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Identification of Better Pharmacokinetic Benzothiazinone Derivatives as New Antitubercular Agents

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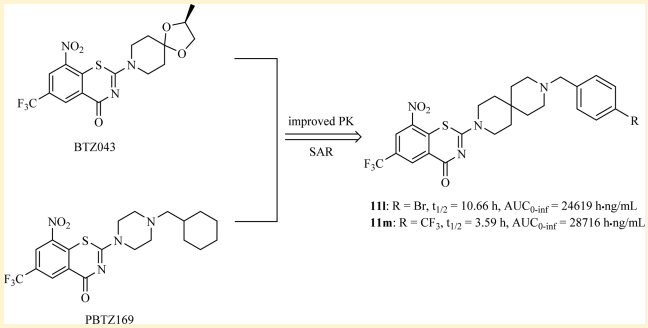
Supporting Information

ABSTRACT: A series of new 8-nitro-6-(trifluoromethyl)-1,3-benzothiazin-4-one(BTZ) derivatives containing a C-2 nitrogen spiro-heterocycle moiety based on the structures of BTZ candidates BTZ043 and PBTZ169 were designed and synthesized as new antitubercular agents. Many of them were found to have excellent *in vitro* activity (MIC < 0.15 μM) against the drug susceptive *Mycobacterium tuberculosis* H37Rv strain and two clinically isolated multidrug-resistant strains. Compounds **11l** and **11m** display acceptable safety, greater aqueous solubility, and better pharmacokinetic profiles than PBTZ169, suggesting their promising potential to be lead compounds for future antitubercular drug discovery.



KEYWORDS: *Antitubercular agents, benzothiazinones, spiro-heterocycles, structure−activity relationships, pharmacokinetics*

Tuberculosis (TB), one of the leading causes of death from an infectious disease ranking above HIV/AIDS, is caused mainly by *Mycobacterium tuberculosis* (MTB).[1] The World Health Organization (WHO) estimated that approximately 10.4 million people were infected and 1.4 million died from TB worldwide in 2015.[2] Recently, the spread of multidrug-resistant (MDR) TB and the emergence of extensively drug-resistant (XDR) TB have revitalized drug discovery efforts in search of new drugs.[3−5] Although Bedaquiline (inhibition of mitochondrial ATP synthase) and Delamanid (inhibition of mycolic acid biosynthesis) were approved for the treatment of MDR-TB, over a huge gap of over 40 years,[6,7] both of them have pronounced issues, including hERG toxicity concerns, as well as multiple ADME issues due to their high lipophilicity.[3] Moreover, there are only three new chemical entities TBA-354 (nitroimidazole), PBTZ169 (benzothiazinone), and Q203 (imidazopyridine)[8] currently in Phase 1 clinical trials. Therefore, there is a significant unmet medical need for safer, more effective TB drugs with novel mechanisms.

Decaprenyl phosphoryl-β-D-ribose 2′-epimerase (DprE1), an essential aspect of MTB survival and a novel mechanism of antitubercular activity, has been identified as a potential target for developing potent and safer anti-TB agents.[9−11] Some new chemical entities (NCE) were found to have excellent activity against MDR/XDR-MTB as covalent or noncovalent inhibitors of the DprE1 enzyme.[12−17] As the most advanced scaffold among these NCEs, nitrobenzothiazinones (BTZs) have garnered great interest recently, and many series of BTZ derivatives were reported.[18−26] The structure−activity relationship (SAR) and mechanistic studies suggest that the $-NO_2$ group at position 8 and the sulfur atom at position 1 are critical for activity, that the $-CF_3$ at position 6 plays an important role in maintaining activity,[18,19,27,28] and that structural modifications at position 2 are the most successful and efficient. For example, BTZ043 (Figure 1), a 2-spiroketal BTZ nearing phase I clinical trials, displays nanomolar bactericidal activity both *in vitro* and *in ex vivo* models of TB.[18,24] PBTZ169(Figure 1), a 2-piperazino BTZ with slightly worse pharmacokinetic (PK) properties relative to BTZ043, has improved *in vivo* potency because of its greater solubility through protonation of the tertiary amino nitrogen on the piperazine.[20] According to the latest report, a Phase I trial of PBTZ169 was completed in Russia, and a second Phase I trial will be undertaken in Switzerland in 2017.[2]

In our previous study, the spiroketal moiety of BTZ043 was replaced by various cyclic ketoximes or the terminal nitrogen on the piperazine ring of BPTZ169 was shifted outside the ring, and some of the resulting compounds were found to have

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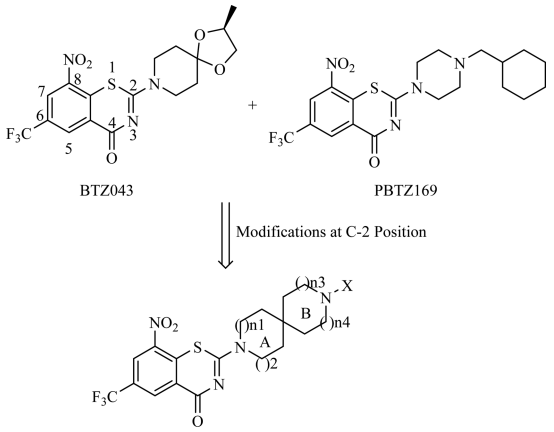


Figure 1. Design of the BTZ derivatives.

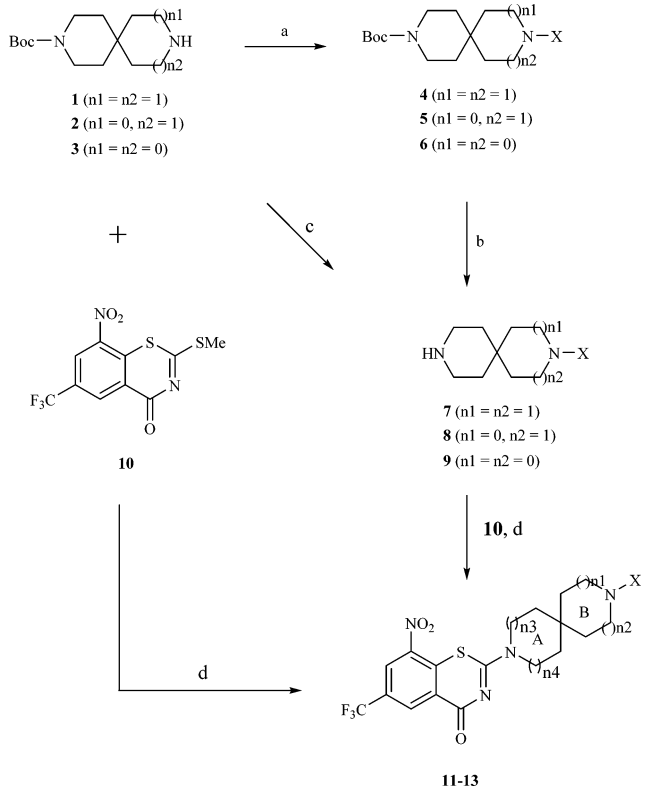
improved activity and aqueous solubility, or acceptable PK profiles.[26] In this study, we intended to integrate the structural features of BTZ043 and PBTZ169, and construct structurally unique compounds, nitrogen spiro-heterocycles as side chains at the 2-position of BTZs (Figure 1). Clearly, these side chains combine the characteristics of both the spiro-cycle of BTZ043 and the alkaline tertiary-amine of BTZ169. The antimycobacterial activity, solubility, toxicity, and PK properties of these target compounds were evaluated, aiming to identify alternative groups at position 2 of BTZs and find optimized potent anti-TB drug candidates with improved drug-like properties through SAR study.

Detailed synthetic pathways to target compounds **11**−**13** are outlined in Scheme 1. Introduction of various R groups to Boc-protected spiro-heterocycles **1**−**3** by reductive amination with aldehydes,[29] nucleophilic substitution with bromides, or amidation with carboxylic acid in the presence of EDC and HOBt[30] gave compounds **4**−**6**, which upon removal of the Boc protecting group afforded the desired side chain compounds **7**−**9** by TFA in DCM in good yields. Additionally, the Boc group of **3** was also reduced by $LiALH_4$ to produce the methide **9**.[31] The target compounds **11**−**13** were conveniently obtained from **10**[20] by nucleophilic substitution with spiro-heterocycle derivatives **7**−**9** or **1**−**3**.

The target compounds **11a**−**i**, **12a**−**d**, and **13a**−**c** bearing different kinds of substituent groups to ensure side chain flexibility and structure diversity were first synthesized (Table 1). They were preliminarily screened for *in vitro* activity against MTB H37Rv ATCC27294 strain, using the Microplate Alamar Blue Assay (MABA).[32] The minimum inhibitory concentration (MIC) is defined as the lowest concentration effecting a reduction in fluorescence of >90% relative to the mean of replicate bacterium-only controls. The MIC values of the compounds along with PBTZ169, isoniazid (INH), and rifampicin (RFP) for comparison were obtained from three independent experiments and presented in μM in Table 1.

The data reveal that with a few exceptions (**11e**−**h**, **12d**, **13c**), all the BTZ derivatives show considerable activity against this strain (MIC < 1 μM). Among them, compounds **12b** and **12c** (MIC = 0.062 and 0.060 μM) are more active than INH (MIC = 0.284 μM) and RFP (MIC = 0.092 μM), and slightly less active than PBTZ169 (MIC < 0.035 μM). The potency of the BTZ derivatives is related to the sizes of both cycles A and B in the spiro-heterocycle moiety, as well as the nature of substituent groups. When ring A is piperidine (**11**−**13**), the contribution of ring B to the activity seems to be as follows:

Scheme 1. Synthesis of Compounds 11−13[a]



[a]Regents and conditions: (a) (i) RCHO, MeOH, AcOH, $NaCNBH_3$, rt−50 °C, 60−85%; or (ii) RBr, MeCN, K_2CO_3, rt, 55−65%; or (iii) RCOOH, EDC, HOBt, Et_3N, DCM, rt, 67%; (b) TFA, DCM, rt, 80−95%; (c) $LiAlH_4$, THF, 60 °C, 65%; (d) MeOH, rt−50 °C, 43−70%.

pyrrolidine > azetidine > piperidine (**11a** vs **12a** vs **13a**; **11b** vs **12b** vs **13b**); whereas, when ring B is piperidine, the contribution of ring A is in this order: piperidine > azetidine > pyrrolidine (**11i** vs **12d** vs **13c**). However, replacement of the cyclohexylmethyl group derived from PBTZ169 with benzyl or acetate moieties leads to increased activity (**11a** vs **11b**−**d**; **12a** vs **12b**−**c**; **13a** vs **13b**). Removal of the cyclohexyl group (**11e**) or replacement of the cyclohexylmethyl with benzoyl (**11h**) abolishes the potency against this strain. Moreover, *N*-acetamide was also found to be much less active than the corresponding *N*-acetate (**11b** vs **11g**; **11d** vs **11f**). Consequently, this set of modifications suggests that the spiro-heterocycle (piperidine as ring A) with *N*-benzylic group might be important for antimycobacterial activity and worthy of further optimizations, considering the probable instability *in vivo* of the *N*-acetate moiety.

Next, the optimizations were focused on R group (Table 2) of the benzene ring. An additional set of *N*-benzylated analogues listed in Table 2 were designed and synthesized. Considering the commercial availability of the spiro-heterocycles, compounds with piperidine as B ring synthesized from the cheapest starting material **1** were selected for the preliminary SAR study. Our results indicate that most of them show potent activity against MTB H37Rv strain (MIC < 0.15 μM) and that the most active compounds, **11m**, **11o** and **12f**, were found to have the same MIC values of <0.035 μM as PBTZ169. Exploration of SAR at the para-position of phenyl group with piperidine as ring B was first conducted by substitution of various functional groups (**11j**−**m**). Compared

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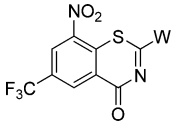
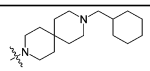
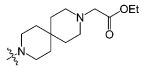
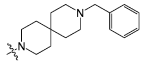
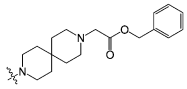
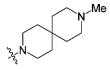
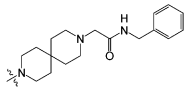
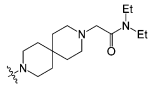
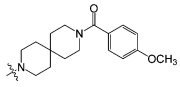
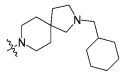
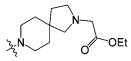
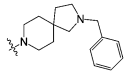
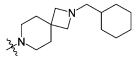
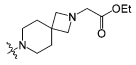
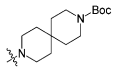
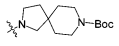
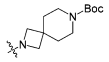
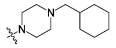
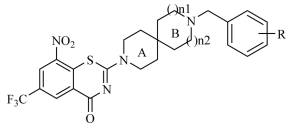
Table 1. Structures and Activities of 11a–i, 12a–d, and 13a–c against MTB H37Rv



Compd.	W	MIC (μM)
11a		0.944
11b		0.542
11c		0.847
11d		0.352
11e		33.54
11f		6.419
11g		6.316
11h		26.610
12a		0.220
12b		0.062
12c		0.060
13a		0.468
13b		0.444
11i		0.219
12d		3.264
13c		1.924
PBTZ169		<0.035
INH		0.284
RFP		0.092

with the lead compound **11c**, introduction of an electron-withdrawing group (F, Br, CF_3) offers more potency (**11k–m**), whereas the presence of an electron-donating one (CH_3O) results in slightly decreased activity (**11j**). Moreover, a *meta*-fluoro atom leads to increased activity (**11k** vs **11n**), and an additional *meta*-fluoro one is even more favorable (**11n** vs **11o**). The SAR applies equally to the analogues with pyrrolidine ring as ring B. For example, introduction of an electron-donating group (CH_3O) is detrimental to the potency (**12c** vs **12e**; **12c** vs **12g**). Conversely, an electron-withdrawing one (F) causes improved activity (**12c** vs **12f**). Additionally, a compound with azetidine ring as ring B (**13c**) was also synthesized to investigate the potential impact of the size of ring B on activity. It is shown that azetidine ≈ pyrrolidine ≫ piperidine (**11j** vs **12e** vs **13d**), suggesting that BTZs with azetidine or pyrrolidine as ring B appears to be more active than piperidine. Inspired by this, more *N*-benzylated analogues with azetidine or pyrrolidine as B ring are now being synthesized in our lab, and the results will be reported in due course.

Table 2. Structures and Activity of *N*-Benzylated Analogues against MTB H37Rv



compd.	n1	n2	R	LogP	MIC (μM)
11c	1	1	H	5.78	0.847
11j	1	1	4-methoxyl	5.91	1.005
11k	1	1	4-fluoro	5.92	0.210
11l	1	1	4-bromo	6.67	0.057
11m	1	1	4-trifluoromethyl	6.76	<0.035
11n	1	1	3-fluoro	5.92	0.054
11o	1	1	3,5-difluoro	6.06	<0.035
11p	1	1	3,5-dimethoxyl	6.05	0.825
12c	0	1	H	5.33	0.059
12e	0	1	4-methoxyl	5.46	0.112
12f	0	1	4-fluoro	5.46	<0.035
12g	0	1	2-methoxyl	5.46	1.676
13d	0	0	4-methoxyl	5.00	0.113
PBTZ 169					<0.035

It was reported there is a strong correlation between MIC and lipophilicity of *N*-alkylpiperazyl-BTZs.[20] The logP values of the *N*-benzylated spiro-heterocycle series were calculated by Chemdraw 15.0 and listed in Table 2. However, no clear correlation between logP and antimycobacterial activity was found in this study.

Encouraged by their strong potency against the drug sensitive MTB H37Rv strain (MIC < 0.15 μM), compounds **11l–o**, **12b-c**, **12e–f**, and **13d** were further evaluated against two clinical isolated MTB-MDR (12525 and14231) strains resistant to both INH and RFP. As shown in Table 3, all of them exhibit excellent activity (MIC < 0.035–0.123 μM) comparable to or slightly less potent than PBTZ169, suggesting their promising potential for both drug-sensitive and resistant MTB strains (Tables 1–3).

Considering acidic gastrointestinal environments, compounds **11l–o**, **12b–c**, **12e–f**, and **13d** were evaluated for their water solubility at pH 2 (0.01 M HCl solution) by using an HPLC-UV method.[33] As expected, the compounds containing a basic nitrogen spiro-heterocycle moiety display good solubility (0.67–2.04 mg/mL). All of them are, with the exception of **11o**, more water-soluble than PBTZ169 (0.90 mg/mL). Compound **12b** with an additional acetate moiety was

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Table 3. Activity, Solubility, Cytotoxicity and Acute Toxicity of Selected Compounds

compd.	MIC (μM) MDR-MTB 12525	MIC (μM) MDR-MTB 14231	water solubility[a] (mg/mL)	CC_{50}[b] Vero cells (μM)	acute toxicity[c]
11l	0.092	0.086	1.17	1677.85	5/5
11m	<0.035	<0.035	1.24	145.35	5/5
11n	<0.035	<0.035	1.13	1398.13	5/5
11o	<0.035	<0.035	0.67	1169.75	5/5
12b	0.080	0.060	2.04	266.34	5/5
12c	<0.035	<0.035	1.56	289.26	3/5
12e	0.056	0.095	1.40	86.20	NT
12f	<0.035	0.034	1.12	810.61	4/5
13d	0.119	0.123	1.20	78.86	NT
PBTZ-169	<0.035	<0.035	0.90	1402.82	NT
INH	>291.7	34.48			
RFP	27.24	>48.60			

[a]The water solubility was tested in 0.01 M HCl solution (approximate pH 2.0). [b]The 50% cytotoxic concentration. [c]No. of animals that survived/total no. of animals; NT, not tested.

found to have the highest solubility (2.04 mg/mL), which is more than two times that of PBTZ169 (Table 3).

Compounds **11l–o**, **12b-c**, **12e-f**, and **13d** were examined for cytotoxicity (CC_{50}) in a mammalian Vero cell line by MTT assay,[34] and the results are reported in Table 3. A comparison demonstrates that only three compounds **11l**, **11n**, and **11o** (CC_{50}: 1169.75–1677 μM) display comparable cytotoxic to PBTZ169 (CC_{50}: 1402.82 μM), and compounds **12e** and **13d** containing a *N*-(4-methoxyl)benzyl group are the most cytotoxic (CC_{50}: 86.20 and 78.86 μM). Moreover, all but one (**11m**) of the derivatives with piperidine as ring B have higher CC_{50} than those with pyrrolidine or azetidine as ring B.

Based on the measured activity levels against all of the tested strains and cytotoxicity, compounds **11l–o**, **12b–c**, and **12f** were further tested for *in vivo* tolerability by recording the number of survivors after a single oral dose in mice of 500 mg/kg, followed by a 7-day observation. As shown in Table 3, all of the compounds with piperidine as ring B (**11l–o**) and compound **12b** with an acetate moiety display the lowest oral acute lethal toxicity, followed by pyrrolidine (ring B)-containing derivatives **12f** and then **12c**.

The *in vivo* PK profiles of compounds **11l–o**, **12b**, and **12f** were evaluated in mice after a single oral administration of 50 mg/kg. As shown in Table 4, all the tested compounds display similar or significantly longer $T_{1/2}$ (3.11–10.66 h) and MRT (4.00–15.9 h) than PBTZ169 (2.87 and 3.73 h). Among the compounds with piperidine as ring B (**11l–o**), compounds **11l** and **11m** with a substituent at the para-position on benzene ring display the best PK profiles, their $T_{1/2}$, C_{max}, $AUC_{0\text{-inf}}$, and MRT are comparable to or significantly longer/higher than PBTZ169, whereas the C_{max} and $AUC_{0\text{-inf}}$ of compounds **11n–o** with a substituent at the meta-position are similar to or significantly lower than PBTZ169. The results suggest that the presence of a substituent at the para-position on benzene ring is more favorable for the PK profiles than the meta-position. In addition, compound **12f** with a *para*-fluoro atom on benzene ring has similar PK profiles to PBTZ169. However, compound **12b** has very poor drug exposures as expected; its C_{max} (17.6 ng/mL) and $AUC_{0\text{-inf}}$ (41.8 ng/mL) are significantly lower than PBTZ169, and the hydrolysis of the acetate moiety in acidic conditions may account for this.

In summary, a series of new BTZ derivatives with a nitrogen spiro-heterocycle moiety at position 2 were designed as new TB agents through combining the structural features of both BTZ043 and PBTZ169. Many of them exhibit excellent *in vitro* inhibitory activity against both drug-sensitive MTB strain H37Rv and drug-resistant clinical isolates (MIC< 0.15 μM). Compounds **11l** and **11m**, with *N*-(4-bromobenzyl)- and *N*-(4-trifuorobenzyl)-3,9-diazaspiro[5,5]undecane moieties, respectively, display acceptable safety, higher aqueous solubility and better PK properties than PBTZ169, and both of them may serve as new and promising lead compounds for further antitubercular drug discovery. Studies to determine the *in vivo* efficacy of **11l** and **11m** are currently underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00106.

Experimental procedures, analytical data, ^{1}H NMR and ^{13}C NMR copies for compounds **11–13**, and HPLC copies of compound **11o** for solubility determination (PDF)

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M.L.L., Y.L., X.F.Y., and H.Y.G. conceived and designed the project. K.L., B.W., Z.W., Y.C., B.W., and A.W. conducted the experiments, K.L. and M.L. analyzed the data and prepared the

Table 4. PK Profiles of 11k–n and 12b, 12e Dosed Orally in Mice at 50 mg/kg (n = 3)[a]

compd.	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-inf}}$ (h·ng/mL)	MRT (h)
11l	10.66 ± 5.62*	1.00 ± 0	1682 ± 307	24619 ± 3478**	15.9 ± 8.66*
11m	3.59 ± 0.64	1.33 ± 0.58	2700 ± 933*	28716 ± 9878*	6.07 ± 0.35**
11n	3.53 ± 1.63	0.67 ± 0.29	901 ± 110	4871 ± 935	4.83 ± 0.66
11o	4.68 ± 0.92*	0.50 ± 0	426 ± 243*	2599 ± 1220	5.80 ± 0.84*
12b	3.46 ± 3.23	0.67 ± 0.29	17.6 ± 5.41**	41.8 ± 33.0**	5.20 ± 4.50
12f	3.11 ± 0.35	1.08 ± 0.88	1234 ± 159	6024 ± 379	4.00 ± 0.85
PBTZ169	2.87 ± 1.03	0.83 ± 0.29	1300 ± 422	5478 ± 1730	3.73 ± 0.94

[a]Note: noncompartmental calculations gave these parameter estimates. *P < 0.05. **P < 0.01, one-tail t test compared with PBTZ169.

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Notes

All animal experiments were carried out in accordance with the guidelines of the Chinese Association for Laboratory Animal Sciences and approved by the institutional ethical committee (IEC) of Peking Union Medical College.

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■ ABBREVIATIONS

ADME, absorption, distribution, metabolism, excretion, toxicity; hERG, human ether-a-go-go related gene; PK, pharmacokinetic; TB, tuberculosis; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; MTB, *Mycobacterium tuberculosis*; MDR, multidrug-resistant; XDR, extensively drug-resistant; WHO, World Health Organization; ATP, adenosine triphosphate; DprE1, decaprenyl phosphoryl-β-D-ribose 2′-epimerase; NCE, new chemical entities; BTZs, nitrobenzothiazinones; SAR, structure−activity relationship; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; THF, tetrahydrofuran; HOBt, hydroxybenzotriazole; TFA, trifluoroacetic acid; DCM, methylene chloride; MIC, minimum inhibitory concentration; MABA, microplate alamar blue assay; INH, isoniazid; RFP, rifampicin; logP, lipophilicity; HPLC, high-performance liquid chromatography; UV, ultraviolet; CC, cytotoxicity; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $T_{1/2}$, half-life; C_{max}, maximum serum concentration; T_{max}, time at which the C_{max} is observed; AUC, area under the curve; MRT, mean residence time

■ REFERENCES

(1) Nusrath Unissa, A.; Hanna, L. E.; Swaminathan, S. A Note on Derivatives of Isoniazid, Rifampicin, and Pyrazinamide Showing Activity Against Resistant Mycobacterium tuberculosis. *Chem. Biol. Drug Des.* **2016**, *87* (4), 537−50.

(2) World Health Organization. Global Tuberculosis Report 2016. http://www.who.int/tb/publications/global_report/en/.

(3) Hoagland, D. T.; Liu, J.; Lee, R. B.; Lee, R. E. New agents for the treatment of drug-resistant Mycobacterium tuberculosis. *Adv. Drug Delivery Rev.* **2016**, *102*, 55−72.

(4) Jones, D. Tuberculosis success. *Nat. Rev. Drug Discovery* **2013**, *12* (3), 175−6.

(5) Schraufnagel, D.; Abubaker, J. Global action against multidrug-resistant tuberculosis. *JAMA, J. Am. Med. Assoc.* **2000**, *283* (1), 54−5.

(6) Cohen, J. Infectious disease. Approval of novel TB drug celebrated–with restraint. *Science* **2013**, *339* (6116), 130.

(7) Ryan, N. J.; Lo, J. H. Delamanid: first global approval. *Drugs* **2014**, *74* (9), 1041−5.

(8) Pethe, K.; Bifani, P.; Jang, J.; Kang, S.; Park, S.; Ahn, S.; Jiricek, J.; Jung, J.; Jeon, H. K.; Cechetto, J.; Christophe, T.; Lee, H.; Kempf, M.; Jackson, M.; Lenaerts, A. J.; Pham, H.; Jones, V.; Seo, M. J.; Kim, Y. M.; Seo, M.; Seo, J. J.; Park, D.; Ko, Y.; Choi, I.; Kim, R.; Kim, S. Y.; Lim, S.; Yim, S. A.; Nam, J.; Kang, H.; Kwon, H.; Oh, C. T.; Cho, Y.; Jang, Y.; Kim, J.; Chua, A.; Tan, B. H.; Nanjundappa, M. B.; Rao, S. P.; Barnes, W. S.; Wintjens, R.; Walker, J. R.; Alonso, S.; Lee, S.; Kim, J.; Oh, S.; Oh, T.; Nehrbass, U.; Han, S. J.; No, Z.; Lee, J.; Brodin, P.; Cho, S. N.; Nam, K.; Kim, J. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat. Med.* **2013**, *19* (9), 1157−60.

(9) Manina, G.; Pasca, M. R.; Buroni, S.; De Rossi, E.; Riccardi, G. Decaprenylphosphoryl-beta-D-ribose 2′-epimerase from Mycobacterium tuberculosis is a magic drug target. *Curr. Med. Chem.* **2010**, *17* (27), 3099−108.

(10) Riccardi, G.; Pasca, M. R.; Chiarelli, L. R.; Manina, G.; Mattevi, A.; Binda, C. The DprE1 enzyme, one of the most vulnerable targets of Mycobacterium tuberculosis. *Appl. Microbiol. Biotechnol.* **2013**, *97* (20), 8841−48.

(11) Brecik, M.; Centarova, I.; Mukherjee, R.; Kolly, G. S.; Huszar, S.; Bobovska, A.; Kilacskova, E.; Mokosova, V.; Svetlikova, Z.; Sarkan, M.; Neres, J.; Kordulakova, J.; Cole, S. T.; Mikusova, K. DprE1 Is a Vulnerable Tuberculosis Drug Target Due to Its Cell Wall Localization. *ACS Chem. Biol.* **2015**, *10* (7), 1631−36.

(12) Crellin, P. K.; Brammananth, R.; Coppel, R. L. Decaprenylphosphoryl-beta-D-Ribose 2 ′-Epimerase, the Target of Benzothiazinones and Dinitrobenzamides, Is an Essential Enzyme in Mycobacterium smegmatis. *PLoS One* **2011**, *6* (2), e16869.

(13) Stanley, S. A.; Grant, S. S.; Kawate, T.; Iwase, N.; Shimizu, M.; Wivagg, C.; Silvis, M.; Kazyanskaya, E.; Aquadro, J.; Golas, A.; Fitzgerald, M.; Dai, H. Q.; Zhang, L. X.; Hung, D. T. Identification of Novel Inhibitors of M. tuberculosis Growth Using Whole Cell Based High-Throughput Screening. *ACS Chem. Biol.* **2012**, *7* (8), 1377−84.

(14) Wang, F.; Sambandan, D.; Halder, R.; Wang, J. N.; Batt, S. M.; Weinrick, B.; Ahmad, I.; Yang, P. Y.; Zhang, Y.; Kim, J.; Hassani, M.; Huszar, S.; Trefzer, C.; Ma, Z. K.; Kaneko, T.; Mdluli, K. E.; Franzblau, S.; Chatterjee, A. K.; Johnson, K.; Mikusova, K.; Besra, G. S.; Futterer, K.; Jacobs, W. R.; Schultz, P. G. Identification of a small molecule with activity against drug-resistant and persistent tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (27), 2510−17.

(15) Naik, M.; Humnabadkar, V.; Tantry, S. J.; Panda, M.; Narayan, A.; Guptha, S.; Panduga, V.; Manjrekar, P.; Jena, L. K.; Koushik, K.; Shanbhag, G.; Jatheendranath, S.; Manjunatha, M. R.; Gorai, G.; Bathula, C.; Rudrapatna, S.; Achar, V.; Sharma, S.; Ambady, A.; Hegde, N.; Mahadevaswamy, J.; Kaur, P.; Sambandamurthy, V. K.; Awasthy, D.; Narayan, C.; Ravishankar, S.; Madhavapeddi, P.; Reddy, J.; Prabhakar, K. R.; Saralaya, R.; Chatterji, M.; Whiteaker, J.; McLaughlin, B.; Chiarelli, L. R.; Riccardi, G.; Pasca, M. R.; Binda, C.; Neres, J.; Dhar, N.; Signorino-Gelo, F.; McKinney, J. D.; Ramachandran, V.; Shandil, R.; Tommasi, R.; Iyer, P. S.; Narayanan, S.; Hosagrahara, V.; Kavanagh, S.; Dinesh, N.; Ghorpade, S. R. 4-Aminoquinolone Piperidine Amides: Noncovalent Inhibitors of DprE1 with Long Residence Time and Potent Antimycobacterial Activity. *J. Med. Chem.* **2014**, *57* (12), 5419−34.

(16) Panda, M.; Ramachandran, S.; Ramachandran, V.; Shirude, P. S.; Humnabadkar, V.; Nagalapur, K.; Sharma, S.; Kaur, P.; Guptha, S.; Narayan, A.; Mahadevaswamy, J.; Ambady, A.; Hegde, N.; Rudrapatna, S. S.; Hosagrahara, V. P.; Sambandamurthy, V. K.; Raichurkar, A. Discovery of Pyrazolopyridones as a Novel Class of Noncovalent DprE1 Inhibitor with Potent Anti-Mycobacterial Activity. *J. Med. Chem.* **2014**, *57* (11), 4761−71.

(17) Shirude, P. S.; Shandil, R.; Sadler, C.; Naik, M.; Hosagrahara, V.; Hameed, S.; Shinde, V.; Bathula, C.; Humnabadkar, V.; Kumar, N.; Reddy, J.; Panduga, V.; Sharma, S.; Ambady, A.; Hegde, N.; Whiteaker, J.; McLaughlin, R. E.; Gardner, H.; Madhavapeddi, P.; Ramachandran, V.; Kaur, P.; Narayan, A.; Guptha, S.; Awasthy, D.; Narayan, C.; Mahadevaswamy, J.; Vishwas, K. G.; Ahuja, V.; Srivastava, A.; Prabhakar, K. R.; Bharath, S.; Kale, R.; Ramaiah, M.; Choudhury, N. R.; Sambandamurthy, V. K.; Solapure, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Azaindoles: Noncovalent DprE1 Inhibitors from Scaffold Morphing Efforts, Kill Mycobacterium tuberculosis and Are Efficacious in Vivo. *J. Med. Chem.* **2013**, *56* (23), 9701−8.

(18) Neres, J.; Pojer, F.; Molteni, E.; Chiarelli, L. R.; Dhar, N.; Boy-Rottger, S.; Buroni, S.; Fullam, E.; Degiacomi, G.; Lucarelli, A. P.; Read, R. J.; Zanoni, G.; Edmondson, D. E.; De Rossi, E.; Pasca, M. R.; McKinney, J. D.; Dyson, P. J.; Riccardi, G.; Mattevi, A.; Cole, S. T.;

DOI: 10.1021/acsmedchemlett.7b00106
ACS Med. Chem. Lett. 2017, 8, 636−641

Binda, C. Structural basis for benzothiazinone-mediated killing of Mycobacterium tuberculosis. *Sci. Transl. Med.* **2012**, *4* (150), 150ra121.
(19) Makarov, V.; Manina, G.; Mikusova, K.; Mollmann, U.; Ryabova, O.; Saint-Joanis, B.; Dhar, N.; Pasca, M. R.; Buroni, S.; Lucarelli, A. P.; Milano, A.; De Rossi, E.; Belanova, M.; Bobovska, A.; Dianiskova, P.; Kordulakova, J.; Sala, C.; Fullam, E.; Schneider, P.; McKinney, J. D.; Brodin, P.; Christophe, T.; Waddell, S.; Butcher, P.; Albrethsen, J.; Rosenkrands, I.; Brosch, R.; Nandi, V.; Bharath, S.; Gaonkar, S.; Shandil, R. K.; Balasubramanian, V.; Balganesh, T.; Tyagi, S.; Grosset, J.; Riccardi, G.; Cole, S. T. Benzothiazinones kill Mycobacterium tuberculosis by blocking arabinan synthesis. *Science* **2009**, *324* (5928), 801−4.
(20) Makarov, V.; Lechartier, B.; Zhang, M.; Neres, J.; van der Sar, A. M.; Raadsen, S. A.; Hartkoorn, R. C.; Ryabova, O. B.; Vocat, A.; Decosterd, L. A.; Widmer, N.; Buclin, T.; Bitter, W.; Andries, K.; Pojer, F.; Dyson, P. J.; Cole, S. T. Towards a new combination therapy for tuberculosis with next generation benzothiazinones. *EMBO Mol. Med.* **2014**, *6* (3), 372−83.
(21) Trefzer, C.; Rengifo-Gonzalez, M.; Hinner, M. J.; Schneider, P.; Makarov, V.; Cole, S. T.; Johnsson, K. Benzothiazinones: Prodrugs That Covalently Modify the Decaprenylphosphoryl-beta-D-ribose 2′-epimerase DprE1 of Mycobacterium tuberculosis. *J. Am. Chem. Soc.* **2010**, *132* (39), 13663−65.
(22) de Jesus Lopes Ribeiro, A. L.; Degiacomi, G.; Ewann, F.; Buroni, S.; Incandela, M. L.; Chiarelli, L. R.; Mori, G.; Kim, J.; Contreras-Dominguez, M.; Park, Y. S.; Han, S. J.; Brodin, P.; Valentini, G.; Rizzi, M.; Riccardi, G.; Pasca, M. R. Analogous Mechanisms of Resistance to Benzothiazinones and Dinitrobenzamides in Mycobacterium smegmatis. *PLoS One* **2011**, *6* (11), e26675.
(23) Karoli, T.; Becker, B.; Zuegg, J.; Mollmann, U.; Ramu, S.; Huang, J. X.; Cooper, M. A. Identification of Antitubercular Benzothiazinone Compounds by Ligand-Based Design. *J. Med. Chem.* **2012**, *55* (17), 7940−7944.
(24) Pasca, M. R.; Degiacomi, G.; Ribeiro, A. L.; Zara, F.; De Mori, P.; Heym, B.; Mirrione, M.; Brerra, R.; Pagani, L.; Pucillo, L.; Troupioti, P.; Makarov, V.; Cole, S. T.; Riccardi, G. Clinical isolates of Mycobacterium tuberculosis in four European hospitals are uniformly susceptible to benzothiazinones. *Antimicrob. Agents Chemother.* **2010**, *54* (4), 1616−8.
(25) Batt, S. M.; Jabeen, T.; Bhowruth, V.; Quill, L.; Lund, P. A.; Eggeling, L.; Alderwick, L. J.; Futterer, K.; Besra, G. S. Structural basis of inhibition of Mycobacterium tuberculosis DprE1 by benzothiazinone inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (28), 11354−59.
(26) Zhang, R.; Lv, K.; Wang, B.; Li, L. H.; Wang, B.; Liu, M. L.; Guo, H. Y.; Wang, A. P.; Lu, Y. Design, synthesis and antitubercular evaluation of benzothiazinones containing an oximido or amino nitrogen heterocycle moiety. *RSC Adv.* **2017**, *7* (3), 1480−83.
(27) Kloss, F.; Krchnak, V.; Krchnakova, A.; Schieferdecker, S.; Dreisbach, J.; Krone, V.; Mollmann, U.; Hoelscher, M.; Miller, M. J. In Vivo Dearomatization of the Potent Antituberculosis Agent BTZ043 via Meisenheimer Complex Formation. *Angew. Chem., Int. Ed.* **2017**, *56* (8), 2187−91.
(28) Tiwari, R.; Miller, P. A.; Chiarelli, L. R.; Mori, G.; Sarkan, M.; Centarova, I.; Cho, S. H.; Mikusova, K.; Franzblau, S. G.; Oliver, A. G.; Miller, M. J. Design, Syntheses, and Anti-TB Activity of 1,3-Benzothiazinone Azide and Click Chemistry Products Inspired by BTZ043. *ACS Med. Chem. Lett.* **2016**, *7* (3), 266−70.
(29) Baxter, E. W. Reductive aminations of carbonyl compounds with borohydride and borane reducing agents. *Organic Reactions* **2001**, *59*, 1−714.
(30) Mahmoud, K. A.; Long, Y. T.; Schatte, G.; Kraatz, H. B. Rearrangement of the active ester intermediate during HOBt/EDC amide coupling. *Eur. J. Inorg. Chem.* **2005**, *1*, 173−180.
(31) Corruble, A.; Davoust, D.; Desjardins, S.; Fressigne, C.; Giessner-Prettre, C.; Harrison-Marchand, A.; Houte, H.; Lasne, M. C.; Maddaluno, J.; Oulyadi, H.; Valnot, J. Y. A NMR and theoretical study of the aggregates between alkyllithium and chiral lithium amides: control of the topology through a single asymmetric center. *J. Am. Chem. Soc.* **2002**, *124* (51), 15267−79.
(32) Lu, Y.; Zheng, M.; Wang, B.; Fu, L.; Zhao, W.; Li, P.; Xu, J.; Zhu, H.; Jin, H.; Yin, D.; Huang, H.; Upton, A. M.; Ma, Z. Clofazimine analogs with efficacy against experimental tuberculosis and reduced potential for accumulation. *Antimicrob. Agents Chemother.* **2011**, *55* (11), 5185−93.
(33) Sun, L. Q.; Zhu, L.; Qian, K.; Qin, B.; Huang, L.; Chen, C. H.; Lee, K. H.; Xie, L. Design, synthesis, and preclinical evaluations of novel 4-substituted 1,5-diarylanilines as potent HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTI) drug candidates. *J. Med. Chem.* **2012**, *55* (16), 7219−29.
(34) Chai, Y.; Wan, Z. L.; Wang, B.; Guo, H. Y.; Liu, M. L. Synthesis and in vitro antibacterial activity of 7-(4-alkoxyimino-3-amino-3-methylpiperidin-1-yl)fluoroquinolone derivatives. *Eur. J. Med. Chem.* **2009**, *44* (10), 4063−9.

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