v**: (17 mg, 23%).^{1}H NMR (300 MHz, DMSO) δ 7.93 (br s, 1H), 7.83 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.29 (br m, 3H), 4.79 (s, 2H), 2.64 (s, 3H), 2.49 (s, 3H). LC-MS (ESI): m/z 393.1 $[M + H]^+$. HPLC purity 96%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(4-((trifluoromethyl)thio)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5w**). Yield **5w**: (73 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 8.1 Hz, 2H), 7.48 (d, J = 8.2 Hz, 2H), 4.84 (s, 2H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 450.3 $[M + H]^+$. HPLC purity 99%.

7-Amino-2-(4-(hydroxymethyl)benzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5x**). Yield **5x**: (51 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.82 (s, 2H), 4.67 (s, 2H), 2.81 (s, 3H), 2.54 (s, 3H), 1.64 (br s, 1H). LC-MS (ESI): m/z 380.3 $[M + H]^+$. HPLC purity 98%.

7-Amino-2-(4-(dimethylamino)benzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5y**). Yield **5y**: (18 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 8.7 Hz, 2H), 6.67 (d, J = 8.7 Hz, 2H), 4.72 (s, 2H), 2.92 (s, 6H), 2.80 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 393.4 $[M + H]^+$. HPLC purity 95%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(4-(2-oxopyrrolidin-1-yl)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5z**). Yield **5z**: (36 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.6 Hz, 2H), 7.44 (d, J = 8.6 Hz, 2H), 4.79 (s, 2H), 3.83 (t, J = 7.0 Hz, 2H), 2.81 (s, 3H), 2.59 (t, J = 8.1 Hz, 2H), 2.54 (s, 3H), 2.22−2.07 (m, 2H). LC-MS (ESI): m/z 433.4 $[M + H]^+$. HPLC purity 99%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(4-(pyridin-4-yl)benzyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5aa**). Yield **5aa**: (43 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68−8.62 (m, 2H), 7.68−7.41 (m, 6H), 4.88 (s, 2H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 427.3 $[M + H]^+$. HPLC purity 97%.

7-Amino-2-(4-(4-fluorophenoxy)benzyl)-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5ab**). Yield **5ab**: (44 mg, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.6 Hz, 2H), 7.10−6.85 (m, 6H), 4.78 (s, 2H), 2.81 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 460.2 $[M + H]^+$. HPLC purity 99%.

2-(4-(1H-Pyrazol-1-yl)benzyl)-7-amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5ac**). Yield **5ac**: (73 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 2.5 Hz, 1H), 7.71 (d, J = 1.5 Hz, 1H), 7.67 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 6.45 (t, J = 2.1 Hz, 1H), 4.84 (s, 2H), 4.74 (s, 1H), 2.82 (s, 3H), 2.54 (s, 3H). LC-MS (ESI): m/z 416.2 $[M + H]^+$. HPLC purity 98%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-(piperidin-4-ylmethyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5ad**). *tert*-Butyl 4-((7-amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1,3-dioxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)methyl)piperidine-1-carboxylate yield (Boc-**5ad**): Yield Boc-**5ad**: (88 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (m, 2H), 3.56 (d, J = 7.1 Hz, 2H), 2.82 (s, 3H), 2.67 (t, J = 11.4 Hz, 2H), 2.54 (s, 3H), 2.02−1.86 (m, 1H), 1.65 (d, J = 12.6 Hz, 2H), 1.48−1.40 (m, 11H). LC-MS (ESI): m/z 457.5 $[M + H]^+$. HPLC purity 99%. To a solution of *tert*-butyl 4-((7-amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-1,3-dioxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)methyl)piperidine-1-carboxylate (Boc-**5ad**) (88 mg, 0.20 mmol) in DCM (2.0 mL) was added trifluoroacetic acid (2 mL) and the mixture stirred for 1 h at rt. The reaction mixture was concentrated and dissolved in DCM (30 mL). The organics were washed with satd $NaHCO_3$ (3 × 20 mL). Filtration and concentration gave **5ad** (54 mg, 75%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (d, J = 7.1 Hz, 2H), 3.11 (d, J = 12.3 Hz, 2H), 2.82 (s, 3H), 2.65−2.50 (m, 5H), 2.01−1.82 (m, 1H), 1.68 (d, J = 12.7 Hz, 2H), 1.26−1.17 (m, 2H). LC-MS (ESI): m/z 357.4 $[M + H]^+$. HPLC purity 98%.

7-Amino-4-methyl-6-(3-methyl-1,2,4-oxadiazol-5-yl)-2-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**5ae**). Yield **5ae**: (65 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (dd, J = 11.4, 3.3 Hz, 2H), 3.57 (d, J = 7.1 Hz, 2H), 3.35 (td, J = 11.7, 1.6 Hz, 2H), 2.82 (s, 3H), 2.55 (s, 3H), 2.12−1.92 (m, 1H), 1.64−1.56 (m, 2H), 1.50−1.32 (m, 2H). LC-MS (ESI): m/z 358.2 $[M + H]^+$. HPLC purity 97%.

Ethyl 7-Amino-4-methyl-1,3-dioxo-2-phenyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**6**). The reaction was performed according to the procedure of Shimada et al. to yield the title compound (36 mg, 7%).[8] The ^{1}H NMR data were in agreement with the reported spectra. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57−7.37 (m, 5H), 4.51 (q, J = 7.1 Hz, 2H), 2.83 (s, 3H), 1.48 (t, J = 7.1 Hz, 3H). LC-MS (ESI): m/z 326.0 $[M + H]^+$. HPLC purity 95%.

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Ethyl 7-Amino-2-benzyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo[3,4-c]-pyridine-6-carboxylate (7). To a cooled suspension of ethyl (*E*)-2-cyano-2-(hydroxyimino)acetate (0.50 g, 3.52 mmol) and acetic anhydride (1.40 g, 14.1 mmol) in formic acid (5 mL) was added zinc dust (0.700 g, 10.7 mmol) in small portions. The suspension was stirred at rt for 4 days. The reaction was diluted with water (10 mL), and the pH was adjusted to 8−9 using a saturated sodium carbonate solution. The product was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (2 × 50 mL) and brine, dried over Na_2SO_4, and evaporated. Crude ethyl 2-cyano-2-formamidoacetate (0.25 g, 46%) was taken to the next step without further purification. LC-MS APCI: calculated for $C_6H_8N_2O_3$ 156.14; observed m/z $[M + H]^+$ 157.4. Trifluoroacetic acid (0.020 g, 0.16 mmol) was added to a mixture of 1-benzyl-1*H*-pyrrole-2,5-dione (0.250 g, 1.60 mmol) and ethyl 2-cyano-2-formamidoacetate (0.360 g, 1.92 mmol) in DCE. The reaction mixture was heated at reflux for 48 h. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (10−20% EtOAc/petroleum ether) to afford **7** (0.23 g, 44%) as a yellow solid. ^1H NMR (400 MHz, DMSO) δ 8.34 (s, 1H), 7.27−7.35 (m, 5H), 7.19 (s, 2H), 4.74 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 168.41, 167.17, 165.89, 142.49, 136.72, 134.90, 129.95, 129.47, 129.01, 127.93, 127.89, 120.06, 61.81, 41.34, 14.49. LC-MS (APCI): m/z 326.2 $[M + H]^+$. HPLC purity 97%

*Methyl 7-Amino-2-benzyl-1-hydroxy-4-methyl-3-oxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**8a**) and Ethyl 7-Amino-2-benzyl-3-hydroxy-4-methyl-1-oxo-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine-6-carboxylate (**8b**).* To a cooled suspension of **1** (1.0 g, 2.94 mmol) in methanol (25 mL) and DCM (15 mL) was added sodium borohydride (0.11 g, 2.94 mmol) in small portions over a period of 30 min and stirred for 1 h at the same temperature. Solvents were evaporated under reduced pressure, and the crude product was purified by preparative HPLC to afford **8a** (0.075 g, 8%) and **8b** (0.11 g, 11%). **8a**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.37−7.45 (m, 5H), 6.91 (s, 2H), 5.62 (s, 1H), 5.10 (d, J = 14.7 Hz, 1H), 4.39 (d, J = 14.8 Hz, 1H), 3.98 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 167.09, 166.58, 159.38, 158.87, 158.18, 142.56, 140.62, 136.83, 128.98, 128.22, 127.93, 127.69, 122.46, 79.82, 52.21, 42.37, 19.91. LC-MS (APCI): m/z 328.2 $[M + H]^+$. HPLC purity 99%. **8b**: ^1H NMR (400 MHz, CD_3OD) δ 8.13−8.06 (m, 5H), 7.39 (s, 2H), 6.57 (s, 1H), 5.67 (d, J = 15.4 Hz, 1H), 5.11−5.17 (m, 3H), 3.21 (s, 3H), 2.13 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 167.46, 167.41, 159.86, 159.49, 159.12, 143.19, 141.39, 138.54, 129.78, 129.31, 129.12, 129.09, 129.04, 128.71, 128.49, 123.33, 80.63, 61.82, 43.19, 20.70, 15.47. LC-MS (APCI): m/z 342.2 $[M + H]^+$. HPLC purity 99%

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01542.

Chemistry, biology assays, in vitro ADMET assays, metabolite identification studies, mouse pharmacokinetic studies with **5h**, references (PDF)

Molecular formula strings (CSV)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

TB, tuberculosis; Mtb, *Mycobacterium tuberculosis*; INH, isoniazid; RIF, rifampicin; MDR, multidrug-resistant; ADME, absorption, distribution, metabolism and excretion; HTS, high throughput screening; HLM, human liver microsomes; RLM, rat liver microsomes; MLM, mouse liver microsomes; EDC, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole hydrate; T3P, propylphosphonic anhydride; TsCl, *p*-toluenesulfonyl chloride; FaSSIF, fasted state simulated intestinal fluid; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PS-BEMP, polymer-supported 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine; PS-PhSH, thiophenol on polystyrene; ADMET, absorption, distribution, metabolism, excretion, and toxicity; SAWC, South Africa Western Cape; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography−mass spectrometry; MIC_{90}, lowest concentration of drug that inhibits growth of more than 90% of the bacterial population; RFLP, restriction fragment length polymorphism; PAMPA, parallel artificial membrane permeability assay; PPB, plasma protein binding; ABT, aminobenzotriazole; CYP cytochrome P450 enzymes; SRM, spontaneous resistant mutants

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