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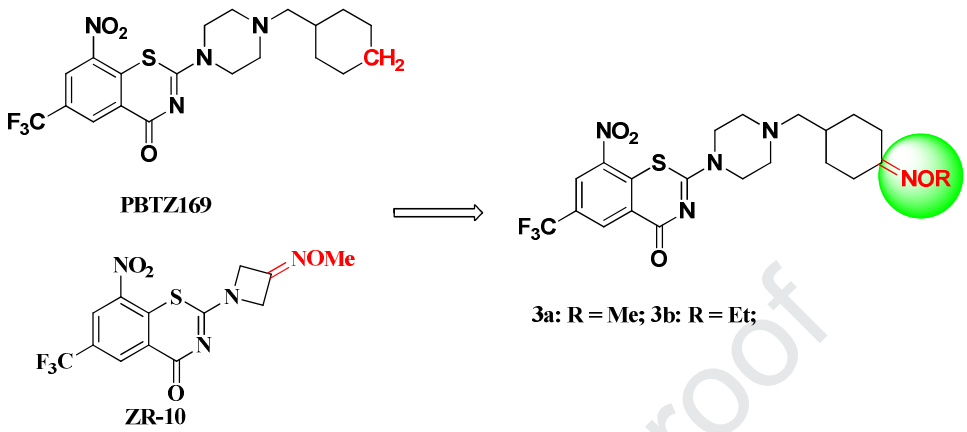
Graphic Abstract



Identification of Benzothiazinones Containing an Oxime Functional Moiety as New Anti-tuberculosis Agents

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ABSTRACT: A series of benzothiazinones (BTZs) containing an oxime moiety, based on the structure of ZR-10 discovered in our lab, were designed and synthesized. Most of the compounds with alkoxyimino groups attached to the piperazine or cyclohexyl ring of PBTZ169, exhibit excellent *in vitro* activity against both drug-sensitive and clinically isolated multidrug-resistant *Mycobacterium tuberculosis* (MTB) strains (MIC: < 0.016 -0.037 μg/mL) and low cell cytotoxicity. Two close PBTZ169-analogues **3a** and **3b** with proper ADME/T and PK properties show potent *in vivo* efficacy in an acute mouse model of tuberculosis. Compound **3a** is under evaluation as a potential clinical candidate for treatment of tuberculosis.

KEYWORDS*:* Benzothiazinones; Oximes; Antimycobacterial activity; Synthesis

1.Introduction

Tuberculosis (TB) affects one-third of the world's population, with approximately 10.0 million new TB cases and 1.6 million TB-related deaths estimated by the World Health Organization (WHO) in 2017 [1]. This disease is mainly caused by *Mycobacterium tuberculosis* (MTB), currently treated with a standard therapy combination of four drugs, during a six months course that does not favour patient compliance. Recently, resistance to this therapy has risen, creating the new labels multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, which pose a serious global health threat [2-5]. Although bedaquiline and delamanid were approved for the treatment of MDR-TB, some adverse events have been noted [6]. Hence, the development of more effective and safer anti-TB agents with novel mechanisms cannot be overemphasized [7].

As a novel class of anti-TB agents targeting decaprenyl phosphoryl-β-D-ribose 2′-epimerase (DprE1), benzothiazinones (BTZs), have garnered great interest recently. Two candidates BTZ043 and PBTZ169 (Figure 1), are in Phase I [8] and II [9] clinical trials at present, respectively, for the treatment of both drug-susceptible TB and MDR-TB. Structure-activity relationship (SAR) studies of BTZs demonstrate that the crucial moieties for potent activity are the S atom and carbonyl group on the thiazinone ring, a strong electron-withdrawing one (CF_3, CN, etc.) at C-6 position, and more importantly the NO_2 group at C-8 position [10, 11]. Many series of new BTZs were reported to have potent antimycobacterial activity [12-23]. Some BTZs containing oxime-functionalized N-heterocycles exampled by ZR-10 (Fig. 1), were identified as new anti-TB agents in our lab [14], highlighting the importance of oximes with respect to antimycobacterial activity of these compounds. Further, the oximino group is frequently employed in the most popular antibiotics - third /fouth generation cephalosporins and some quinolones (gemifloxacin, zabofloxacin). Thus, we intended to design and synthesize a series of novel BTZ derivatives, wherein oximes are attached to nitrogen heterocycles through a methylene or an ethylene, or the

cyclohexyl ring of PBTZ169 is replaced with a symmetric cyclic ketoxime moiety in this study (Fig. 1). Our primary objective was to find new BTZ derivatives with potent antimycobacterial activity and facilitate the further development of these compounds.

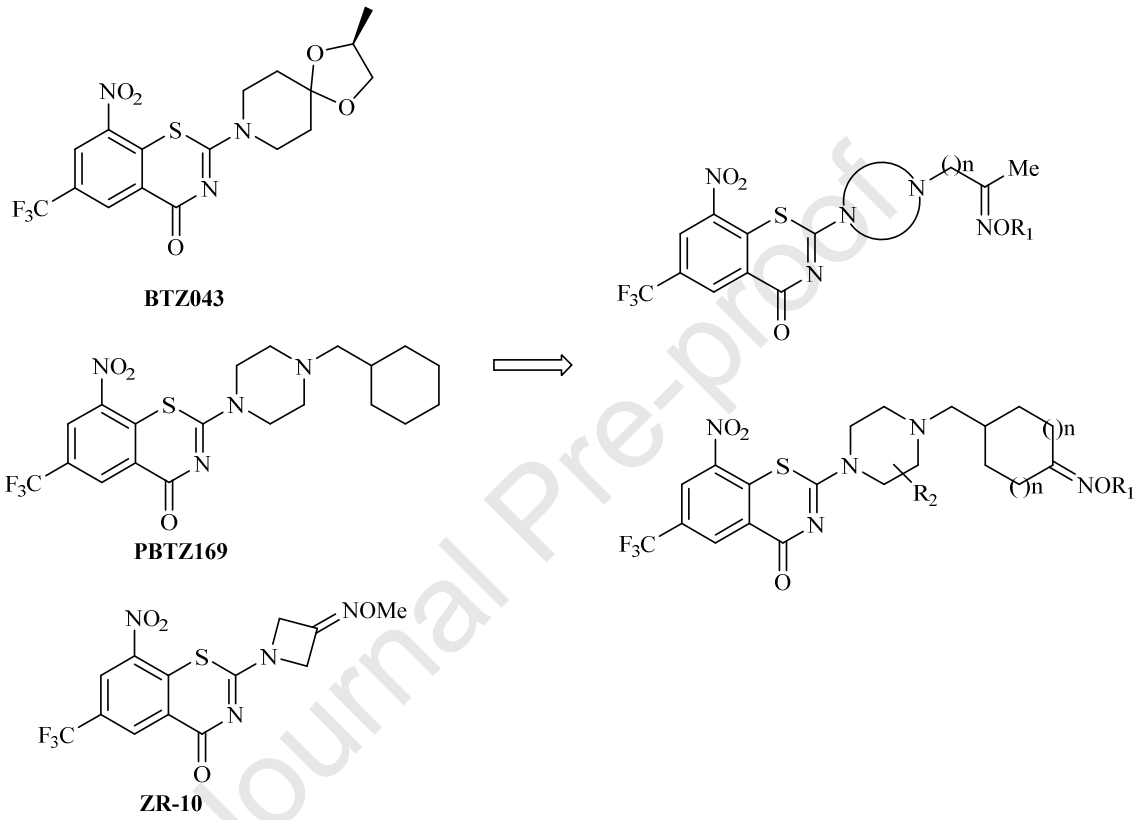


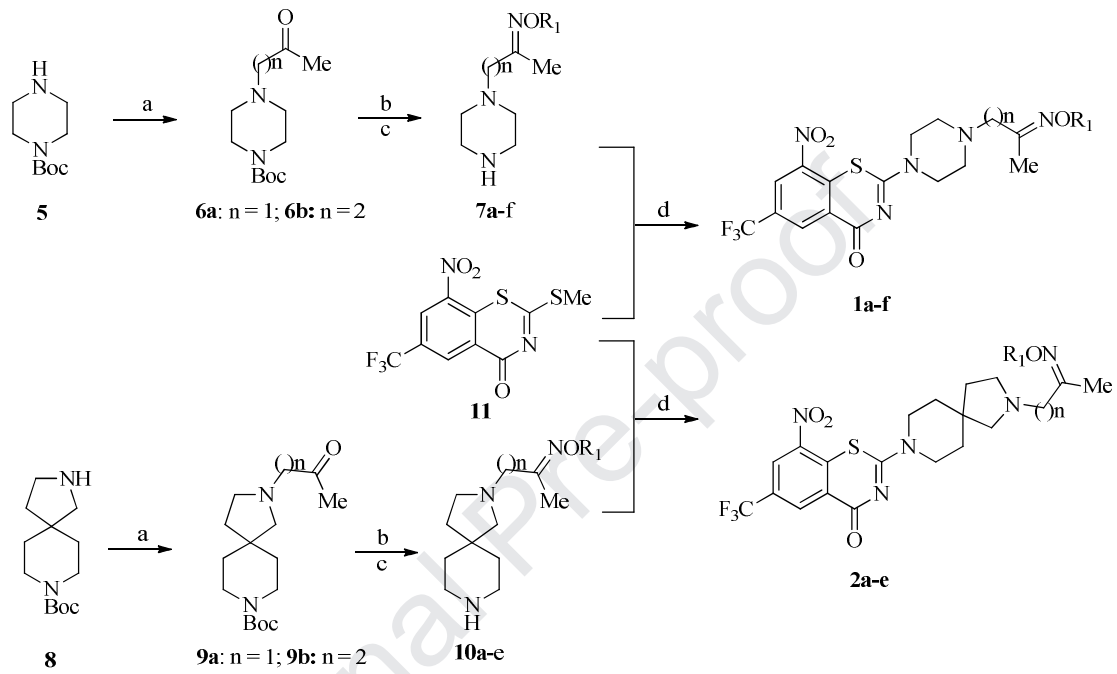
Figure 1. Design of new BTZ derivatives

2. Results and discussion

2.1. Chemistry

The syntheses of target compounds **1-2** and **3-4** are shown in **Schemes 1** and **2**, respectively. Nucleophilic substitution of *N*-Boc-piperazine **5** with 1-bromopropan-2-one and Michael addition with but-3-en-2-one gave ketones **6a** and **6b**, respectively. Oximation of the ketones **6a** and **6b** with *O*-methyl/ ethyl/ benzylhydroxylamine, and then removal of the Boc protecting group with

trifluoracetic acid (TFA) yielded side chain compounds **7a-f**. Coupling of BTZ core compound **11** with **7a-f** in the presence of Et_3N (TEA) afforded the target compounds **1a-f**. Compounds **2a-e** were conveniently obtained from **8** in a similar manner as compounds **1a-f** (**Scheme 1**).

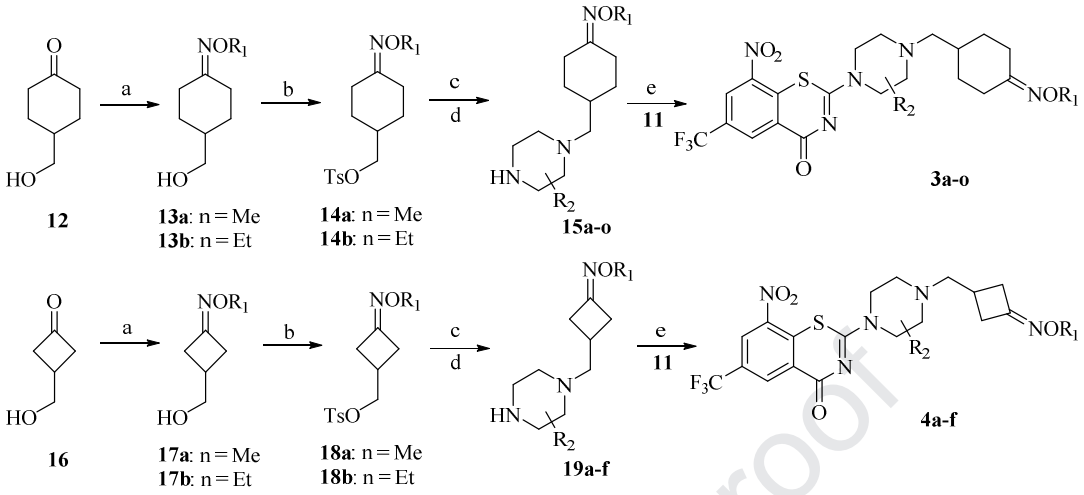


Reagents and conditions: a) 1-bromopropan-2-one or but-3-en-2-one, K_2CO_3, CH_3CN, rt, 3-4h, 52% - 61%; b) R_1ONH_2, Pyridine, MeOH, rt, 5-7h; c) TFA, DCM, rt, 3-4 h, 48%-72%, two steps; d) Et_3N, MeOH, 40℃, 1-3 h, 27%-45%.

Scheme 1. Synthesis of compounds **1a-f** and **2a-e**

Oximation of 4-(hydroxymethyl)cyclohexanone **12** with *O*-methylhydroxylamine ($MeONH_2$) or *O*- ethylhydroxylamine ($EtONH_2$) gave oximes **13a** and **13b**, respectively. Tosylation of the alcohols **13a** and **13b** with *p*-toluenesulfonyl chloride (TsCl) in the presence of pyridine yielded **14a** and **14b**, respectively. Nucleophilic substitution of the esters **14a** and **14b** with *N*-Boc-piperazine derivatives, and then removal of the Boc protecting group with TFA afforded side chain compounds **15a-o**. Coupling of **11** with **15a-o** in the presence of Et_3N afforded the target compounds **3a-o**. Compounds **4a-f** were furnished as a similar procedure for compounds **3a-o**

using 3-(hydroxymethyl)cyclobutanone **16** as the starting material (**Scheme 2**).



Reagents and conditions: a) MeOH, $MeONH_2$ or $EtONH_2$, Pyridine, rt, 5-7h; b) Pyridine, TsCl, rt, overnight, 56%-64%; c) N-Boc-piperazine derivatives, K_2CO_3, KI, 18-crown-6, CH_3CN, 80℃, overnight; d) TFA, DCM, rt, 3-4h, 35%-42%, two steps; e) Et_3N, MeOH, 40℃, 1-3 h, 25%-41%.

Scheme 2. Synthesis of compounds **3a-o** and **4a-f**

2.2. In-vitro anti-MTB activity

The target compounds **1-4** were initially screened for *in vitro* activity against MTB H37Rv ATCC 27294 strain using the Microplate Alamar Blue Assay (MABA) [24]. 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 isoniazid (INH), rifampicin (RFP) and PBTZ169 for comparison are presented in Table 1.

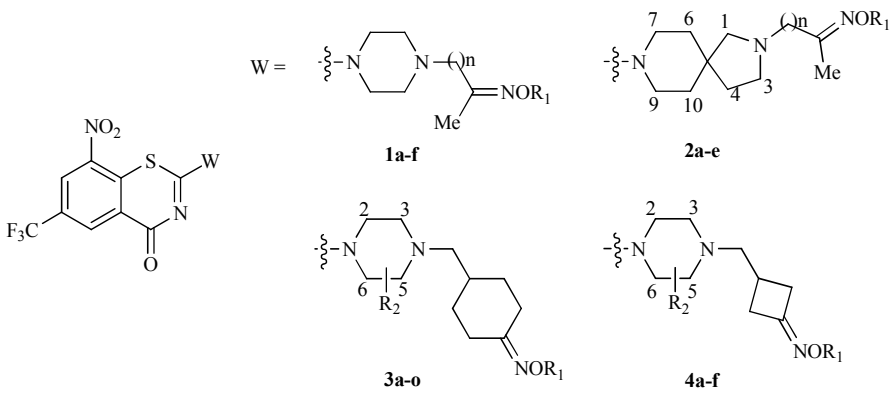
The data reveal that with a few exceptions (**2c-e**), all the BTZ derivatives show potent in vitro activity against this strain (MIC: < 0.1 μg/mL). 19 compounds were found to have the same excellent potency (MIC: <0.016 μg/mL) as PBTZ169, while being more active than RFP and INH (MIC: 0.031-0.078 μg/mL). Overall, the potency of the derivatives is related to the whole structures of the side chains. Moreover, exception for **1f**, **2e** and **3l**, all of them have lower ClogP values (3.25 –

4.96) than PBTZ169 (5.10), indicating that they should have slightly less lipophilicity than PBTZ169. However, no clear correlation between ClogP and antimycobacterial activity was found in this study.

We are pleased to find that the first designed series **1a-f** exhibit roughly equal MIC values of <0.016 – 0.037 μg/mL to PBTZ169, suggesting that oxime-functionalities on the terminal nitrogen of the piperazine ring are well acceptable, and the carbon chain length (n) and the size of the alkyl group (R_1) on the oxime moiety have little effects on antimycobacterial activity. However, replacement of the piperazine ring with 2,8-diazaspiro[4.5]decanes leads to decreased activity (**1** *vs* **2**), probably due to the rigid structures of the spiro-heterocycles.

In further modifications, oximes were directly introduced on the *para*-position of the cyclohexane ring of PBTZ169 (CLogP: 5.10), the corresponding compounds **3a** (CLogP: 3.70) and **3b** (CLogP: 4.23) with less lipophilicity remain excellent potency (MIC: <0.016 μg/mL). The effect of R_2 group (s) on the piperazine ring of **3a, b** was also investigated. All of the methylated derivatives **3c-o** show potent activity (MIC: <0.016 – 0.031 μg/mL) comparable to **3a, b**, indicating that the position, number and chirality of the methyl group(s) have hardly influence on antimycobacterial activity. Yet surprisingly, exception for **4b** (**3b** *vs* **4b**), replacement of the cyclohexanone-oximes with symmetric cyclobutanone-oximes leads to decreased activity (**3a** *vs* **4a**, **3n** *vs* **4c**, **3o** *vs* **4d**, **3k** *vs* **4e**, **3l** *vs* **4f**), maybe due to the ring tension of the cyclobutanone-oximes.

Table 1. Structures and activity of compounds **1-4** against MTB H37Rv



Cpds.[a]	R_1	n / R_2	ClogP[b]	MIC (μg/mL)	Cpds.[a]	R_1	n / R_2	ClogP[b]	MIC (μg/mL)
1a	Me	1	3.25	0.037	**3h**	Et	(R)-2-Me	4.75	<0.016
1b	Et	1	3.78	<0.016	**3i**	Me	(S)-2-Me	4.22	<0.016
1c	Bn	1	4.96	<0.016	**3j**	Et	(S)-2-Me	4.75	<0.016
1d	Me	2	3.42	<0.016	**3k**	Me	3,5-di-Me	4.74	<0.016
1e	Et	2	3.95	<0.016	**3l**	Et	3,5-di-Me	5.27	<0.016
1f	Bn	2	5.15	<0.016	**3m**	Me	2,5-di-Me	4.74	0.031
2a	Me	1	3.53	0.085	**3n**	Me	3-Me	4.22	<0.016
2b	Et	1	4.06	0.029	**3o**	Et	3-Me	4.75	<0.016
2c	Me	2	3.70	0.469	**4a**	Me	H	2.98	0.023
2d	Et	2	4.23	0.241	**4b**	Et	H	3.51	<0.016
2e	Bn	2	5.43	0.119	**4c**	Me	3-Me	3.50	0.056
3a	Me	H	3.70	<0.016	**4d**	Et	3-Me	4.02	0.052
3b	Et	H	4.23	<0.016	**4e**	Me	3,5-di-Me	4.01	0.066
3c	Me	(S)-3-Me	4.22	<0.016	**4f**	Et	3,5-di-Me	4.54	0.056
3d	Et	(S)-3-Me	4.75	<0.016	RFP				0.078
3e	Me	(R)-3-Me	4.22	<0.016	INH				0.031
3f	Et	(R)-3-Me	4.75	<0.016	PBTZ169			5.10	<0.016
3g	Me	(R)-2-Me	4.22	0.023					

[a] Cpds are Compounds; [b] ClogP is caculated in ChemOffice 2017.

Encouraged by their potent activity (MIC: < 0.016 μg/mL) against drug sensitive MTB H37Rv strain, 19 compounds were evaluated against two clinical isolated MTB-MDR strains (16995 and 16833) resistant to both INH and RFP. As shown in Table 2, all of them exhibit comparable activity (MIC: < 0.016 - 0.017 μg/mL) to PBTZ169, suggesting their promising potential for both drug-sensitive and resistant MTB strains (Tables 1 and 2).

2.3. Cell cytotoxicity

The above 19 compounds were tested for mammalian cell cytotoxicity using Vero cells measured as a concentration inhibiting 50% growth (CC_{50}) as compared to a no-treatment control, and the results are reported in Table 2. Exception for **3l**, all the BTZ derivatives show low cytotoxicity (CC_{50}: >64 μg/mL).

Table 2. Anti-MDR-MTB activity and cytotoxicity of selected compounds

Cpds.	MIC (μg/mL)		Vero cells CC_{50}[b] (μg/mL)	Compd.	MIC (μg/mL)		Vero cells CC_{50}[b] (μg/mL)
	MDR-1[a]	MDR-2[a]			MDR-1[a]	MDR-2[a]	
1b	0.010	<0.016	>64	**3h**	<0.016	<0.016	>64
1c	0.010	<0.016	>64	**3i**	<0.016	<0.016	>64
1d	<0.016	0.017	>64	**3j**	<0.016	<0.016	>64
1e	<0.016	<0.016	>64	**3k**	<0.016	<0.016	>64
1f	<0.016	<0.016	>64	**3l**	<0.016	<0.016	60.45
3a	<0.016	<0.016	>64	**3n**	<0.016	<0.016	>64
3b	<0.016	<0.016	>64	**3o**	<0.016	<0.016	>64
3c	<0.016	<0.016	>64	**4b**	<0.016	<0.016	>64
3d	<0.016	<0.016	>64	PBTZ169	<0.016	<0.016	>64
3e	<0.016	<0.016	>64	RFP	>40	>40	NT
3f	<0.016	<0.016	>64	INH	>40	>40	NT

[a] MDR-1 is MDR-MTB 16995 and MDR-2 is MDR-MTB 16833, which were isolated from patients in Beijing Chest Hospital; NT, not tested. [b] The 50% cytotoxic concentration. NT: not tested.

2.4. In vivo pharmacokinetic profiles

Among of the above compounds with potent activity against all the tested strains and low cytotoxicity, two closest PBTZ169-analogues **3a** and **3b** were further evaluated for their *in vivo* pharmacokinetic (PK) profiles in ICR mice after a single oral administration of 25 mg/kg. Although shorter $T_{1/2}$ (1.65 - 2.29 h), As shown in

Table 3, **3a** and **3b** display excellent drug exposures with C_{max} (8757 - 15221 ng·mL^{-1}) and $AUC_{0-\infty}$ (27527 - 40601 h·ng·mL^{-1}) which are significantly higher than PBTZ169.

Table 3. *In vivo* PK profiles of compounds **3a** and **3b**

Cpds.	**3a**	**3b**	PBTZ169
$T_{1/2}$ (h)	1.65±0.9	2.29±1.04	2.78±1.85
T_{max} (h)	0.25±0	0.25±0	0.25±0
C_{max} (ng·mL^{-1})	8573±2232	15221±2009	912±156
$AUC_{0-\infty}$ (h·ng·mL^{-1})	27527±3581	40601±6976	1657±711
MRT (h)	2.46±0.5	2.30±0.18	3.17±1.42

2.5. In vivo efficacy

The preliminary *in vivo* efficacy evaluation of compounds **3a** and **3b** along with PBTZ169 and INH was conducted in a murine model of acute infection with MTB H37Rv strain. All of them were given orally at the same dose of 25 mg/kg, once daily for 5 days a week for a total of 3 weeks in BALB/c mice, as described in published protocols [24]. As shown in Table 4, compounds **3a** and **3b** appear highly active after 3 weeks of treatment, which results in a significant reduction of MTB colony-forming unit (CFU) in lungs by 4.04 and 4.44 Logs, respectively, as compared to the untreated control group. To our satisfaction, both of **3a** and **3b** demonstrate slightly stronger *in vivo* efficacy compared to PBTZ169, although poorer than INH (25 mg/kg). Compared to the previous compounds [12-23], the identification of **3a** and **3b** with potent *in vivo* efficacy was a progress in the modification of BTZs derivatives.

Table 4. *In vivo* efficacy of **3a** and **3b** against MTB H37Rv in a BALB/c mouse model

Cpds.	Log_{10} CFU/lung
Untreated	6.44±0.34

3a	2.40±0.20
3b	2.00±0.07
PBTZ169	2.65±0.54
INH	1.76±0.49

2.6. Further Appraisal

Based on the potent *in vitro* activity and *in vivo* efficacy described above, additional studies were also conducted focusing on compounds **3a** and **3b**. As presented in Table 5, **3a** and **3b** are not active against MTB H37Rv strain under LORA condition (MIC > 32 μg/mL), although they show slightly higher inhibition ratios (86% and 79%, respectively) than PBTZ169 (78%) at the same concentration of 32 μg/mL. Compounds **3a** and **3b** (IC_{50}: 7.80 μM and >10μM, respectively) are less active than PBTZ169 (IC_{50}: 3.77 μM) against cytochrome P450 enzyme 2C19, and they do not, like PBTZ169, inhibit other four enzymes (1A2, 2D6, 3A4, 2C9) with IC_{50}s > 10 μM, suggesting a very low potential for drug-drug interactions. Low inhibition of hERG (IC_{50} > 30 μM) of **3a** and **3b** suggest a low risk of blocking the cardiac potassium channel and causing QT prolongation. Compounds **3a** and **3b**, like PBTZ169, have not mutate effects on TA100 and TA102 strains, in the presence or absence of metabolic activation (S9 fraction), in the salmonella typhimurium reverse mutation assay (mini-Ames). A preliminary *in vivo* tolerability study was carried out in SD rats with a single dose at 2 g/kg. All animals survived after oral administration followed by a 7-day observation.

Table 5. Additional Comparative Data for **3a** and **3b**

Cpds.		**3a**	**3b**	**PBTZ169**
LORA[a]	% Inh[b]	86	79	78
	MIC (μg/mL)	>32	>32	>32
CYP inhibition	1A2	>10	>10	>10
IC_{50} (μM)	2D6	>10	>10	>10

	3A4	>10	>10	>10
	2C9	>10	>10	>10
	2C19	7.80	>10	3.77
hERG IC_{50} (μM)		47.98±5.51	38.92±12.78	101.7±24.09
Mutagenic effect (Ames test)		No	No	No
Acute Toxicity Study[c]		10/10[d]	10/10[d]	10/10[d]

[a]Low-oxygen-recovery assay. [b]The inhibition ratio at 32 μg/mL. [c]Single oral dose in SD rats. [d]No. of animals that survived/total no. of animals after 7 days.

The PK profiles of compound **3a** was also further studied in SD rats, following a single intravenous dose (2.5 mg/kg) and oral multi-dose administrations (25, 50, 200 mg/kg, n = 6, bisexual each half). As shown in Table 6, **3a** shows excellent oral PK profiles, and its major parameters (C_{max}, $AUC_{0-\infty}$, F%) are significantly greater than PBTZ169 at the same doses. Thus far, all the above results support compound **3a** to be worth of further investigation.

Table 6. *In vivo* PK profiles of compound **3a**

Cpds.	route	Dose (mg/kg)	$T_{1/2}$ (h)	Tmax (h)	C_{max} ($ng \cdot mL^{-1}$)	$AUC_{0-\infty}$ ($h \cdot ng \cdot mL^{-1}$)	F (%)
3a	IV	2.5	1.77±0.43	-	2591±1322	5721±1402	
	PO	25	3.20±0.71	1.25±0.61	6227±2122	26779±12301	46.1±19.4
		50	4.13±1.27	1.67±0.52	10085±4509	45909±15262	40.4±12.5
		200	6.73±1.41	2.83±1.33	18247±4848	128143±66556	30.3±14.0
PBTZ169	IV	2.5	1.64±0.48	-	1445±234	1543±376	
	PO	25	4.23±2.58	0.91±0.64	463±258	1604±942	10.7±6.63
		50	4.86±0.74	1.33±0.75	694±104	3117±842	10.2±2.99
		200	5.76±2.03	2.50±1.76	804±442	4725±967	3.82±0.73

3. Conclusion

In summary, we designed and synthesized a series of novel BTZ derivatives containing an oxime functional moiety as new anti-TB agents based on the structure feature of ZR-10 discovered in our lab. With a few exceptions (**2c-e**), all of them exhibit considerable activity against MTB H37Rv strain (MIC: < 0.1 μg/mL). All of those compounds (**1a-f**, **3a-o**), wherein oximes are attached to the piperazine or cyclohexyl ring of PBTZ169, show comparable activity to PBTZ169 against MTB H37Rv and MDR-MTB strains (MIC: < 0.016 – 0.037 μg/mL). Two close PBTZ169-analogues **3a** and **3b** with proper ADME/T and PK properties were identified to have potent *in vivo* efficacy in an acute mouse model of tuberculosis which is a breakthrough respect to the previous benzothiazinones [12-23]. Compound **3a** with significantly greater major parameters than PBTZ169 at the same doses after oral multi-dose administrations (25, 50, 200 mg/kg), has been selected as a potential clinical development candidate for treatment of TB. Further evaluation including combination study and deep understanding of druggability of compound **3a** are ongoing to fully assess its potential as a new agent to treat MDR-TB.

Experimental protocols

4.1. Chemistry

All commercially available solvents and reagents were obtained from commercial suppliers and used without further purification. Melting points were determined in open capillaries and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury-600/500/400 spectrometer in DMSO-d6 or $CDCl_3$ using tetra-methylsilane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectra and high-resolution mass spectra (HRMS) were obtained on an MDSSCIEX Q-Tap mass spectrometer. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F254).

4.2. Synthesis

4.2.1. General synthesis procedure of compounds 6a-b and 9a-b.

To a stirring solution of tert-butyl piperazine-1-carboxylate **5** (3 mmol) or tert-butyl 2,8-diazaspiro[4.5]decane-8-carboxylate **8** or in CH_3CN (50 mL) was added

1-bromopropan-2-one or but-3-en-2-one (4 mmol) and K_2CO_3 (5 mmol) at room temperature. The mixture was stirred for 3-4 hours at room temperature and filtered. The filtrate was diluted by H_2O (250 mL) and extracted by DCM (100 mL × 3). The combined organic layer was washed by brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified over silica gel column (DCM: MeOH = 40: 1) to yield oils compounds **6a-b** or **9a-b** (yield, 52%-61%).

4.2.1.1. tert-butyl 4-(2-oxopropyl)piperazine-1-carboxylate 6a

Yellow oil; yield: 61%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 3.37 (t, *J* = 5.1 Hz, 4H, H-2, H-6), 3.11 (s, 2H, -CH_2C(O)-), 2.33 (t, *J* = 5.1 Hz, 4H, H-3, H-5), 2.05 (s, 3H, Me), 1.35 (s, 9H, 1-NBoc).

4.2.1.2. tert-butyl 4-(3-oxobutyl)piperazine-1-carboxylate 6b

Yellow oil; yield: 57%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 3.38 (brs, 4H, H-2, H-6), 2.64 (d, *J* = 13.3 Hz, 4H, -CH_2-CH_2-C(O)-), 2.37 (brs, 4H, H-3, H-5), 2.14 (s, 3H, Me), 1.42 (s, 9H, 1-NBoc).

4.2.2. General synthesis procedure of compounds for 1a-f and 2a-e

To a stirred solution of **6a-b** or **9a-b** (0.5 mmol) in MeOH (15 mL) was added pyridine (2 mL) and $RONH_2$·HCl (0.6 mmol) at room temperature. The mixture was stirred for 5-7 hours and then concentrated. The residue was dissolved with DCM and washed by brine. The organic phase was added TFA (3 mL) at room temperature. The mixture was stirred for 3-4 hours and concentrated to afford the crude products **7a-f** or **10a-e** which was used directly in the next step without further purification. To a stirred solution of above crude products **7a-f** or **10a-e** in anhydrous MeOH (10 mL) was added BTZ core compound **11** (0.3 mmol) and Et_3N (0.6 mmol) at room temperature. The mixture was stirred overnight at 40 ^{o}C and concentrated. The residue was purified by silica gel column (DCM: MeOH = 20: 1) to yield the yellow oils or yellow solids, which were further treated by n-hexane to give compounds **1a-f** and **2a-e** (yield, 27%-45%).

4.2.2.1. 2-(4-(2-(methoxyimino)propyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl) -4H-benzo[e][1,3]thiazin-4-one (1a)

Yellow solid; yield: 43%; mp: 105-107 ^{o}C; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.17-4.06 (m, 4H, piperazine(H-2, H-6)), 3.87 (s, 3H, CH_3, -NOMe), 3.06 (s, 2H, CH_2, -*CH_2*C(NOMe)), 2.60 (brs, 4H, piperazine (H-3, H-5)), 1.91 (s, 3H, Me, -C(NOMe)*CH_3*). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.8, 162.6, 144.2, 134.1, 133.8 (q, *J* = 3.3 Hz), 130.0, 127.0, 126.4 (q, *J* = 3.3 Hz), 122.7 (q, *J* = 271.4 Hz), 52.8, 52.4, 46.5, 30.5, 29.6, 27.6, 8.9. MS-ESI (m/z): 446.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{17}H_{19}F_3N_5O_4S$ $(M+H)^+$: 446.1104; Found: 446.1108.

4.2.2.2.
2-(4-(2-(ethoxyimino)propyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**1b**)

Yellow solid; yield, 40%; mp: 112-114 °C; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.11(q, *J* = 7.5 Hz, 2H, -$NOCH_2$-), 4.18-4.06 (m, 4H, piperazine (H-2, H-6)), 3.06 (s, 2H, CH_2, -*CH_2*C(NOMe)), 2.59 (t, *J* = 5.0 Hz, 4H, piperazine (H-3, H-5)), 1.92 (s, 3H, Me, -C(NOMe)*CH_3*), 1.25 (t, *J* = 7.5 Hz, 3H, Me, -$NOCH_2$*CH_3*). ^{13}C NMR (150 Mz, $CDCl_3$) δ 166.6, 162.3, 154.0, 144.1, 133.5 (q, *J* = 3.6 Hz), 130.0 (q, *J* = 35.4 Hz), 127.0, 126.1 (q, *J* = 3.6 Hz), 122.5 (q, *J* = 273.3 Hz), 69.4, 62.0, 52.6, 14.8, 13.3.MS-ESI (m/z): 460.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{18}H_{21}F_3N_5O_4S$ $(M+H)^+$: 460.1261; Found: 460.1269.

4.2.2.3.
2-(4-(2-((benzyloxy)imino)propyl)piperazin-1-yl)-8-nitro-6-(trifluorome-thyl)-4H-benzo[e][1,3]thiazin-4-one (**1c**)

Yellow solid; yield: 27%; mp: 101-103 °C; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 7.35-7.26 (m, 5H, -$NOCH_2$*Ph*), 5.11 (s, 2H, -NO*CH_2*Ph), 4.10-3.88 (m, 4H, piperazine(H-2, H-6)), 3.06 (brs, 2H, CH_2, -*CH_2*C(NOMe)), 2.54 (brs, 4H, piperazine (H-3, H-5)), 1.95 (s, 3H, Me, -C(NOMe)*CH_3*). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.3, 155.0, 144.0, 138.1, 134.1, 133.5 (q, *J* = 3.6 Hz), 129.9 (q, *J* = 35.5 Hz), 128.5, 128.1, 127.9, 126.9, 126.2 (q, *J* = 3.6 Hz), 122.5 (q, *J* = 271.6 Hz), 75.8, 61.8, 52.5, 29.8, 13.6. MS-ESI (m/z): 522.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{23}F_3N_5O_4S$ $(M+H)^+$: 522.1417; Found: 522.1423.

4.2.2.4.
2-(4-(3-(methoxyimino)butyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**1d**)

Yellow solid; yield: 45%; mp: 131-133 °C; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.00-3.81 (m, 4H, piperazine(H-2, H-6)), 3.82 (s, 1.74H, OMe), 3.81 (s, 1.08H, OMe), 2.63-2.52 (m, 7H, piperazine(H-3, H-5), -*CH_2CHHC*(NOMe)), 2.39 (t, *J* = 7.25 Hz, 1H, -*CH*HC(NOMe)), 1.89 (s, 1.22 H, Me), 1.85 (s, 1.74 H, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.5, 162.2, 156.1, 155.6, 144.0, 134.2, 133.5 (q, *J* = 3.4 Hz), 123.0 (q, *J* = 35.0 Hz), 126.8, 126.2 (q, *J* = 3.4 Hz), 122.5 (q, *J* = 271.8 Hz), 61.4, 54.5, 53.7, 52.5,

33.4, 26.9, 20.3, 14.2. MS-ESI (m/z): 460.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{18}H_{21}F_3N_5O_4S$ $(M+H)^+$: 460.1261; Found: 460.1271.

4.2.2.5. 2-(4-(3-(ethoxyimino)butyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**1e**)

Yellow solid; yield: 45%; mp: 115-117 ^oC; ^1H NMR (500 Mz, $CDCl_3$): δ 9.07 (s, 1H, benzothiazinone H-7), 8.74 (s, 1H, benzothiazinone H-5), 4.12-3.89 (m, 6H, piperazine(H-2, H-6), -$NOCH_2$-), 2.54-2.63 (m, 7H, piperazine(H-3, H-5), -*CH_2*C*H*-*HC*(NOMe)), 2.40-2.37 (m,1H, -*CH*HC(NOMe)), 1.85 (s, 3H, Me), 1.23 (t, *J* = 7.0 Hz, 3H, Me, -$NOCH_2$*CH_3*). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.5, 162.2, 155.2, 144.0, 134.1, 133.5 (q, *J* = 3.4 Hz), 129.7 (q, *J* = 35.0 Hz) 126.8, 126.1 (q, *J* = 3.4 Hz), 122.5 (q, *J* = 271.8 Hz), 69.0, 54.5, 53.7, 52.4, 33.5, 26.9, 20.3, 14.8, 14.3.; MS-ESI (m/z): 474.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{23}F_3N_5O_4S$ $(M+H)^+$: 474.1417; Found: 474.1423.

4.2.2.6. 2-(4-(3-((benzyloxy)imino)butyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**1f**)

Yellow solid; yield: 37%; mp: 110-112 ^oC; ^1H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 7.36-7.26 (m, 5H, -$NOCH_2$*Ph*), 5.07 (s, 1.16H, -C*H*H*Ph*), 5.06 (s, 0.79H, -CH*HPh*), 4.08-3.77 (m, 4H, piperazine(H-2, H-6)), 2.65-2.56 (m, 7H, piperazine(H-3,H-5), -*CH_2*C*H*-*HC*(NOBn)), 2.42-2.39 (m,1H, -*CH*HC(NOBn)), 1.91 (s, 1.63H, Me), 1.90 (s, 1.51H, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.2, 156.6, 156.2, 144.0, 138.5, 138.2, 134.2, 133.5 (q, *J* = 3.4 Hz), 129.5 (q, *J* = 35.5Hz), 128.5, 128.4, 128.3, 127.9, 127.8, 126.8, 126.2 (q, *J* = 3.4 Hz), 122.4 (q, *J* = 271.8 Hz), 75.5, 54.1, 53.7, 52.4, 46.2, 33.4, 29.8, 27.0, 20.3, 14.6, 8.7. MS-ESI (m/z): 536.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{25}F_3N_5O_4S$ $(M+H)^+$: 536.1574; Found: 536.1578.

4.2.2.7. 2-(2-(2-(methoxyimino)propyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (2a)

Yellow solid; yield: 35%; mp: 113-115 ^oC; ^1H NMR (500 Mz, $CDCl_3$) δ 9.10 (d, *J* = 1.0 Hz, 1H, benzothiazinone H-7), 8.75 (d, *J* = 1.0 Hz, 1H, benzothiazinone H-5), 3.99-3.81 (m, 7H, diazaspiro[4.5]decan (H-7, H-9), NOMe), 3.14 (s, 2H, -*CH_2* C(NOMe)), 2.68 (brs, 2H, diazaspiro[4.5]decan (H-1)), 2.48 (brs, 2H, diazaspiro[4.5]decan (H-3)), 1.90 (s, 3H, Me), 1.79 (brs, 6H, diazaspiro[4.5]decan

(H-4, H-6, H-10)); ^{13}C NMR (125 Mz, $CDCl_3$) δ166.5, 161.7, 144.0, 134.2, 133.4, 128.8 (q, *J* = 34.5 Hz), 126.7, 126.0, 122.4 (q, *J* = 272.4 Hz), 61.5, 59.4, 58.5, 53.2, 44.7, 40.9, 37.1, 36.2, 18.4, 12.9; MS-ESI (m/z): 500.1 $(M+H)^+$.

4.2.2.8. 2-(2-(2-(ethoxyimino)propyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (2b)

Yellow solid; yield: 27%; mp: 109-111 ^{o}C; ^{1}H NMR (500 Mz, $CDCl_3$) δ 9.15 (s, 1H, benzothiazinone H-7), 8.80 (s, 1H, benzothiazinone H-5), 4.16-3.89 (m, 6H, diazaspiro[4.5]decan (H-7, H-9), -$NOCH_2$-), 3.16 (s, 2H, -*CH_2* C(NOEt)), 2.71 (s, 2H, diazaspiro[4.5]decan (H-1)), 2.51 (s, 2H, diazaspiro[4.5]decan (H-3)), 1.94 (s, 3H, Me), 1.79 (brs, 6H, diazaspiro[4.5]decan (H-4, H-6, H-10)), 1.24 (t, *J* = 7.0 Hz, 3H, Me, -$NOCH_2$*CH_3*); ^{13}C NMR (125 Mz, DMSO-d6) δ165.7, 161.4, 144.9, 134.9, 131.7, 127.8 (q, *J* = 34.5 Hz), 126.6, 123.1 (q, *J* = 272.4 Hz), 68.6, 65.3, 59.3, 53.3, 40.9, 37.1, 36.1, 15.1, 13.3; MS-ESI (m/z): 514.1 $(M+H)^+$.

4.2.2.9. 2-(2-(3-(methoxyimino)butyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**2c**)

Yellow solid; yield: 38%; mp: 113-115 ^{o}C; ^{1}H NMR (500 Mz, $CDCl_3$) δ 9.10 (d, *J* = 1.5 Hz, 1H, benzothiazinone H-7), 8.75 (d, *J* = 1.5 Hz, 1H, benzothiazinone H-5), 3.94-3.82 (m, 7H, diazaspiro[4.5]decan (H-7, H-9), -$NOCH_3$), 2.65-2.58 (m, 4H, -N*CH_2CH_2*C(NOMe)), 2.52-2.50 (m, 2H, diazaspiro[4.5]decan (H-1)), 2.36 (t, J = 7.6 Hz, 2H, diazaspiro[4.5]decan (H-3)), 1.88 (s, 3H, Me), 1.79-1.73 (m, 6H, diazaspiro[4.5]decan (H-4, H-6, H-10)); ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.5, 161.7, 156.6, 156.1, 144.0, 134.3, 131.7 (q, *J* = 3.5 Hz), 130.1, 127.7 (q, *J* = 35.5 Hz), 126.6, 123.1 (q, *J* = 273.2 Hz), 65.2, 61.2, 53.4, 44.7, 40.6, 37.5, 36.3, 35.0, 14.1; MS-ESI (m/z): 514.1 $(M+H)^+$.

4.2.2.10. 2-(2-(3-(ethoxyimino)butyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**2d**)

Orange oil; yield: 43%; ^{1}H NMR (500 Mz, $CDCl_3$) δ 9.14 (s, 1H, benzothiazinone H-7), 8.80 (s, 1H, benzothiazinone H-5), 4.12-3.85 (m, 6H, diazaspiro[4.5]decan (H-7, H-9), -$NOCH_2$-), 2.81-2.50 (m, 8H, -N*CH_2CH_2*C(NOEt), diazaspiro[4.5]decan (H-1, H-3)), 1.93-1.80 (m, 9H, diazaspiro[4.5]decan (H-4, H-6, H-10), Me), 1.30-1.28 (m, 3H, $NOCH_2$*CH_3*); ^{13}C NMR (100 Mz, $CDCl_3$) δ 165.6, 161.3, 156.4, 155.9, 144.8, 134.8, 131.7 (q, *J* = 3.5 Hz), 130.1, 127.7 (q, *J* = 35.5 Hz), 126.6, 123.1 (q, *J* = 273.2 Hz), 68.3, 68.3, 53.2, 52.8, 45.2, 43.4, 37.2, 36.2, 34.8, 15.1, 15.1; MS-ESI (m/z): 527.1 $(M+H)^+$.

4.2.2.11.
2-(2-(3-((benzyloxy)imino)butyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**2e**)

Orange oil; yield: 45%; ^{1}H NMR (500 Mz, $CDCl_3$) δ 9.15 (s, 1H, benzothiazinone H-7), 8.80 (s, 1H, benzothiazinone H-5), 7.40-7.31 (m, 5H, $NOCH_2$*Ph*), 5.11 (s, 2H, $NOCH_2$Ph), 4.16-3.83 (m, 4H, diazaspiro[4.5]decan (H-7, H-9)), 2.72-2.42 (m, 8H, -NCH_2CH_2C(NOBn), diazaspiro[4.5]decan (H-1, H-3)), 1.94 (s, 3H, Me), 1.75 (brs, 6H, diazaspiro[4.5]decan (H-4, H-6, H-10)); ^{13}C NMR (125 Mz, DMSO-d6) δ 165.7, 161.3, 156.9, 144.8, 139.0, 134.8, 131.7, 128.7, 128.0, 127,9, 127.8 (q, *J* = 34.5 Hz), 126.6, 123.1 (q, *J* = 272.4 Hz), 74.8, 65.0, 53.2, 52.5, 51.7, 45.1, 40.7, 36.0, 34.6, 14.4; MS-ESI (m/z): 590.2 $(M+H)^+$.

4.2.3. General synthesis procedure of compounds 13a-b, 17a-b.

To a stirred solution of compound **12** or **16** (1.0 mmol) in MeOH (20 mL) was added pyridine (10 mL) and $MeONH_2$·HCl or $EtOHNH_2$.HCl (2.5 mmol) at room temperature. The mixture was stirred for 5-7 hours and washed by brine, then concentrated for next step without purification.

To a stirred solution of the above compounds (1.0 mmol) in pyridine (20 mL) was added TsCl (285.7 mg, 1.5 mmol) at room temperature. The mixture was stirred overnight and concentrated. The residue was purified over silica gel column (EtOAc: n-hexane = 1: 5) to give compounds **13a-b**, **17a-b** in a yield of 56%-64%.

4.2.3.1. (4-(methoxyimino)cyclohexyl)methyl 4-methylbenzenesulfonate (13a)

Yellow oil; yield: 64%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 7.77 (d, *J* = 8.0 Hz, 2H, -$S(O)_2$Ph(H)), 7.34 (d, *J* = 8.0 Hz, 2H, -MePh(H)), 3.86 (d, *J* = 6.0 Hz, 2H, -$S(O)_2OCH_2$), 3.79 (s, 3H, -OMe), 3.17 (d, *J* = 14.5 Hz, 1H, H-3), 2.45 (s, 3H, -PhMe), 2.37 (d, *J* = 14.0 Hz, 1H, H-5), 2.05 (dt, *J* = 4.0 Hz, *J* = 13.5 Hz, 1H, H-3), 1.94-1.83 (m, 3H, H-5, H-2, H-6), 1.73 (dt, *J* = 4.0 Hz, *J* = 13.5 Hz, 1H, H-2), 1.20-1.06 (m, 2H, H-6, H-1).

4.2.3.2. (4-(ethoxyimino)cyclohexyl)methyl 4-methylbenzenesulfonate (13b)

Yellow oil; yield: 61%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 7.75 (d, *J* = 6.0 Hz, 2H, -$S(O)_2$Ph(H)), 7.32 (d, *J* = 6.0 Hz, 2H, -MePh(H)), 4.00 (d, *J* = 5.0 Hz, 2H, -$NOCH_2$), 3.83 (brs, 2H, -$S(O)_2OCH_2$), 3.17 (d, *J* = 14.0 Hz, 1H, H-3), 2.42 (s, 3H, -PhMe), 2.34 (d, *J* = 14.0 Hz, 1H, H-5), 2.03 (t, *J* = 13.0 Hz, 1H, H-3), 1.90-1.81 (m, 3H, H-5, H-2, H-6), 1.71 (t, *J* = 13.0 Hz, 1H, H-2), 1.19 (t, *J* = 7.00 Hz, 3H,-$NOCH_2CH_3$), 1.15-1.06 (m, 2H, H-6, H-1).

4.2.3.3. (3-(methoxyimino)cyclobutyl)methyl 4-methylbenzenesulfonate (17a)

Yellow oil; yield: 60%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 7.78 (d, *J* = 7.0 Hz, 2H, $S(O)_2Ph(H)$), 7.35 (d, *J* = 8.0 Hz, 2H, -MePh(H)), 4.05 (brs, 2H, $-S(O)_2OCH_2$), 3.79 (s, 3H, OMe), 3.00-2.94 (m, 2H, H-2, H-4), 2.69-2.67 (m, 1H, H-2), 2.62-2.53 (m, 2H, H-4, H-1), 2.45 (s, 3H, -PhMe).

4.2.3.4. (3-(ethoxyimino)cyclobutyl)methyl 4-methylbenzenesulfonate (17b)

Yellow oil; yield: 56%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 7.77 (d, *J* = 7.0 Hz, 2H, $S(O)_2Ph(H)$), 7.34 (d, *J* = 7.0 Hz, 2H, -MePh(H)), 4.05-4.01 (m, 4H, $-S(O)_2OCH_2$, $NOCH_2$), 3.01-2.91 (m, 2H, H-2, H-4), 2.69-2.64 (m, 1H, H-2), 2.59-2.54 (m, 2H, H-4, H-1), 2.44 (s, 3H, -PhMe), 1.21 (t, *J* = 7.0 Hz, 3H, $-NOCH_2\mathit{CH_3}$).

4.2.4. General synthesis procedure of compounds 3a-o, 4a-f.

To a stirred solution of compound **13a-b, 17a-b** (1.0 mmol) in CH_3CN (20 mL) were added N-Boc-piperazine (180.9 mg, 0.9 mmol), K_2CO_3 (205.9 mg, 1.5 mmol), KI (166.0 mg, 1.0 mmol) and 18-crown-6 (26.4 mg, 0.1 mmol) at room temperature. The mixture was stirred overnight at 80^{o}C and filtered. The filtrate was diluted by DCM and washed by brine. The organic layer was concentrated for next step. To a stirred solution of the above compounds in DCM (20 mL) was added TFA (3 mL) at room temperature. The mixture was stirred for 3-4 hours and concentrated to afford the crude products **15a-o** and **19a-f** in a yield of 35%-42%. To a stirred solution of above crudes in anhydrous MeOH (10 mL) was added BTZ core compound **11** (403.2 mg, 1.0 mmol) and Et_3N (0.2 mL, 1.5 mmol|) at room temperature. The mixture was stirred for 1-3 hours at 40 ^{o}C and concentrated. The residue was purified by silica gel column (DCM: MeOH = 20: 1) to give **1a-f** and **2a-e** (25%-41% for two steps),

4.2.4.1. 2-(4-((4-(methoxyimino)cyclohexyl)methyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3a)

Yellow solid; yield: 41%; mp: 192-193□; HPLC purity: 98.68%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.14-3.82 (m, 4H, piperazine(H-2, H-6)), 3.82 (s, 3H, NOMe), 3.19 (d, *J* = 14.5 Hz, 1H, N-*C*H*H*CH-), 2.56 (brs, 4H, piperazine(H-3, H-5)), 2.42 (d, *J* = 14.0 Hz, 1H, N-*CH*HCH-), 2.23 (d, *J* = 7.5 Hz, 2H, cyclohexane (H-3)), 2.10 (dt, *J* = 4.5 Hz, *J* = 13.5 Hz, 1H, cyclohexane (H-1)), 2.00 (d, *J* = 12.0 Hz, 1H, cyclohexane (H-5)), 1.94 (d, *J* = 11.0 Hz, 1H, cyclohexane (H-5)), 1.83-1.69 (m, 2H, cyclohexane (H-2)), 1.20-1.04 (m, 2H, cyclohexane (H-6)). ^{13}C NMR (125 Mz, $CDCl_3$) δ 166.5, 162.1, 159.6, 144.0, 134.2, 133.4, 129.7 (q, *J* = 35.3 Hz), 126.8, 126.1, 122.5 (q, *J* = 274.7 Hz), 63.8, 61.1, 53.2, 46.7, 34.4, 31.3, 31.2, 30.1, 24.1. MS-ESI (m/z): 500.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{21}H_{25}F_3N_5O_4S$ $(M+H)^+$: 500.1574; Found: 500.1578.

4.2.4.2. 2-(4-((4-(ethoxyimino)cyclohexyl)methyl)piperazin-1-yl)-8-nitro-6-(tri-

fluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3b)

Yellow solid; yield: 40%; mp: 197-199□; HPLC purity: 97.78%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.27-3.81 (m, 4H piperazine(H-2, H-6)), 4.06 (q, *J* = 6.5 Hz, 2H, $NOCH_2$-), 3.22 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 2.56 (brs, 4H, piperazine(H-3, H-5)), 2.42 (d, *J* = 14.0 Hz, 1H, N-*CH**H*CH-), 2.24 (d, *J* = 6.5 Hz, 2H, cyclohexane (H-3)), 2.11 (t, *J* = 13.0 Hz, 1H, cyclohexane (H-1)), 2.04-1.93 (m, 2H, cyclohexane (H-5)), 1.83-1.66 (m, 2H, cyclohexane (H-2)), 1.25 (t, *J* = 7.0 Hz, 3H, $NOCH_2CH_3$), 1.20-1.09 (m, 2H, cyclohexane (H-6)). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.2, 159.3, 144.0, 134.2, 133.5 (q, *J* = 4.0 Hz), 129.8 (q, *J* = 35.3 Hz), 126.8, 126.1 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 273.7 Hz), 68.8, 63.8, 53.1, 46.8, 34.4, 31.3, 30.1, 24.2, 14.7. MS-ESI (m/z): 514.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{22}H_{27}F_3N_5O_4S$ $(M+H)^+$: 514.1730; Found: 514.1738.

4.2.4.3.

(S)-2-(4-((4-(methoxyimino)cyclohexyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3c)

Yellow oil; yield: 31%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.71-4.36 (m, 1H, piperazine(H-2)), 4.10-3.90 (m, 1H, piperazine(H-6)), 3.81 (s, 3H, NOMe), 3.81-3.58 (m, 1H, piperazine(H-2)), 3.48-3.23 (m, 1H, piperazine(H-6)), 3.19 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 3.02-2.84 (m, 1H, piperazine(H-3)), 2.70-2.40 (m, 2H, piperazine(H-5)), 2.39 (d, *J* = 14.5 Hz, 1H, N-*CH**H*CH-), 2.35 (brs, 1H, cyclohexane (H-1)), 2.14-2.07 (m, 3H, cyclohexane (H-3, H-5)), 1.92 (d, *J* = 12.0 Hz, 1H, cyclohexane (H-5)), 1.78 (dt, *J* = 5.5 Hz, J = 14.0 Hz, 1H, cyclohexane (H-2)), 1.68 (brs, 1H, cyclohexane (H-6)), 1.20-1.00 (m, 5H, cyclohexane (H-2, H-6), Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.1, 159.8, 144.0, 134.2, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 35.3 Hz), 126.9, 126.1 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 273.7 Hz), 61.2, 58.9, 55.4, 35.1, 31.2, 30.3, 24.2. MS-ESI (m/z): 514.1 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{22}H_{27}F_3N_5O_4S$ $(M+H)^+$: 514.1730; Found: 514.1738.

4.2.4.4.

(S)-2-(4-((4-(ethoxyimino)cyclohexyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3d)

Yellow oil; yield: 35%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.70-4.43 (m, 1H, piperazine(H-2)), 4.05 (q, *J* = 6.4 Hz, 2H, -$NOCH_2$-), 4.07-3.87 (m, 1H, piperazine(H-6)), 3.80-3.60 (m, 1H, piperazine(H-2)), 3.46-3.28 (m, 1H, piperazine(H-6)), 3.22 (d, *J* = 14.0 Hz, 1H, N-*CH*HCH-), 3.06-2.89 (m, 1H, piperazine(H-3)), 2.70-2.45 (m, 2H, piperazine(H-5)), 2.43-2.31 (m, 2H, N-*CH**H*CH-, cyclohexane (H-1)), 2.11-2.00 (m, 3H, cyclohexane (H-3, H-5)), 1.87-1.77 (m, 2H, cyclohexane (H-5, H-2)), 1.70 (brs, 1H, cyclohexane

(H-2)), 1.24 (t, *J* = 7.0 Hz, 3H, -NOCH$_2$*CH$_3$*-), 1.16-1.06 (m, 5H, cyclohexane (H-6), Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.1, 159.4, 144.0, 134.2, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 35.3 Hz), 126.9, 126.1 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 273.7 Hz), 68.8, 58.9, 55.5, 35.2, 31.3, 30.4, 24.3, 14.7. MS-ESI (m/z): 528.2 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{29}F_3N_5O_4S$ $(M+H)^+$: 528.1887; Found: 528.1891.

4.2.4.5.

(R)-2-(4-((4-(methoxyimino)cyclohexyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3e)

Yellow oil; yield: 32%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.65-4.43 (m, 1H, piperazine(H-2)), 4.09-3.87 (m, 1H, piperazine(H-6)), 3.82 (s, 3H, NOMe), 3.82-3.58 (m, 1H, piperazine(H-2)), 3.46-3.30 (m, 1H, piperazine(H-6)), 3.18 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 3.02-2.93 (m, 1H, piperazine(H-3)), 2.68-2.48 (m, 2H, piperazine(H-5)), 2.43-2.35 (m, 2H, N-*C*H*H*CH-, cyclohexane (H-1)), 2.14-2.00 (m, 3H, cyclohexane (H-3, H-5)), 1.92 (d, *J* = 12.0 Hz, 1H, cyclohexane (H-5)), 1.77 (dt, *J* = 5.0 Hz, *J* = 14.0 Hz, 1H, cyclohexane (H-2)), 1.70 (brs, 1H, cyclohexane (H-2)), 1.19-0.99 (m, 5H, cyclohexane (H-6), Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.1, 159.8, 144.0, 134.2, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 35.3 Hz), 126.9, 126.1 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 273.7 Hz), 61.2, 59.0, 55.5, 35.1, 31.6, 31.4, 30.1, 24.1. MS-ESI (m/z): 514.2 $(M+H)^+$.

4.2.4.6.

(R)-2-(4-((4-(ethoxyimino)cyclohexyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3f)

Yellow oil; yield: 36%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.74 (s, 1H, benzothiazinone H-5), 4.68-4.41 (m, 1H, piperazine(H-2)), 4.05 (q, *J* = 7.0 Hz, 2H, NOCH$_2$-), 4.07-3.86 (m, 1H, piperazine(H-6)), 3.82-3.58 (m, 1H, piperazine(H-2)), 3.50-3.29 (m, 1H, piperazine(H-6)), 3.22 (d, *J* = 15.0 Hz, 1H, N-*CH*HCH-), 3.05-2.84 (m, 1H, piperazine(H-3)), 2.66-2.48 (m, 2H, piperazine(H-5)), 2.43-2.31 (m, 2H, N-*C*H*H*CH-, cyclohexane (H-1)), 2.14-2.00 (m, 3H, cyclohexane (H-3, H-5)), 1.92 (d, *J* = 12.0 Hz, 1H, cyclohexane (H-5)), 1.77 (dt, *J* = 5.0 Hz, *J* = 14.0 Hz, 2H, cyclohexane (H-2)), 1.24 (t, *J* = 6.5 Hz, 3H, NOCH$_2$CH$_3$), 1.09-1.01 (m, 5H, cyclohexane (H-6), Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.1, 159.5, 144.0, 134.2, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 35.3 Hz), 126.9, 126.1 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 273.7 Hz), 68.8, 59.0, 55.5, 35.2, 31.6, 31.4, 30.1, 24.2, 14.7. MS-ESI (m/z): 528.2 $(M+H)^+$.

4.2.4.7.

(R)-2-(4-((4-(methoxyimino)cyclohexyl)methyl)-2-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3g)

Yellow oil; yield: 25%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 5.60-4.11 (m, 1H, piperazine(H-2)), 3.81 (s, 3H，NOMe), 3.62-3.31 (m, 1H, piperazine(H-6)), 3.20 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 2.94 (d, *J* = 10.0 Hz, 1H, piperazine(H-6)), 2.79 (d, *J* = 10.5 Hz, 1H, piperazine(H-3)), 2.42 (d, *J* = 13.5 Hz, 1H, N-*CH**H*CH-), 2.30-2.07 (m, 5H, piperazine(H-3, H-5), cyclohexane (H-1, H-3, H-5)), 2.03-1.94 (m, 2H, cyclohexane (H-3, H-5)), 1.83-1.71 (m, 2H, cyclohexane (H-2, H-6)), 1.49-1.43 (m, 3H, Me), 1.20-1.06 (m, 2H, cyclohexane (H-2, H-6)). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.1, 159.8, 144.0, 134.2, 133.5 (q, *J* = 4.0 Hz), 129.8 (q, *J* = 36.4 Hz), 126.9, 126.1 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 274.7 Hz), 61.2, 59.0, 55.5, 35.1, 31.5, 30.1, 24.1. MS-ESI (m/z): 514.2 $(M+H)^+$.

4.2.4.8.

(R)-2-(4-((4-(ethoxyimino)cyclohexyl)methyl)-2-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3h)

Yellow oil; yield: 27%; ^{1}H NMR(500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 5.45-4.45 (m, 1H, piperazine(H-2)), 4.06 (q, *J* = 7.5 Hz, 2H, $NOCH_2$-), 3.76-3.28 (m, 1H, piperazine(H-6)), 3.23 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 2.94 (d, *J* = 9.5 Hz, 1H, piperazine(H-6)), 2.79 (d, *J* = 10.5 Hz, 1H, piperazine(H-3)), 2.42 (d, *J* = 14.5 Hz, 1H, N-*CH**H*CH-), 2.30-2.07 (m, 5H, piperazine(H-3, H-5), cyclohexane (H-1, H-3, H-5)), 2.03-1.90 (m, 2H, cyclohexane (H-3, H-5)), 1.84-1.72 (m, 2H, cyclohexane (H-2, H-6)), 1.50-1.38 (m, 3H, Me), 1.25 (t, *J* = 7.5 Hz, 3H, $NOCH_2CH_3$), 1.20-1.07 (m, 2H, cyclohexane (H-2, H-6)). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.0, 159.5, 144.1, 134.4, 133.5 (q, *J* = 3.0 Hz), 127.0, 126.1 (q, *J* = 3.0 Hz), 68.9, 63.7, 34.6, 31.4, 31.2, 30.2, 30.0, 29.9, 24.3, 14.7. MS-ESI (m/z): 528.2 $(M+H)^+$.

4.2.4.9.

(S)-2-(4-((4-(methoxyimino)cyclohexyl)methyl)-2-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3i)

Yellow oil; yield: 29%; ^{1}H NMR(500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 5.52-4.08 (m, 1H, piperazine(H-2)), 3.83 (s, 3H, NOMe), 3.67-3.27 (m, 1H, piperazine(H-6)), 3.20 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 2.94 (d, *J* = 10.5 Hz, 1H, piperazine(H-6)), 2.79 (d, *J* = 10.5 Hz, 1H, piperazine(H-3)), 2.42 (d, *J* = 14.0 Hz, 1H, N-*CH**H*CH-), 2.28-2.08 (m, 5H, piperazine(H-3, H-5), cyclohexane (H-1, H-3, H-5)), 2.03-1.94 (m, 2H, cyclohexane (H-3, H-5)), 1.84-1.73 (m, 2H, cyclohexane (H-2, H-6)), 1.50-1.31 (m, 3H, Me), 1.30-1.16 (m, 2H, cyclohexane (H-2, H-6)). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.0, 159.8, 144.1, 134.4, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 35.3 Hz), 127.0, 126.1 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 273.7 Hz) , 63.7, 61.2, 57.8, 53.6, 34.5, 34.5, 31.3, 31.2, 30.2, 30.0, 29.8, 29.5, 24.2. MS-ESI (m/z): 514.2 $(M+H)^+$.

4.2.4.10.

(S)-2-(4-((4-(ethoxyimino)cyclohexyl)methyl)-2-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3j)

Yellow oil; yield: 27%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 5.57-4.16 (m, 1H, piperazine(H-2)), 4.06 (q, *J* = 7.0 Hz, 2H, $NOCH_2$-), 3.72-3.30 (m, 1H, piperazine(H-6)), 3.23 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 2.94 (d, *J* = 10.0 Hz, 1H, piperazine(H-6)), 2.79 (d, *J* = 10.5 Hz, 1H, piperazine(H-3)), 2.42 (d, *J* = 14.0 Hz, 1H, N-*C*H*H*CH-), 2.30-2.08 (m, 5H, piperazine(H-3, H-5), cyclohexane (H-1, H-3, H-5)), 2.03-1.93 (m, 2H, cyclohexane (H-3, H-5)), 1.84-1.63 (m, 2H, cyclohexane (H-2, H-6)), 1.50-1.41 (m, 3H, Me), 1.25 (t, *J* = 7.0 Hz, 3H, $NOCH_2$*CH_3*), 1.20-1.02 (m, 2H, cyclohexane (H-2, H-6)). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.0, 159.4, 144.10, 134.4, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 36.4 Hz), 127.0, 126.1 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 273.7 Hz), 68.8, 63.7, 53.6, 34.6, 31.4, 31.3, 31.2, 30.2, 30.0, 29.8, 24.3, 14.7. MS-ESI (m/z): 528.2 $(M+H)^+$.

4.2.4.11.

2-(4-((4-(methoxyimino)cyclohexyl)methyl)-3,5-dimethylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3k)

Yellow oil; yield: 34%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.84 (d, *J* = 10.0 Hz, 1H, piperazine(H-2)), 4.00 (d, *J* = 10.0 Hz, 1H, piperazine(H-6)), 3.81 (s, 3H, NOMe), 3.26-3.15 (m, 2H, piperazine(H-2), N-*CH*HCH-), 2.97 (brs, 1H, piperazine(H-6)), 2.70-2.55 (m, 2H, piperazine(H-3, H-5)), 2.45-2.35 (m, 3H, N-*C*H*H*CH-, cyclohexane (H-3, H-5)), 2.11-1.95 (m, 3H, cyclohexane (H-1, H-3, H-5)), 1.73 (dt, *J* = 5.0 Hz, *J* = 14.0 Hz, 2H, cyclohexane (H-2, H-6)), 1.20-0.98 (m, 8H, cyclohexane (H-2, H-6), Me, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 161.7, 159.7, 144.0, 134.2, 133.6 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 36.4 Hz), 126.9, 126.1 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 273.7 Hz), 61.2, 56.6, 52.3, 51.6, 38.2, 31.8, 31.6, 30.6, 24.4, 19.3, 19.0. MS-ESI (m/z): 528.2 $(M+H)^+$.

4.2.4.12.

2-(4-((4-(ethoxyimino)cyclohexyl)methyl)-3,5-dimethylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3l)

Yellow oil; yield: 36%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.84 (brs, 1H, piperazine(H-2)), 4.05 (q, *J* = 7.0 Hz, 2H, $NOCH_2$-), 4.04-3.96 (m, 1H, piperazine(H-6)), 3.27 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 3.21 (brs, 1H, piperazine(H-2)), 2.99 (brs, 1H, piperazine(H-6)), 2.67 (brs, 2H, piperazine(H-3, H-5)), 2.43-2.34 (m, 3H, N-*C*H*H*CH-, cyclohexane (H-3, H-5)), 2.10-1.96 (m, 3H, cyclohexane (H-1, H-3, H-5)), 1.76-1.69 (m, 2H, cyclohexane (H-2, H-6)), 1.20-0.98 (m, 11H, cyclohexane (H-2, H-6), Me, Me, $NOCH_2$*CH_3*). ^{13}C NMR (150 Mz, $CDCl_3$) δ 166.6, 161.7, 159.4, 144.0, 134.2, 133.5

(q, *J* = 3.0 Hz), 129.8 (q, *J* = 34.7 Hz), 126.9, 126.2 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 271.8 Hz), 68.8, 58.6, 56.6, 51.0, 38.2, 31.8, 31.6, 30.6, 24.6, 18.5, 14.7. MS-ESI (m/z): 542.2 $(M+H)^+$.

4.2.4.13.

2-(4-((4-(methoxyimino)cyclohexyl)methyl)-2,5-dimethylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3m)

Yellow oil; yield: 25%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 5.42 (brs, 0.30 H, piperazine(H-2)), 4.97 (brs, 0.39 H, piperazine(H-2)), 4.50 (brs, 0.39H, piperazine(H-6)), 3.94 (brs, 0.39 H, piperazine(H-6)), 3.82 (s, 3H, NOMe), 3.20 (d, *J* = 14.5 Hz, 1.50H, piperazine(H-6), N-*CH*HCH-), 2.89 (brs, 1.50H, piperazine(H-6), piperazine(H-3)), 2.60 (t, *J* = 11.0 Hz, 1H, piperazine(H-5)), 2.42 (d, *J* = 14.0 Hz, 1H, N-*CH**H*CH-), 2.32 (brs, 2H, piperazine(H-3), cyclohexane (H-3)), 2.15-2.05 (m, 2H, cyclohexane (H-3, H-5)), 1.91-1.65 (m, 4H, cyclohexane (H-1, H-5, H-2)), 1.52-1.39 (m, 3H, Me), 1.17-1.03 (m, 5H, cyclohexane (H-6), Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 161.6, 159.8, 144.1, 134.4, 133.5 (q, *J* = 4.0 Hz), 129.8 (q, *J* = 35.3 Hz), 127.0, 126.1 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 272.0 Hz), 61.2, 58.2, 56.7, 35.3, 31.6, 31.4, 31.2, 31.1, 30.3, 29.9, 29.8, 24.3, 24.1. MS-ESI (m/z): 528.2 $(M+H)^+$.

4.2.4.14.

2-(4-((4-(methoxyimino)cyclohexyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3n)

Yellow oil; yield: 33%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.67-4.48 (m, 1H, piperazine(H-2)), 4.10-3.90 (m, 1H, piperazine(H-6)), 3.82 (s, 3H, NOMe), 3.75-3.67 (m, 1H, piperazine(H-2)), 3.48-3.29 (m, 1H, piperazine(H-6)), 3.19 (d, *J* = 14.5 Hz, 1H, N-*CH*HCH-), 3.02-2.86 (m, 1H, piperazine(H-3)), 2.70-2.50 (m, 2H, piperazine(H-5)), 2.43-2.35 (m, 2H, N-*CH**H*CH-, cyclohexane (H-3)), 2.14-2.01 (m, 3H, cyclohexane (H-1, H-3, H-5)), 1.94-1.67 (m, 3H cyclohexane (H-5, H-2, H-6)), 1.16-1.01 (m, 5H, cyclohexane (H-2, H-6), Me). ^{13}C NMR (150 Mz, $CDCl_3$) δ 166.6, 162.1, 159.8, 144.0, 134.2, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 34.7 Hz), 126.9, 126.2 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 273.3 Hz), 61.2, 55.5, 35.2, 31.6, 31.4, 31.2, 30.3, 30.1, 24.2, 24.1. MS-ESI (m/z): 514.1 $(M+H)^+$.

4.2.4.15. 2-(4-((4-(ethoxyimino)cyclohexyl)methyl)-3-methylpiperazin-1-yl)-8 nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3o)

Yellow oil; yield: 34%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.70-4.45 (m, 1H, piperazine(H-2)), 4.05 (q, *J* = 7.0 Hz, 2H, $NOCH_2$-), 4.00-3.86 (m, 1H, piperazine(H-6)), 3.78-3.67 (m, 1H, , piperazine(H-2)), 3.48-3.30 (m, 1H, piperazine(H-6)), 3.19 (d, *J* = 15.0 Hz, 1H, N-*CH*HCH-), 3.08-2.86 (m, 1H, piperazine(H-3)), 2.67-2.48 (m, 2H, piperazine(H-5)),

2.43-2.27 (m, 2H, N-*CHH*CH-, cyclohexane (H-3)), 2.14-2.00 (m, 3H, cyclohexane (H-1, H-3, H-5)), 1.94-1.70 (m, 3H, cyclohexane (H-5, H-2, H-6)), 1.25 (t, J = 7.0 Hz, 3H, $NOCH_2CH_3$), 1.16-1.01 (m, 5H, , cyclohexane (H-2, H-6), Me). ^{13}C NMR (150 Mz, $CDCl_3$) δ 166.6, 162.1, 159.5, 144.0, 134.2, 133.5 (q, J = 2.9 Hz), 129.8 (q, *J* = 35.5 Hz), 126.9, 126.1 (q, *J* = 3.3 Hz), 122.5 (q, *J* = 273.3 Hz), 68.8, 59.1, 58.6, 55.5, 35.2, 31.6, 31.4, 31.3, 30.4, 30.1, 24.3, 18.6, 14.7. MS-ESI (m/z): 528.2 $(M+H)^+$.

4.2.4.16. 2-(4-((3-(methoxyimino)cyclobutyl)methyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4a)

Yellow solid; yield: 33%; mp: 186-187□; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.19-3.83 (m, 4H, piperazine(H-2, H-6)), 3.83 (s, 3H, NOMe), 3.07-3.02 (m, 2H, -N*CH*$_2$CH-), 2.60-2.54 (m, 9H, piperazine(H-3, H-5), cyclobutene (H-1, H-2, H-4)). ^{13}C NMR (125 Mz, $CDCl_3$) δ 166.5, 162.2, 155.6, 144.0, 134.1, 133.4 (q, *J* = 4.5 Hz), 126.8, 126.2 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 271.9 Hz), 63.2, 61.6, 52.7, 46.7, 36.3, 35.5, 25.9. MS-ESI (m/z): 472.2 $(M+H)^+$.

4.2.4.17. 2-(4-((3-(ethoxyimino)cyclobutyl)methyl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4b)

Yellow solid; yield: 31%; mp: 182-184□; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.10 (s, 1H, benzothiazinone H-7), 8.76 (s, 1H, benzothiazinone H-5), 4.26-3.76 (m, 4H, piperazine(H-2, H-6)), 4.06 (q, *J* = 7.0 Hz, 2H, $NOCH_2$-), 3.06-3.03 (m, 2H, -N*CH*$_2$CH-), 2.62-2.54 (m, 9H piperazine(H-3, H-5), cyclobutene (H-1, H-2, H-4)), 1.25 (t, *J* = 7.0 Hz, 3H, $NOCH_2CH_3$). ^{13}C NMR (150 Mz, $CDCl_3$) δ 166.6, 162.2, 155.3, 144.1, 133.6 (q, *J* = 3.0 Hz), 129.9 (q, *J* = 36.2 Hz), 126.9, 126.2 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 273.3 Hz), 69.4, 63.4, 52.8, 36.4, 35.8, 29.8, 26.0, 14.8. MS-ESI (m/z): 486.2 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{20}H_{23}F_3N_5O_4S$ $(M+H)^+$: 486.1417; Found: 486.1419.

4.2.4.18. 2-(4-((3-(methoxyimino)cyclobutyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4c)

Yellow oil; yield: 29%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.76-4.40 (m, 1H, piperazine(H-2)), 4.12-3.87 (m, 1H, piperazine(H-6)), 3.82 (s, 3H, NOMe), 3.82-3.57 (m, 1H, piperazine(H-2)), 3.46-3.20 (m, 1H, piperazine(H-6)), 3.13-2.87 (m, 3H, piperazine(H-3), N*CH*$_2$CH-), 2.87-2.67 (m, 1H, cyclobutene (H-1)), 2.61-2.48 (m, 4H, cyclobutene (H-2, H-4)), 2.42-2.35 (m, 2H, piperazine(H-5)), 1.14 (brs, 3H, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.2, 155.7, 144.0, 134.1, 133.5 (q, *J* = 3.0 Hz),

129.8 (q, *J* = 35.3 Hz), 126.9, 126.2 (q, *J* = 4.0 Hz), 122.5 (q, *J* = 273.7 Hz), 61.7, 58.4, 55.2, 53.6, 36.3, 35.6, 35.5, 26.1, 26.1. MS-ESI (m/z): 486.2 $(M+H)^+$.

4.2.4.19. 2-(4-((3-(ethoxyimino)cyclobutyl)methyl)-3-methylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4d)

Yellow oil; yield: 31%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.08 (s, 1H, benzothiazinone H-7), 8.74 (s, 1H, benzothiazinone H-5), 4.71-4.38 (m, 1H, piperazine(H-2)), 4.05 (q, *J* = 7.0 Hz, 2H, $NOCH_2$-), 4.05-3.82 (m, 1H, piperazine(H-6)), 3.82-3.55 (m, 1H, piperazine(H-2)), 3.46-3.23 (m, 1H, piperazine(H-6)), 3.03-2.87 (m, 3H, piperazine(H-3), N*CH$_2$*CH-), 2.74 (brs, 1H, cyclobutene (H-1)), 2.61-2.48 (m, 4H, cyclobutene (H-2, H-4)), 2.43-2.38 (m, 2H, piperazine(H-5)), 1.24 (t, *J* = 7.00 Hz, 3H, $NOCH_2CH_3$), 1.13 (brs, 3H, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 162.2, 155.3, 144.0, 134.2, 133.6 (q, *J* = 3.0 Hz), 129.9 (q, *J* = 35.3 Hz), 126.9, 126.2 (q, *J* = 3.0 Hz), 122.5 (q, *J* = 273.7 Hz), 69.4, 58.5, 55.2, 36.3, 35.8, 29.8, 26.2, 14.8. MS-ESI (m/z): 500.1 $(M+H)^+$.

4.2.4.20. 2-(4-((3-(methoxyimino)cyclobutyl)methyl)-3,5-dimethylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4e)

Yellow oil; yield: 27%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.09 (s, 1H, benzothiazinone H-7), 8.75 (s, 1H, benzothiazinone H-5), 4.85 (brs, 1H, piperazine(H-2)), 4.03 (brs, 1H, piperazine(H-6)), 3.81 (s, 3H, NOMe), 3.20 (brs, 1H, piperazine(H-2)), 3.03-2.96 (m, 3H, piperazine(H-6), N*CH$_2$*CH-), 2.82 (d, *J* = 4.5 Hz, 2H, cyclobutene (H-2, H-4)), 2.74 (brs, 2H, cyclobutene (H-2, H-4)), 2.58-2.52 (m, 3H, cyclobutene (H-1), piperazine(H-3, H-5)), 1.24-1.18 (m, 6H, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.6, 161.8, 155.2, 144.0, 134.1, 133.5 (q, *J* = 3.0 Hz), 1329.8 (q, *J* = 35.3 Hz), 126.9, 126.1 (q, *J* = 4.0 Hz), 123.0 (q, *J* = 273.7 Hz), 61.7, 54.9, 54.0, 52.4, 51.6, 36.5, 35.8, 29.8, 26.5, 18.8, 18.6. MS-ESI (m/z): 500.2 $(M+H)^+$.

4.2.4.21. 2-(4-((3-(ethoxyimino)cyclobutyl)methyl)-3,5-dimethylpiperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4f)

Yellow oil; yield: 30%; ^{1}H NMR (500 Mz, $CDCl_3$): δ 9.06 (s, 1H, benzothiazinone H-7), 8.73 (s, 1H, benzothiazinone H-5), 4.84 (brs, 1H, piperazine(H-2)), 4.03 (q, *J* = 6.50 Hz, 3H, $NOCH_2$-, piperazine(H-6)), 3.19 (brs, 1H, piperazine(H-2)), 3.04-2.94 (m, 3H, piperazine(H-6), N*CH$_2$*CH-), 2.81 (d, *J* = 6.0 Hz, 2H, cyclobutene (H-2, H-4)), 2.73 (brs, 2H, cyclobutene (H-2, H-4)), 2.56-2.52 (m, 3H, cyclobutene (H-1), piperazine(H-3, H-5)), 1.22 (t, *J* = 7.0 Hz, 3H, $NOCH_2CH_3$), 1.24-1.04 (m, 6H, Me). ^{13}C NMR (100 Mz, $CDCl_3$) δ 166.5, 161.7, 154.8, 144.0, 134.1, 133.5 (q, *J* = 3.0 Hz), 129.8 (q, *J* = 35.3 Hz), 126.8, 126.1 (q, *J* = 3.0 Hz), 122.4 (q, *J* = 274.7 Hz), 69.3, 54.8, 54.0, 52.4, 51.6, 36.5, 35.9, 26.4, 18.8, 18.5, 14.7. MS-ESI (m/z): 514.2 $(M+H)^+$.

4.3. MIC determination.

MICs against replicating *M. tuberculosis* were determined by the microplate Alamar blue assay (MABA). RIF and INH were included as positive controls. *M. tuberculosis* H37Rv (ATCC27294) and clinical isolate strains were grown to late log phase (70 to 100 Klett units) in Difco Middlebrook 7H9 Broth (catalog no. 271310) supplemented with 0.2% (vol/vol) glycerol, 0.05% Tween 80, and 10% (vol/vol) albumin dextrosecatalase (BBL Middlebrook ADC Enrichment, catalog no. 212352) (7H9-ADCTG). Cultures were centrifuged, washed twice, and then suspended in phosphate phosphate-buffered saline. Suspensions were then passed through an 8 µm-pore-size filter to remove clumps, and aliquots were frozen at -80 °C. Two folds dilutions of test compounds and positive controls were prepared in 7H9-ADC-TG in a volume of 100 µl in 96-well, black, clear-bottom microplates (BD Biosciences, Franklin Lakes, NJ). *M. tuberculosis* (100 µl containing 2×10^5 CFU) was added, yielding a final testing volume of 200 µl. The plates were incubated at 37°C; on day 7 of incubation, 12.5 µl of 20% Tween 80 and 20 µl of Alamar blue were added to all wells. After incubation at 37 °C for 16 to 24 h, the fluorescence was read at an excitation of 530 nm and an emission of 590 nm. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacterium-only controls.

4.4. Inhibition evaluation on hERG K^+ channel

Inhibition evaluation on hERG K+ channel. The electrophysiology recording of hERG channel current was carried out following the standard protocol as described previously. HEK 293 cells were stably transfected with human Ether-a-gogo related gene (hERG) channel. The voltage-gated hERG potassium channel current was recorded at room temperature (25 ^oC) from randomly selected transfected cells using whole-cell recording technique with an EPC10 USB (HEKA) or Multiclamp 700B amplifier (Molecular Devices), while electrical data was digitalized by Digidata1440A with acquisition rate of 10 kHz and signals filtered at 2.5 KHz using Patchmaster or pClamp10 respectively. Dofetilide was included as a positive control to ensure the accuracy and sensitivity of the test system. All experiments were performed in 3-4 times.

4.5. Pharmacokinetic Profiles determination

Pharmacokinetic Profiles determination of compounds **3a** and **3b**. SPF female ICR mice (obtained from JOINN (Suzhou) weighing 18–22 g were used in the pharmacokinetic study. The ICR mice were fasted overnight before dosing. Every treatment group contain 3 mice. Mice were dosed with the tested compounds

suspension at 25 mg/kg (p.o.). Compounds were suspended in 0.5% CMC for oral administration. Blood was collected from the jugular vein of each animal at the following times after administration of drugs: 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after a single oral dosing. All blood samples were centrifuged at 3000 r/min for 10 min to obtain serum which was then stored at −20 °C. 150 μL of the serum was added to 500 μL of acetonitrile and the mixture was centrifuged at 13000 r/min for 10 min to remove protein. The supernatant was dried and dissolve in 100 μL of acetonitrile, the solution was centrifuged at 13000 r/min for 10 min. The supernatant was moved to a sample bottle for HPLC analysis. Total area under the concentration time curve (AUC), the elimination half-time (t1/2), the peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of samples were determined directly from the experimental data using WinNonlin V6.2.1. The SD female rats obtained from JOINN (Suzhou) weighing 180 - 220 g were also used in the pharmacokinetic study of compound **3a** as a similar procedure.

4.6. Acute toxicity determination

The toxicity study was carried out using SD rats weighting 180 - 220 g each. The animals were randomly distributed to control group and treated groups, containing ten animals per group. They were maintained on animal cubes, provided with water and foods. After depriving the animals' food overnight, the control group received 5% CMC solution orally whereas each treated group received orally the agents suspended in 5% CMC solution (2 g /kg). The animals were observed continuously for the first 4 hours and then each hour for the next 24 h and at 8 hourly intervals for the following 7 days after administering, to observe any death or changes.

4.7. *In vivo* activity determination

In vivo TB infection assay of compounds **3a**, **3b** and PBTZ169. SPF BALB/c mice (female) weighing 18-20 g were used in this study. Each group was composed of 6 mice. Mice were infected via aerosol with a suspension of 5×10^6 CFU/mL *M. tuberculosis* (H37Rv) using a Glas-Col inhalation system, to deposit 50-100 bacilli into the lungs of each animal. The course of infection was followed by plating homogenates of harvested organs [n = 3] on 7H11 agar plates (7H11 plates containing 10% oleic acid albumin-dextrose-catalase (OADC) enrichment and 50 μg/mL cycloheximide, 200 U/mL polymyxin B, 50 mg/mL carbenicillin, and 20 mg/mL trimethoprim) and determining CFU on days 3, 10, and 30 post infection. compounds **3a**, **3b** and PBTZ169 were dissolved or suspended in 0.5% CMC and administered by oral gavage in a maximum volume of 200 mL such that a dose of 25 mg/kg body weight was achieved. The control group was received only 0.5% CMC. Mice were treated on day 10 post infection and 5 times per week. The treatment period was 3 weeks. Mice were sacrificed the day after the last day of treatment, lungs removed, homogenized, and serially diluted in 10-fold steps in HBSS. One hundred mL were

spread on 7H11 agar in duplicate. The plates were incubated at 37 ^{o}C for 3 weeks. Data are expressed as the log10 (and as log10 reduction) provided by a given dose of the compound against the growth of the organism in the untreated control group. Mean log10 values were calculated from bacterial burden counts.

4.8. Purity determination

The purity of compounds **3a** and **3b** was checked by HPLC analyzing using Agilent 1260 HPLC-UV system with the Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm, PN: 883975-902) as our previous method [22]. Compounds were dissolved in MeOH with a concentration of 0.3 mg / mL, the mobile phase contains solvent A (methanol) and solvent B (0.1% TFA + H_2O). Gradient elution procedure was set up as: 0 min, 10% A; 3 min, 50% A; 10 min, 100% A; 12 min, 10% A (or 12min, 50% A and then 18min, 10% A). The Ultraviolet absorption wavelength is 254 nm and the column temperature is 25 □. The injection volume is 10 μL and the flow rate is 1.3 mL/min. As the above procedure, the HPLC purity of **3a** and **3b** is 98.68% and 97.78%, respectively.

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Notes

The authors declare no competing financial interest.

References

[1] World Health Organization. Global Tuberculosis Report 2018. http://www. who. int/tb/.

[2] A. N. Unissa, L. E. Hanna, S. Swaminathan, A Note on Derivatives of Isoniazid, Rifampicin, and Pyrazinamide Showing Activity Against Resistant Mycobacterium tuberculosis, Chem. Biol. Drug Des. 87 (2016) 537-550.

[3] A. Campaniço, R. Moreira, F. Lopes, Drug discovery in tuberculosis. New drug targets and antimycobacterial agents, Eur. J. Med. Chem. 150 (2018) 525-545.

[4] R.S. Wallis, M. Maeurer, P. Mwaba, J. Chakaya, R. Rustomjee, G.B. Migliori, B. Marais, M. Schito, G. Churchyard, S. Swaminathan, M. Hoelscher, A. Zumla, Tuberculosis-advances in development of new drugs, treatment regimens, host-directed

therapies, and biomarkers, Lancet Infect. Dis. 16 (2016) e34-e46.

[5.] A. Wang, Y. Yang, Y. Jun, B. Wang, K. Lv, M. Liu, H. Guo, Y. Lu, Synthesis, evaluation and CoMFA/CoMSIA study of nitrofuranyl methyl N-heterocycles as novel antitubercular agents, Bioorg. Med. Chem. Lett. 26 (2018) 2073-2084.

[6] D.T. Hoagland, J. Liu, R.B. Lee, R.E. Lee, New agents for the treatment of drug resistant Mycobacterium tuberculosis, Adv. Drug Deliv. Rev. 102 (2016) 55-72.

[7] S. Tiberi, R. Buchanan, J.A. Caminero, R. Centis, M.A. Arbex, M. Salazar, J. Potter, G.B. Migliori, The challenge of the new tuberculosis drugs, Presse Med. 46 (2017) e41-51.

[8]. A Single Ascending Dose Study of BTZ043, https://www.clinicaltrials.gov/ct2/show/NCT 03590600

[9] Phase 2a Study of PBTZ169, https://clinicaltrials.gov/ct2/show/NCT03334734

[10] V. Makarov, G. Manina, K. Mikusova, U. Mollmann, O. Ryabova, B. Saint-Joanis, N. Dhar, M. R. Pasca, S. Buroni, A. P. Lucarelli, A. Milano, E. De Rossi, M. Belanova, A. Bobovska, P. Dianiskova, J. Kordulakova, C. Sala, E. Fullam, P. Schneider, J. D. McKinney, P. Brodin,T. Christophe, S. Waddell, P. Butcher, J. Albrethsen, I. Rosenkrands, R. Brosch, V. Nandi, S. Bharath, S. Gaonkar, R. K. Shandil, V. Balasubramanian, T. Balganesh, S. Tyagi, J. Grosset, G. Riccardi and S. T. Cole, Benzothiazinones kill Mycobacterium tuberculosis by blocking arabinan synthesis, Science, 324 (2009) 801–804.

[11] V. Makarov, B. Lechartier, M. Zhang, J. Neres, A. M. van der Sar, S. A. Raadsen, R. C. Hartkoorn, O. B. Ryabova, A. Vocat, L. A. Decosterd, N. Widmer, T. Buclin, W. Bitter, K. Andries, F. Pojer, P. J. Dyson and S. T. Cole, Towards a new combination therapy for tuberculosis with next generation benzothiazinones, EMBO Mol. Med. 6 (2014) 372–383.

[12] C. Gao, T.H. Ye, N.Y. Wang, X.X. Zeng, L.D. Zhang, Y. Xiong, X.Y. You, Y. Xia, Y. Xu, C.T. Peng, W.Q. Zuo, Y. Wei, L.T. Yu, Synthesis and structure activity relationships evaluation of benzothiazinone derivatives as potential antitubercular agents, Bioorg. Med. Chem. Lett. 23 (2013) 4919-4922.

[13] C.T. Peng, C. Gao, N.Y. Wang, X.Y. You, L.D. Zhang, Y.X. Zhu, Y. Xu, W.Q. Zuo, K. Ran, H.X. Deng, Q. Lei, K.J. Xiao, L.T. Yu, Synthesis and antitubercular evaluation of 4-carbonyl piperazine substituted 1,3-benzothiazin-4-one derivatives, Bioorg. Med. Chem. Lett. 25 (2015) 1373-1376.

[14] R. Zhang, K. Lv, B. Wang, L. Li, B. Wang, M. Liu, H. Guo, A. Wang, Y. Lu, Design, synthesis and antitubercular evaluation of benzothiazinones containing an oximido or amino nitrogen heterocycle moiety, RSC Adv. 7 (2017) 1480-1483.

[15] K. Lv, X. You, B. Wang, Z. Wei, Y. Chai, B. Wang, A. Wang, G. Huang, M. Liu, Y. Lu, Identification of better pharmacokinetic benzothiazinone derivatives as new antitubercular agents, ACS Med. Chem. Lett, 8 (2017) 636-641.

[16] K. Lv, Z. Tao, Q. Liu, L. Yang, B. Wang, S. Wu, A. Wang, M. Huang, M. Liu, Y. Lu, Design, synthesis and antitubercular evaluation of benzothiazinones containing a piperidine moiety, Eur. J. Med. Chem. 151 (2018) 1-8.

[17] L. Li, K. Lv, Y. Yang, J. Sun, Z. Tao, A. Wang, B. Wang, H. Wang, Y. Geng, M. Liu, H. Guo, Y. Lu, Identification of N-benzyl 3,5-dinitrobenzamides derived from PBTZ169 as antitubercular agents, ACS Med. Chem. Lett. 9 (2018) 741-745.

[18] J. Piton, A. Vocat, A. Lupien, C. Foo, O. Riabova, V. Makarov, S.T. Cole, Structure based drug design and characterization of sulfonyl-piperazine benzothiazinone inhibitors of DprE1 from Mycobacterium tuberculosis, Antimicrob. Agents Chemother. 62 (2018) e00681-18,

[19] C. Gao, C. Peng, Y. Shi, X. You, K. Ran, L. Xiong, T.H. Ye, L. Zhang, N. Wang, Y. Zhu, K. Liu, W. Zuo, L. Yu, Y. Wei, Benzothiazinethione is a potent preclinical candidate for the treatment of drug-resistant tuberculosis, Sci. Rep. 6 (2016) 29717.

[20] R. Tiwari, P.A. Miller, S. Cho, S.G. Franzblau, M.J. Miller, Syntheses and antituberculosis activity of 1,3-benzothiazinone sulfoxide and sulfone derived from BTZ043, ACS Med. Chem. Lett. 6 (2015) 128-133.

[21] P. Li, B. Wang, X. Zhang, S. M. Batt, G. S. Besra, T. Zhang, C. Ma, D. Zhang, Z. Lin, G. Li, H. Huang, Y. Lu, Identification of novel benzothiopyranone compounds against Mycobacterium tuberculosis through scaffold morphing from Benzothiazinones. Eur. J. Med. Chem. 160 (2018) 157-170.

[22] G. Zhang, M. Howe, C. C.Aldrich, Spirocyclic and Bicyclic 8‑Nitrobenzothiazinones for Tuberculosis with Improved Physicochemical and Pharmacokinetic Properties, ACS Med. Chem. Lett. 10 (2019) 348−351

[23] K.Lv, A.Wang, Z. Tao, L. Fu, H. Liu, B. Wang, C. Ma, H. Wang, X. Ma, B. Han, A. Wang, K. Zhang, M. Liu, Y. Lu, hERG optimizations of IMB1603, discovery of alternative benzothiazinones as new antitubercular agents, Eur. J. Med. Chem. 179 (2019) 208-217.

[24] Y. Lu, M. Zheng, B. Wang, L. Fu, W. Zhao, P. Li, J. Xu, H. Zhu, H. Jin, D. Yin, H. Huang, A.M. Upton, Z. Ma, Clofazimine analogs with efficacy againstexperimental tuberculosis and reduced potential for accumulation, Antimicrob. Agents Chemother. 55 (2011) 5185-5193.

Highlights

1, Four series of BTZs with oxime functional moiety were synthesized;

2, Most of the compounds show excellent *in vitro* activity and low cell cytotoxicity;

3, **3a** and **3b** with proper ADME/T and PK profiles show potent *in vivo* efficacy.