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Synthesis and Biological Evaluation of Polar Functionalities Containing Nitrodihydroimidazooxazoles as Anti-TB Agents

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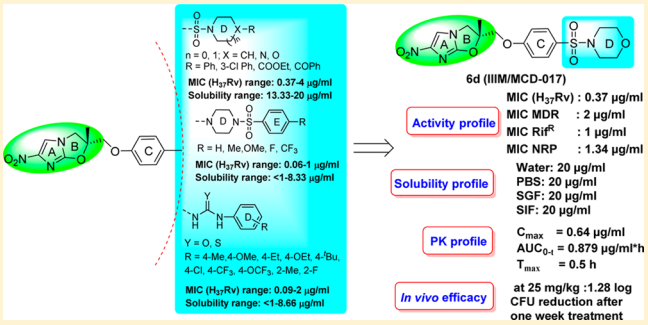
Supporting Information

ABSTRACT: Novel polar functionalities containing 6-nitro-2,3-dihydroimidazooxazole (NHIO) analogues were synthesized to produce a compound with enhanced solubility. Polar functionalities including sulfonyl, uridyl, and thiouridyl-bearing NHIO analogues were synthesized and evaluated against *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$. The aqueous solubility of compounds with MIC values ≤0.5 μg/mL were tested, and six compounds showed enhanced aqueous solubility. The best six compounds were further tested against resistant (Rif^R and MDR) and dormant strains of MTB and tested for cytotoxicity in HepG2 cell line. Based on its overall *in vitro* characteristics and solubility profile, compound **6d** was further shown to possess high microsomal stability, solubility under all tested biological conditions (PBS, SGF and SIF), and favorable oral *in vivo* pharmacokinetics and *in vivo* efficacy.



KEYWORDS: *Mycobacterium tuberculosis, MTB $H_{37}Rv$, multidrug resistant-TB, 6-nitro-2,3-dihydroimidazooxazole, structure−activity relationship*

Tuberculosis is a deadly infectious disease that has infected approximately one-third of the world's population. The current lengthy treatment for TB and the development of resistance toward existing drugs have further complicated the treatment of this disease.[1] New TB drugs that can shorten and simplify current treatment regimes and demonstrate potency against both drug-sensitive and drug-resistant TB are urgently needed.[2] In the past decade, the nitroimidazole skeleton has been of great interest to TB researchers,[3] which led to the identification of one drug, delamanid or OPC-67683[4−6] (a nitrodihydroimidazooxazole analogue approved by the European union for MDR-TB) and two clinical candidates, pretomanid or PA-824[7] and TBA-354[8,9] (nitroimidazooxazine analogues in phase-II/III and phase-I clinical trials, respectively) (Figure 1). Both of the advanced candidates, delamanid and pretomanid, are lipophilic, which may aid their entry through the highly lipophilic cell wall of MTB and lead to their high potency.[10−12] The highly lipophilic nature of these compounds has presented absorption issues during clinical trials, which have been managed by altering the formulation of the compound. To address these absorption issues, several groups have studied the nitroimidazooxazine scaffold (PA-824)[13−19] and synthesized a newer generation analogue, TBA-354, with improved physicochemical properties and *in vivo* efficacy.[8] Despite the comparatively better anti-TB profile of OPC-67683, no efforts were made to improve the physicochemical properties of OPC-67683, and delamanid was approved for MDR-TB with a recommended dose of 100 mg twice daily.[6] The recommendation of a twice-daily dose might limit its introduction in a first-line drug regimen and efforts to improve its physicochemical properties are needed.

We therefore generated novel triazolyl- and isoxazolyl-containing nitrodihydroimidazooxazole (NHIO) analogues, of which the molecule IIIM/MCD-019 demonstrated good *in vitro* and *in vivo* profiles but high lipophilicity and poor aqueous solubility.[20] In this study, we synthesized polar functionalities containing nitrodihydroimidazooxazole (NHIO) analogues to improve the aqueous solubility. The present study also provides further insight into existing structure−activity relationships (SARs) of this class, as well as a new compound with a good activity profile, improved aqueous solubility, and favorable oral PK and *in vivo* efficacy.

Three series of analogues were synthesized with different polar functional moieties (sulfonyl, uridyl, and thiouridyl) (Figure 2). In Series 1, a sulfonyl moiety was placed between ring C and ring D. In Series 2, a sulfonyl moiety was introduced

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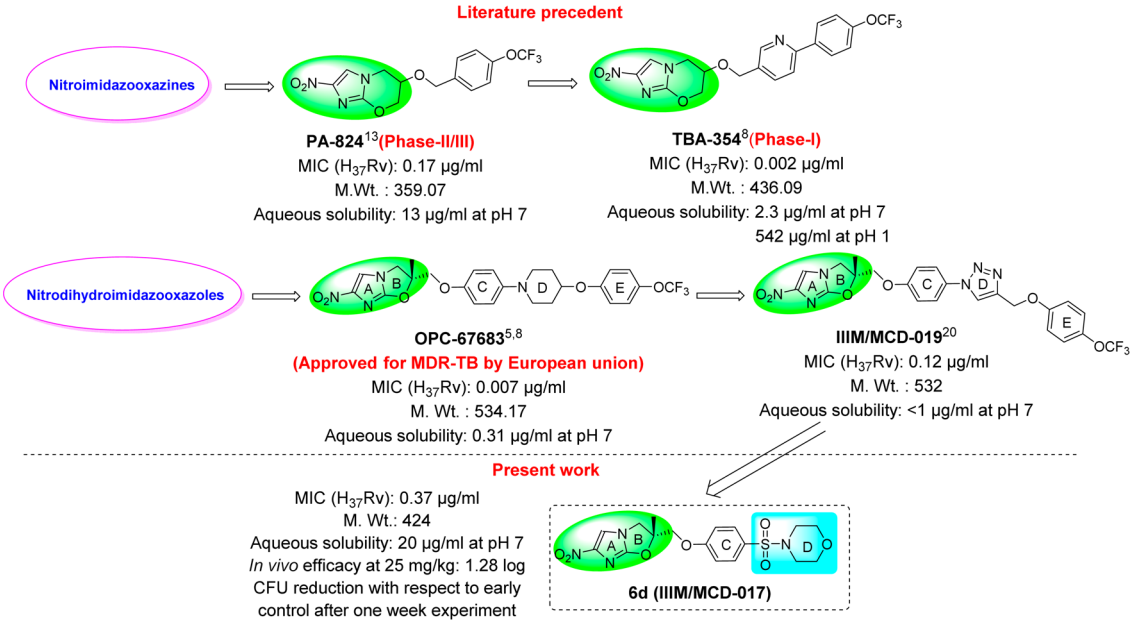


Figure 1. Nitroimidazole-containing anti-TB agents.

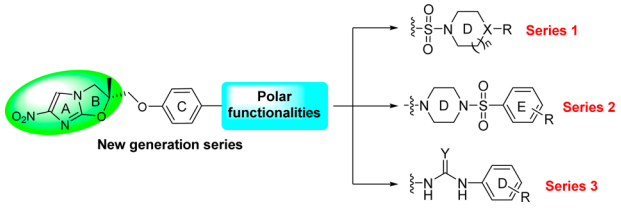


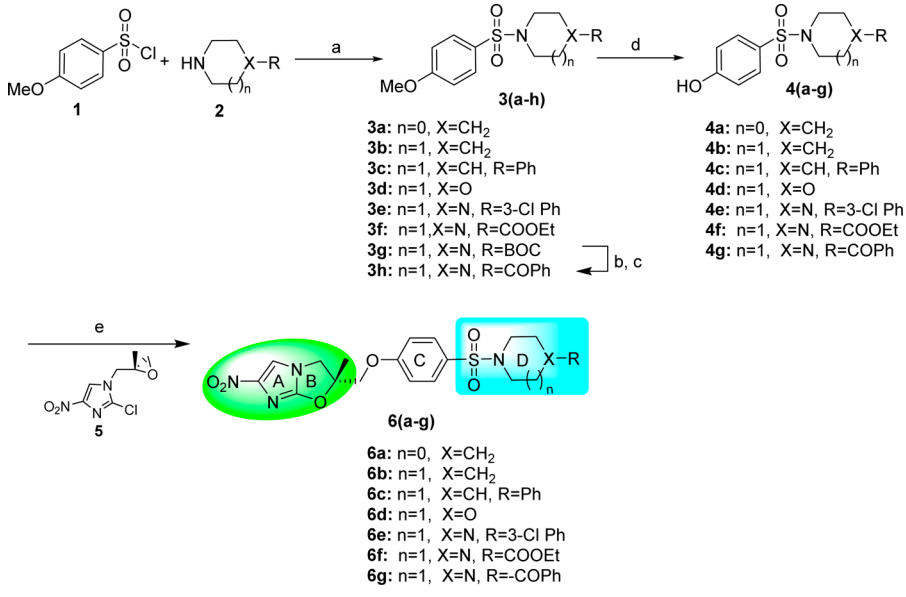
Figure 2. Medicinal chemistry approach.

between ring D and ring E. In Series 3, uridyl/thiouridyl moieties were introduced between rings C and D.

Results and Discussion. *Chemistry.* First, the key phenolic intermediate **4** was synthesized from 4-methoxybenzenesulfonyl chloride **1** in two steps by coupling with secondary amines **2** in the presence of triethylamine and DMAP followed by demethylation using boron tribromide.[21] Then, a key epoxy intermediate **5** was synthesized from 4-nitroimidazole in seven steps, as previously reported.[20] Finally, phenolic intermediate **4**, when treated with epoxide **5** in the presence of sodium hydride, afforded NHIO analogues **6a−g** of Series 1 (Scheme 1).

Next, NHIO analogues **13a−e** of Series 2 were synthesized as illustrated in Scheme 2. The key phenolic intermediate **10** for this series was synthesized in two steps wherein (i) *N*-Boc piperazine **8** coupled with THP protected 4-bromophenol **7** under Buchwald conditions[22] gave product **9** and; (ii) in the second step, THP-deprotection in the presence of pyridinium

Scheme 1. Synthesis of Sulfonyl-Containing NHIO Analogues of Series 1[a]



[a]Reagents and conditions: (a) Et_3N, DMAP, DCM, overnight, rt, 85−95%; (b) TFA, DCM, rt, 1 h; (c) benzoyl chloride, TEA, DMAP, DCM, rt, 3 h, 90%; (d) BBr_3 DCM, 12h, rt, 60−80%; (e) NaH, DMF, 0 to 50 °C, 6 h, 25−35%.

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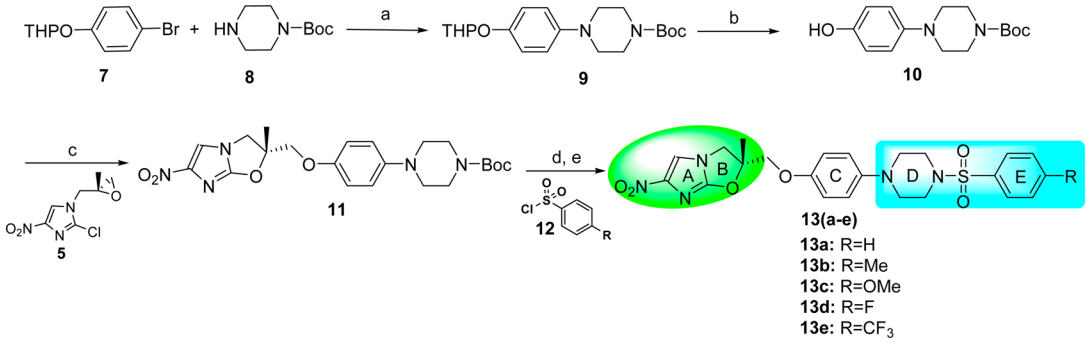
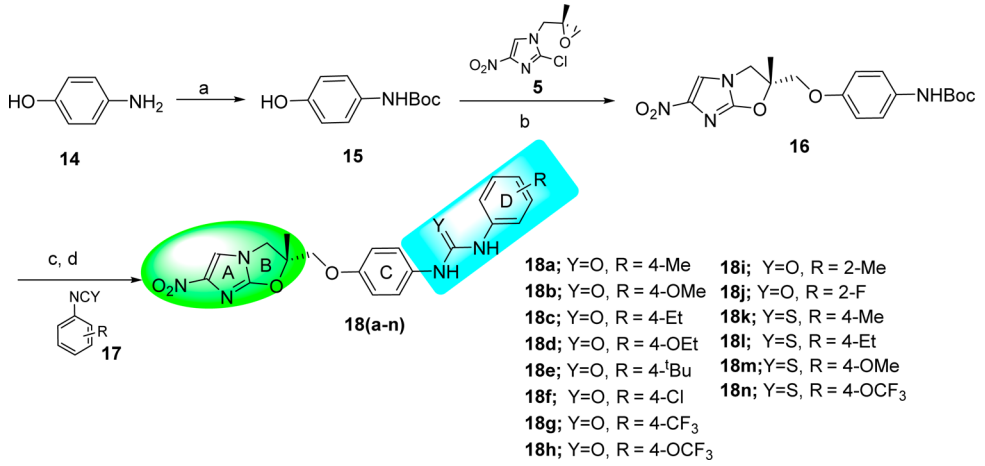
Scheme 2. Synthesis of Sulfonyl-Containing NHIO Analogues of Series 2[a]



[a]Reagents and conditions: (a) $Pd(OAc)_2$, *rac*-BINAP, Cs_2CO_3, toluene, reflux, 1 h, 65%; (b) pyridinium *p*-toluenesulfonate, EtOH, 70 °C, 24 h, 85%; (c) NaH, DMF, 50 °C, 2 h, 32%; (d) TFA, DCM, 1 h; (e) TEA, DMAP, DCM, rt, 12 h, 80−90%.

Scheme 3. Synthesis of Uridyl/Thiouridyl-Containing NHIO Analogues of Series 3[a]



[a]Reagents and conditions: (a) Boc_2O, NaOH, H_2O, rt, 4 h, 95%; (b) NaH, DMF, 0 °C to 50 °C, 12 h, 35%; (c) TFA, DCM, rt, 2 h; (d) TEA, DCM, rt, 5 h, 60−80%.

Table 1. *In Vitro* Activity and Aqueous Solubility of 6a−g, 11, 13a−e, 16, and 18a−n[a]

compd	MIC ($H_{37}R_V$) (μg/mL)	solubility[b] (μg/mL)	ClogP[c]	compd	MIC ($H_{37}R_V$) (μg/mL)	solubility[b] (μg/mL)	ClogP[c]
6a	1		1.43	**18c**	0.5	<1	3.47
6b	0.5	13.33	1.8	**18d**	0.5	6.66	3.49
6c	0.67		3.52	**18e**	0.09	1.0	4.20
6d	0.37	20.0	0.92	**18f**	0.25	6.66	3.44
6e	0.83		3.48	**18g**	0.5	<1	3.92
6f	4		1.62	**18h**	0.67		2.90
6g	4		2.02	**18i**	1		3.28
11	6.67		3.93	**18j**	1.67		3.23
13a	1		2.66	**18k**	0.83		4.10
13b	0.5	<1	3.13	**18l**	1		4.61
13c	0.5	<1	2.72	**18m**	0.5	8.66	3.89
13d	0.06	8.33	3.01	**18n**	0.25	<1	5.06
13e	0.25	<1	3.66	IIIM/MCD-019	0.12	<1	5.25
16	1.34		2.88	Delamanid	0.007	0.31	6.19
18a	0.21	<1	3.26	Rifampicin	0.06		
18b	2		4.08				

[a]Values reported are the average of three individual measurements. [b]Solubility was determined at pH 7. [c]ClogP values were calculated by Schrodinger software.

p-toluenesulfonate furnished key phenolic intermediate **10**. Intermediate **10** was coupled with intermediate **5** in the presence of sodium hydride to give compound **11**, followed by Boc deprotection and subsequent coupling with substituted benzenesulfonyl chlorides **12** in the presence of triethyl amine and DMAP to produce NHIO analogues **13a−e**.

The synthesis of uridyl- and thiouridyl-containing NHIO analogues **18a−n** of Series 3 was performed as illustrated in

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Scheme 3. The key phenolic intermediate **15** was synthesized from 4-aminophenol **14**. Treatment of intermediate **15** with intermediate **5** in the presence of sodium hydride gave the coupled product **16**. Boc-deprotection of compound **16** followed by coupling with substituted phenyl isocyanates or isothiocyanates **17** produced the NHIO analogues **18a−n** of Series 3.

Biological Evaluation. A total of 28 new NHIO analogues, **6a−g**, **11**, **13a−e**, **16**, and **18a−n**, were screened for *in vitro* activity against MTB $H_{37}Rv$ (ATCC27294 strain) using the agar dilution method. The MIC was determined as the minimum concentration of the compound required to inhibit 90% of bacterial growth. The aqueous solubility was determined at pH 7. ClogP values were calculated by Schrodinger software. The MIC, aqueous solubility, and ClogP values of all of the synthesized compounds are summarized in Table 1.

In Series 1, all seven synthesized NHIO analogues with varying cyclic secondary amine rings D attached to the sulfonyl group (**6a−g**) were screened against MTB $H_{37}Rv$. The MIC values of these compounds were between 0.37 and 4 μg/mL. Two NHIO analogues, with piperidyl **6b** and morpholinyl **6d** rings attached to the sulfonyl group, possessed MIC values of 0.5 and 0.37 μg/mL, respectively against MTB $H_{37}Rv$. The presence of a piperazine ring (**6e−g**) led to a decrease in activity.

In Series 2, all five synthesized NHIO analogues with varying substitutions on ring E (**13a−e**) were screened against MTB $H_{37}Rv$. The MIC values were between 0.06 and 1 μg/mL. All the 4-substituted ring E analogues had high MIC values and two compounds 4-F **13d** and 4-CF_3 **13e** showed the best activity, with MIC values of 0.06 and 0.25 μg/mL, respectively.

In Series 3, all 14 synthesized NHIO analogues with varied substitutions on ring D and a uridyl/thiouridyl linker between the C and D rings (**18a−n**) were screened against MTB $H_{37}Rv$ and found to have MIC values between 0.09 and 2 μg/mL. Among all of the tested analogues, uridyl-containing NHIO analogues demonstrated comparatively better activity than thiouridyl-containing NHIO analogues. In uridyl-based NHIO analogues, 4-Me **18a**, 4-*tert*-butyl **18e**, and 4-Cl **18f** substitutions on ring D are favored and demonstrated comparatively better activity. Of the thiouridyl-based NHIO analogues, 4-OCF_3 substituted compound **18n** demonstrated the best activity with a MIC value of 0.25 μg/mL.

Based on the screening results of sulfonyl- and uridyl/thiouridyl-containing NHIO analogues, key structural features essential for anti-TB activity have been identified as follows: (i) the introduction of polar functionalities is acceptable, (ii) the presence of hydrophobic groups/atoms at the terminal rings are essential for activity, even in truncated analogues (compounds **6b** and **6d**), (iii) the presence of a sulfonyl group either between rings C and D or between rings D and E is also acceptable, and (iv) among the uridyl/thiouridyl analogues, uridyl-containing NHIO analogues are more favorable (Figure 3).

Based on their preliminary *in vitro* profiles against MTB $H_{37}Rv$, NHIO analogues with MIC values ≤0.5 μg/mL were selected for an aqueous solubility study. The NHIO analogues **6b**, **6d**, **13d**, **18d**, **18f**, and **18m** demonstrated comparatively better aqueous solubility between 6.66 and 20 μg/mL. These analogues were further assessed for activity against resistant strains (i.e., Rif^R and MDR) and nonreplicating strains of MTB, and one analogue, **6d**, was found to have single digit MIC

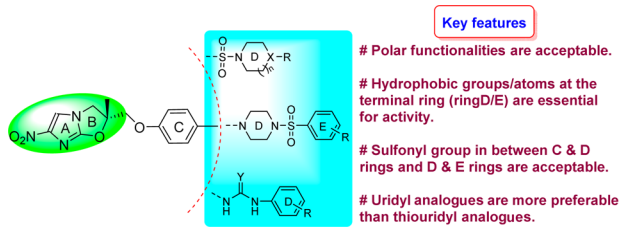

Figure 3. Structural−activity relationship of NHIO analogues and MTB $H_{37}Rv$.

values in all of the tested panels. Cytotoxicity was also evaluated in the HepG2 cell line. None of the tested compounds were toxic in HepG2 cell line, and all of the compounds had acceptable safety indices (Table 2). Among the six selected NHIO analogues, **6d** had a comparatively better profile across the panel of assays performed.

Table 2. *In Vitro* Activity against Nonreplicating and Resistant Strains of MTB[a] and Cytotoxicity

compd	MIC (NRP)[b] (μg/mL)	MIC (Rif^R) (μg/mL)	MIC (MDR) (μg/mL)	CC_{50}[c] (μg/mL)	SI[d]
6b	10.67	3.34	>8	>20	>40
6d	1.34	1	2	>20	>40
13d	>8	1	>8	>20	>333
18d	1	1	>8	>20	>80
18f	4	0.67	>8	>20	>80
18m	>8	2	>8	>20	>80
IIIM/MCD-019	4	0.06	0.12	>20	>166
Delamanid[e]	0.37	0.005	0.02	107.5	10710
Rifampicin	2	256	128		
GATI	1		0.5		

[a]Values reported are the average of three individual measurements. [b]Nonreplicating phase of *M.tb*. [c]Cytotoxicity (concentration causing death of 50% of cells; CC_{50}) to HepG2 cells. [d]Selectivity index: CC_{50}/MIC. [e]Data for the delamanid was reported in ref 8.

The best NHIO analogue, **6d**, was further assessed for solubility, microsomal stability, and *in vivo* oral pharmacokinetics (data shown in Table 3). Compound **6d** demonstrated a promising solubility of 20 μg/mL under all of the tested biological conditions (PBS, SGF, and SIF). In microsomal stability studies, compound **6d** was found to have good stability, and 99.32% of the compound remained after 30 min in rat liver microsomes. Compound **6d** was evaluated by a snapshot *in vivo* oral PK study and was found to have a C_{max} value of 0.64 μg/mL and absorbed quickly into systemic circulation (T_{max} = 0.5 h). This compound also possesses a higher volume of distribution (V_d = 12,970 mL/kg) than both an earlier identified lead (IIIM/MCD-019) and delamanid, moderate exposure with an $AUC_{0\text{-}t}$ of 0.879 μg/mL·h, a 2 h half-life ($t_{1/2}$ = 1.99 h), and moderate clearance (Cl = 75.2 mL/min/kg).

The *in vivo* efficacy of compound **6d** was further evaluated in an intranasal model of acute infection in Balb/C mice (Figure 4). One week post-MTB infection, compound **6d** was orally administered at 25 mg/kg once daily for 5 days. Rifampicin (20 mg/kg) and IIIM/MCD-019 (100 mg/kg) were used as controls. Compound **6d** significantly reduced the bacterial load, demonstrating a 1.28 log CFU reduction compared to the early control (group at the start of treatment) and a 1.61 log CFU

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Table 3. Solubility, Microsomal Stability, and *in Vivo* Pharmacokinetic Studies in Mice

	Solubility (μg/mL)[a]			Microsomal stability[a]	PK parameters[e]					
compd	PBS[b]	SGF[c]	SIF[d]	% remaining after 30 min in RLM	C_{max} (μg/mL)	$AUC_{0\text{-}t}$ (μg/mL·h)	T_{max} (h)	$t_{1/2}$ (h)	V_d (mL/kg)	CI (mL/min/kg)
6d	20	20	20	99.32	0.64	0.879	0.5	1.99	12,970	75.2
IIIM/MCD-019	<1	<1	<1	90.84	0.54	7.428	2.00	4.01	377	0.38

[a]Data were the average of three individual measurements. [b]PBS: phosphate buffer solution (pH 7.4). [c]SGF: simulated gastric fluid (pH 1.2). [d]SIF: simulated intestinal fluid (pH 6.8). [e]P.O. at 5 mg/kg and each value represents average of five determinations. Pharmacokinetic parameters were calculated by WINNONLIN software.

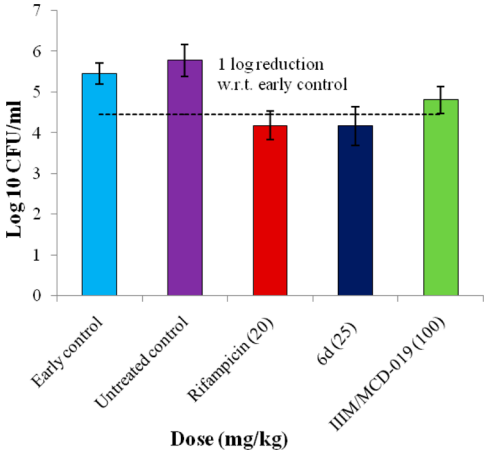

Figure 4. *In vivo* activity of **6d** in intranasal model of acute infection in Balb/c mice. The mice were orally dosed once daily for 5 days ($n = 6$) starting on the day after infection with 10^5 CFU of MTB.

reduction compared to the untreated control (late control group run in parallel without drug treatment). Compound **6d** demonstrated better *in vivo* efficacy than our previously identified lead compound, IIIM/MCD-019. Moreover, all of the mice remained healthy during the course of treatment.

In conclusion, the present study suggests that the introduction of polar functionalities between rings D and C/E is acceptable. Overall, 28 new NHIO-based analogues were synthesized and evaluated against *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$. Potent compounds with MIC values $\leq$0.5 μg/mL were further selected for the determination of aqueous solubility. Based on the MIC values ($H_{37}Rv$) and the aqueous solubility data, six compounds were tested against both resistant (Rif^R and MDR) and dormant strains of MTB, and cytotoxicity was evaluated in the HepG2 cell line. Compound **6d** was found to have a good *in vitro* profile and to exhibit good aqueous solubility. In *in vivo* studies, compound **6d** exhibited a promising PK profile and showed significant log CFU reductions in an intranasal mouse model of acute infection. The present study presents new opportunities for modifications to compounds with poor physicochemical characteristics and provides a novel compound with a simple structure, low molecular weight, and improved aqueous solubility.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00202.

Full experimental details for the synthesized compounds, NMR and MS spectra, and descriptions of biological assays (PDF)

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Author Contributions

#K.R.Y., G.M., and S.Si. contributed equally to this work. K.R.Y. and G.M. performed the chemical synthesis. S.Si. performed *in vitro* and *in vivo* anti-TB experiments. S.Sh. helped with chemical synthesis. S.K. performed cytotoxic experiments. G.K., R.C., P.W., and G.D.S. performed microsomal stability and *in vivo* PK studies. S.S.B. performed the solubility study. P.P.S. and K.R.Y. drafted the manuscript. P.P.S., I.A.K., and R.A.V. participated in the design and execution of this study.

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Notes

The authors declare no competing financial interest.

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