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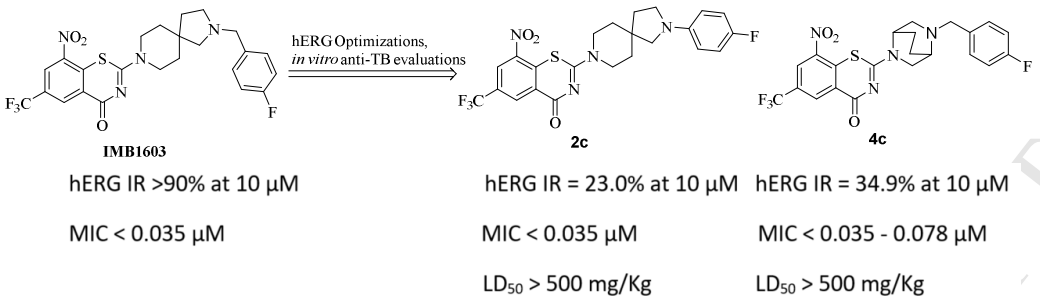
Graphic Abstract



IMB1603, a new benzothiazinone lead discovered by our lab, exhibited potent anti-TB activity *in vitro* and *in vivo*, but significant hERG binding potency (IR > 90% at 10 μM). Thus, we embarked on a hERG optimization program. Multi-series of compounds were designed, synthesized, and evaluated. Among them, compounds **2c** and **4c** were found to have acceptable hERG binding potency, potent anti-TB activity *in vitro*, acceptable safety, and pharmacokinetic properties. Currently, compounds **2c** and **4c** were selected as new leads and subjected for *in vivo* assessment.

hERG optimizations of IMB1603, discovery of alternative benzothiazinones as new antitubercular agents

Kai Lv,[a, †] Apeng Wang,[a, †] Zeyu Tao,[a] Lei Fu,[b] Hongtao Liu,[a, c] Bin Wang,[b] Chao Ma,[a] Hongjian Wang,[a] Xican Ma,[a] Bing Han,[a] Auyu Wang,[d] Kai Zhang,[d] Mingliang Liu,[a, *] Yu Lu[b, *]

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Abstract

IMB1603, a new benzothiazinone lead discovered by our lab, exhibited potent anti-MTB activity *in vitro* and *in vivo*, but significant hERG binding potency (IR > 90% at 10 μM). Thus, we embarked on a lead optimization program with the goal of identifying alternative leads that could reduce the hERG liability without sacrificing antimycobacterial potency. Compounds **2c** and **4c** were identified to maintain the anti-MTB activity (MICs <0.035-0.078 μM), and had lower hERG binding affinity (IR < 50% at 10 μM). Both of them were also found to have acceptable safety and pharmacokinetic properties. Studies to determine the *in vivo* efficacy of **2c** and **4c** are currently underway.

KEYWORDS: *Antitubercular agents, benzothiazinones, structure-activity relationships, hERG*

1. Introduction

Tuberculosis (TB) is an old disease which has existed for millennia. The TB is caused by *Mycobacterium tuberculosis* (MTB), which can spread when people who are

sick with TB expel bacterial into the air, such as coughing.[1] According to World Health Organization (WHO) statistics, TB has been the top 10 leading cause of death in the past decades. Thus, WHO has declared TB a global health emergency since 1993, published a global TB report every year since 1997, and started End TB Strategy at the World Health Assembly in 2014. But nevertheless, TB still caused about 1.6 million deaths including 1.3 million HIV-negative people in 2017.[2] At the current control efforts, the goal of ending the TB epidemic by 2030 will not be achieved.[3] In addition, the emergence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB have intensified the situation.[4-6] The treatment of MDR-TB is extremely difficult, requiring daily administration of a combination of 5−7 drugs for a period of 18−24 months.[7] Some of these existing drugs show significant toxicities. Even now XDR-TB has no guidelines or evidence that may guide its treatment.[8] Although Bedaquiline and Delamanib were approved for the treatment of MDR-TB in recent years, both of them were limited for use in the clinical due to pronounced issues in terms of hERG toxicity concerns and ADME issues.[4] Therefore, there is an urgent need for the discovery of new drugs with novel mechanisms of action for the treatment of TB.

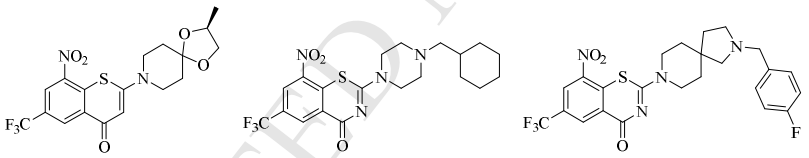


Figure 1. Structures of BTZ043, PBTZ169 and IMB1603

Benzothiazinones (BTZs), a novel class of TB agents targeting decaprenyl phosphoryl-β-D-ribose 2′-epimerase (DprE1), were reported to have strong inhibitory potency against drug-sensitive MTB, MDR-MTB and XDR-MTB strains.[9-14] BTZ043 and PBTZ169 (now known as Macozinone)[15], the most advanced candidates (Figure 1) from this series, are in Phase I[16] and Phase II[17] clinical trials at present, respectively, for the treatment of both drug-susceptible TB and MDR-TB. However, PBTZ169 also have limitations such as its low solubility.

The co-crystal structures of the DprE1 with BTZ043 / PBTZ169 complexes and the structure-activity relationship (SAR) of this series suggested 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] On the other hand, it should be prudent to continue populating the pipeline of antimycobacterial leads due to the high failure rate in new drug development.[18-19] Based on it, we have previously focused on structural modification of the side chain at C-2 position of the BTZ core to found more effective candidates.[20-22] Among of the derivatives, IMB1603 (Figure 1) containing a C-2 nitrogen spiro-heterocycle moiety displayed excellent *in vitro* activity against MTB H37Rv and clinical isolated MDR-MTB strains (MIC: < 0.035 μM), acceptable pharmacokinetic property, greater aqueous solubility than PBTZ169.[20] More importantly, IMB1603 showed strong efficacy in a mouse model of acute MTB H37Rv infection (25 mg/kg, P.O., once daily for 5 days a week for 3 weeks in BALB/c mice), producing 3.20 logs of colony forming unit (CFU) reduction in the lung (no published). However, our subsequent investigations revealed that IMB1603 turned out to inhibit *in vitro* hERG ion channels in mammalian cell lines, with inhibition rate (IR) of >90% at 10 μM, predicting potential electrocardiogram (QT) prolongation risk. Consequently, we embarked on a lead optimization program to identify novel analogues with reduced hERG cardiac toxicity but not at the expense of antimycobacterial potency to make critical compound progression decisions.

2. Results and Discussion

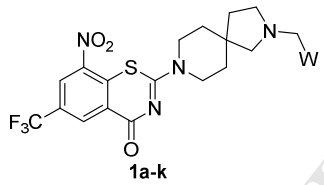
Many efficient strategies to combat the affinity of drugs to hERG have been proposed, but in fact making even subtle changes in the structures of small molecules have been found to cause profound impact on the hERG activity.[23-24] Herein, we described SAR of three moieties (benzene ring, linker, N-heterocycle) on the C-2 side chain of the BTZ core toward the discovery of the promising leads **2c** and **4c**. We believe that the SAR developed could also be applicable to other compound series.

2.1. Substitutions on Benzene Ring

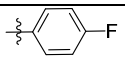
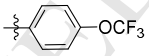
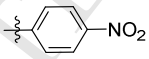
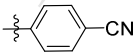
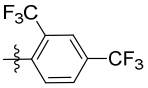
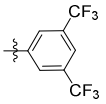
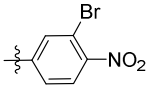
We first examined the influence of benzene substitutions, such as halogen (F, Br) and / or strong electron-withdrawing groups (CF_3, CN, NO_2) in IMB1603 derivatives **1a-i** which were easily synthesized according previous reported procedure.[20] As shown in Table 1, all of them displayed the same excellent *in vitro* antimycobacterial activity (MIC < 0.035 μM) as PBTZ169. Compared with mono substituted compounds (**1a-e**, IR >

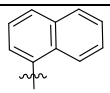
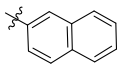
90%), disubstituted ones (**1f-i**) exhibited decreased binding potency against hERG channel, with IR values of < 90% which were still not acceptable. Subsequently, two compounds **1j** and **1k** with bulkier naphthalene rings were also investigated. Interestingly, β-naphthalene compound (**1j**) with potent antimycobacterial activity (MIC < 0.035 μM), displayed a relatively lower level of hERG inhibition (IR = 78%) than all of substituted benzene ones with an exception of **1g**.

Table 1. Activity against MTB H37Rv and hERG inhibition of BTZs with a substituted benzene moiety



1a-k

Compd.	W	MIC (μM)	hERG (IR at 10 μM)
1a (IMB1603)		<0.035	95.7±1.3%
1b		<0.035	94.9±1.5%
1c		<0.035	90.2±1.8%
1d		<0.035	94.1±2.2%
1e		<0.035	90.6±3.4%
1f		<0.035	88.5±3.5%
1g		<0.035	68.6±4.8%
1h		<0.035	83.3±2.3%
1i		<0.035	88.6±3.6%

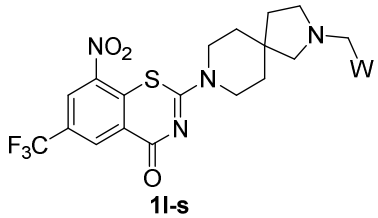
1j		0.055	NT
1k		<0.035	78.1±5.2%
PBTZ169		<0.035	41.6±5.3%
INH		0.269	
RFP		0.164	

INH, isoniazid; RFP, rifampicin.

2.2. Benzene Ring Modifications.

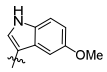
Among the most successful reported approaches for diminishing binding to the hERG channel is reducing the lipophilicity, as measured by cLog P.[23, 25] We probed the consequences of replacing the benzene moiety in the lead IMB1603 with other heteroaromatic rings (**1l-s**, cLog P: 2.32 – 4.65). With a few exceptions, these analogues were found to have much less antimycobacterial activity than IMB1603. For the top 3 most active compounds **1m**, **1q** and **1s**, their antimycobacterial activity and hERG affinity decreased as cLog P values reduced. For example, thiophene derivative **1m** (cLog P = 4.28) maintaining the same potency to IMB1603 (MIC: < 0.035 μM), turned out to be a strong hERG inhibitor at 10 μM (IR = 96.2%). Oxazole derivative **1m** with the lowest CLog P of 2.32 displayed moderate hERG inhibition (IR = 53.2%) (Table 2). It suggested that increasement of the polarity could decrease hERG inhibition, but this also results in reduced antimycobacterial activity possible due to lower membrane permeability and is illustrative of the challenges of multidimensional optimization.

Table 2. Activity against MTB H37Rv and hERG inhibition of BTZs with a modified benzene ring



1l-s

Compd.	W	MIC (μM)	hERG (IR at 10 μM)	cLog P

1a		<0.035	95.7±1.3%	4.78
(IMB1603)				
1l		1.478	NT	4.65
1m		<0.035	96.2±0.8%	4.28
1n		0.479	NT	3.71
1o		0.477	NT	3.14
1p		0.235	NT	3.14
1q		0.122	74.1±1.5%	3.14
1r		1.576	NT	3.24
1s		0.248	53.2±2.4%	2.32
PBTZ169		<0.035	41.6±5.3%	5.10

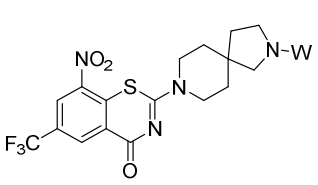
2.3. Linker Modifications.

The effect of the methylene linker between the benzene and spiro-heterocycle in IMB1603 was subsequently investigated. As shown in Table 3, the length of the linker had little impact on antimycobacterial activity, and hERG inhibition slightly decreased as the carbon chain extended (**1a** *vs* **2a** *vs* **2b**). Surprisingly, compound **2c** by removal of the methylene linker, maintained excellent antimycobacterial potency (MIC: < 0.035 μM) and was virtually inactive in hERG inhibition assay at 10 μM with IR value of 23% lower than PBTZ169 (IR = 41%). The significantly decreased hERG binding affinity of **2c** might attribute by the removal of the basic center, or the rigid structure of the C-2 side chain. Based on the above data, compound **2c** merited further study.

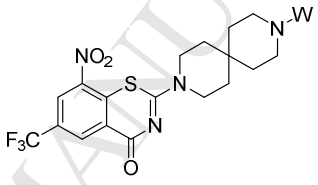
At the same time, an alkyl group was tried to introduced on the methylene linker of IMB1603 to explore the impact of the steric hindrance. However, the synthesis of these alkyl derivatives has low yields, and the products were very hard to purification. Thus, an

alkyl group was introduced to IMB1604 (**3a**) and IMB1605 (**3b**) which has the similar structure feature and pharmacological properties to IMB1603. As shown in Table 3, replacement of a hydrogen atom on the methylene of **3a** with methyl remained the same antimycobacterial potency (MIC: < 0.035 μM), and led to reduced hERG inhibition (**3a** *vs* **3c**), although changes of the halogen atom at *para*-position of the benzene ring had little effect on hERG activity (**3c** *vs* **3d** *vs* **3e**). The same trend was also observed by comparison between **3b** and **3f**. Introduction of ethyl seemed to be preferred over methyl (**3f** *vs* **3g**). Our results suggested that a bulky alkyl on the methylene linker might be favorable for reduction of hERG potency. Currently, more analogues with other bigger substituents were under investigation.

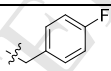
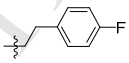
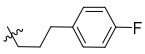
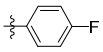
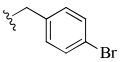
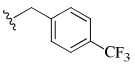
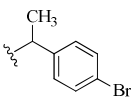
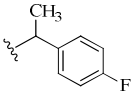
Table 3. Activity against MTB H37Rv and hERG inhibition of linker modified BTZs

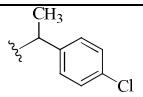
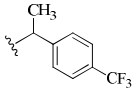
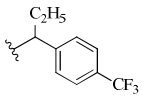


2a-f



3a-g

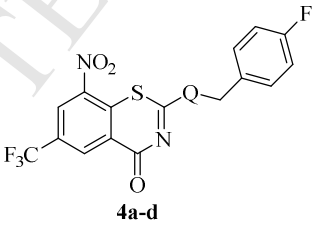
Compd.	W	MIC (μM)	hERG (IR at 10 μM)
1a (IMB1603)		<0.035	95.7±1.3%
2a		0.055	92.7±0.6%
2b		<0.035	85.8±2.6%
2c		<0.035	23.0±3.3%
3a (IMB1604)		<0.035	97.0±1.6%
3b (IMB1605)		<0.035	94.6±2.5%
3c		<0.035	77.2±3.0%
3d		0.107	74.0±4.2%

3e		<0.035	73.4±4.6%
3f		<0.035	72.9±4.0%
3g		<0.035	69.7±2.1%

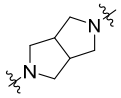
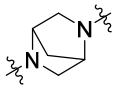
2.4. *N*-Heterocycle Modifications.

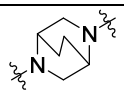
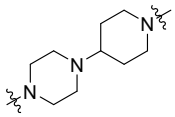
In order to get more candidates with diversity structure features, changes were subsequently focused on the spiro-heterocycle of IMB1603 while the *para*-fluorobenzene moiety was maintained. As shown in table 4, compounds **4b-d** exhibited good antimycobacterial activity (MIC: < 0.035 – 0.078 μM). To our delight, compound **4c** containing an azabicyclo[2.2.2]octane ring showed significantly decreased hERG affinity with IR of 34.9% at 10 μM, even lower than that of PBTZ169, indicating its potential for further development.

Table 4. Activity against MTB H37Rv and hERG inhibition of *N*-heterocycle modified BTZs



4a-d

Compd.	Q	MIC (μM)	Herg (IR at 10 μM)
4a		0.248	NT
4b		<0.035	94.3±0.6%

4c		0.078	34.9±2.9%
4d		0.054	95.6±0.5%

2.5. Additional assessments of selected compounds

On the bases of the above studies, compounds **2c** and **4c** with potent antimycobacterial activity (MIC: <0.035-0.078 μM) and poor hERG inhibition (IR:23.0-34.9%) at 10 μM were evaluated against two clinical isolated MTB-MDR (16833 and 16995) strains resistant to both INH and RFP. As shown in Table 5, both of them exhibited strong activity (MIC: < 0.035-0.062 μM) similar to that against MTB H37Rv, suggesting their promising potential for both drug-sensitive and resistant MTB strains (Tables 3-5).

The above 2 compounds were tested for *in vivo* tolerability by recording the number of survivors after a single oral dose in ICR mice of 500 mg/kg, followed by a 7-day observation. In general, compounds **2c** and **4c** showed good tolerance in mice at 500 mg/kg (Table 6).

Table 5. Anti-MDR-MTB activity and acute toxicity of selected compounds

Compd.	MIC[a] (μM)		Acute Toxicity[b]
	MDR-MTB16883	MDR-MTB16995	
2c	<0.035	<0.035	5/5
4c	0.062	<0.035	5/5
PBTZ169	<0.035	<0.035	5/5
INH	>40	>40	-
RFP	>40	>40	-

[a]MDR-MTB 16883 and MDR-MTB 16995 were isolated from patients in Beijing Chest Hospital. [b]No. of animals that survived / total no. of animals.

Compounds **2c** and **4c** with low oral acute lethal toxicity were evaluated for their *in vivo* PK profiles in ICR mice after a single oral administration of 25 mg/kg. As shown in

Table 6, both of them displayed good drug exposures, although shorter $T_{1/2}$ (1.25-4.79 h) than PBTZ169 (6.33 h). In particular, the AUC_{0-t} of **2c** were significantly higher than PBTZ169. Currently, these two compounds were all subjected to *in vivo* assessment.

Table 6. *In vivo* PK profiles of selected compounds in mice at 25 mg/kg

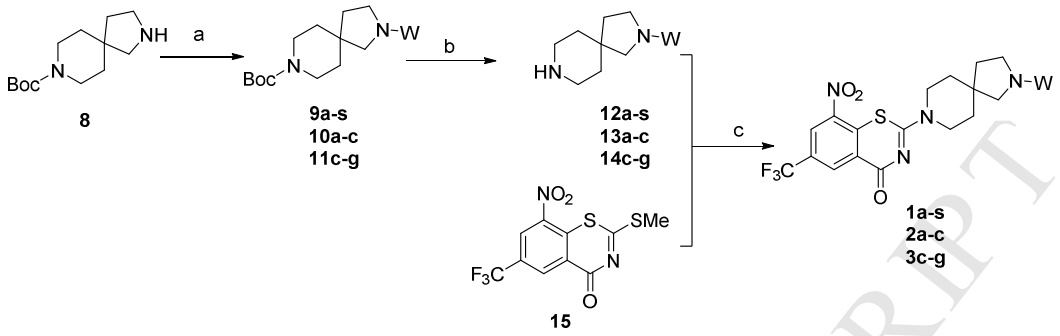
Compd.	$T_{1/2}$ (h)	Tmax (h)	C_{max} ($ng \cdot mL^{-1}$)	AUC_{0-t} ($h \cdot ng \cdot mL^{-1}$)
2c	4.79±1.19	0.50±0	1613±231	5033±311
4c	1.25±0.04	0.25±0	3040±1480	3550±1450
PBTZ169	6.33±1.07	0.33±0	1746±281	2475±702

3. Conclusion

In summary, we reported on optimization of lead compound IMB1603 discovered in our lab, including SAR studies of three moieties (benzene ring, linker, and N-heterocycle) on the C-2 side chain of the BTZ core, to identify alternatives with reduced hERG cardiac toxicity while keeping potent anti-TB activity. The optimization process led to compounds **2c** and **4c** which showed potent antimycobacterial activity ((MIC: <0.035-0.078 μM) against MTB H37Rv as well as two MDR-MTB clinical isolates, acceptable hERG affinity (IR: 23.0-34.9%) at 10 μM. In addition, these two compounds also displayed acceptable safety and good PK properties. Studies to determine the in vivo efficacy of **2c** and **4c** are currently underway.

4. Chemistry

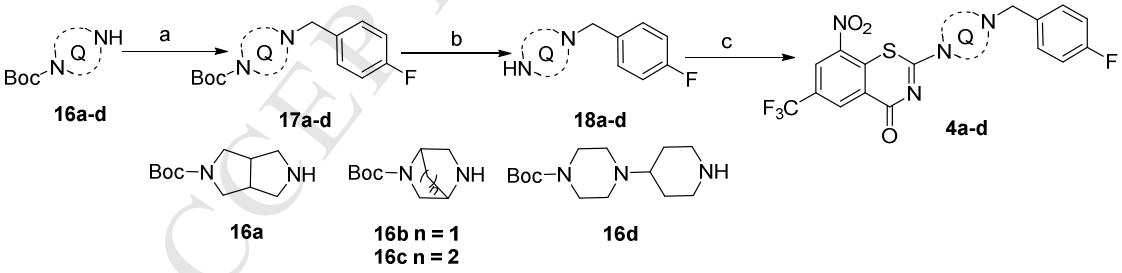
The synthesis of these molecules was carried out as shown in various schemes 1-2. A general synthetic route for compounds **1-3** was shown in scheme 1. Introduction of diverse W groups to 2,8-diazaspiro[4.5]decane **8** by previous reported reductive amination with aldehydes, Buchwald-Hartwig amination with 1-Bromo-4-Flurobenzene, nucleophilic substitution with bromides, or Ti(OiPr) mediated reductive amination with ketone gave compounds **9-11**, which upon removal of Boc protecting group afforded the desired side chain intermediates **12-14**. Coupling compounds **12-14** with benzothiazinone **15** yielded the targets **1-3**.



Reagents and conditions: a): i) ArCHO, $NaCNBH_3$, AcOH, rt-50 ^oC (for **9a-s**); OR iii) K_2CO_3, CH_3CN, 1-(2-Bromoethyl)-4-fluorobenzene or 1-(3-bromopropyl)-4-fluorobenzene (for **10a-b**); OR ii) $Pd(OAc)_2$, BINAP, 1-bromo-4-fluorobenzene, *t*-BuONa, anhydrous toluene (for **10c**); OR iv) $Ti(OiPr)_4$, ketone, MeOH, $NaCNBH_3$ (for **3c-g**); b): DCM, TFA; c): Et_3N, MeOH.

Scheme 1. Synthesis of compounds **1a-s**, **2a-c** and **3c-g**

The synthesis of compounds **4a-d** was shown in scheme 2. Reductive amination of commercially available compounds **16a-d** with 4-fluorobenzaldehyde afforded compounds **17a-d**. Removal of Boc-protecting group followed by coupling with compound **15** gave target compounds **4a-d**.



Reagents and conditions: a) 4-fluorobenzaldehyde OR cyclohexanecarbaldehyde, $NaCNBH_3$, AcOH, rt-50 ^oC; b) DCM, TFA; c) **15**, Et_3N, MeOH.

Scheme 2. Synthesis of compounds **4a-d**

5. Experiment section

MIC determination. MICs against replicating M. tuberculosis were determined by the microplate Alamar blue assay (MABA). PBTZ169 (synthesized by our lab), 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 fold 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.

Inhibition evaluation on hERG K+ channel. The electrophysiology recording of hERG channel current was carried out following the standard protocol as described previously.[30] 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 ^{o}C) 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.

Acute toxicity determination of compounds 2c and 4c. The toxicity study was carried out using female ICR mice weighting 18-21 g each. The animals were randomly

distributed to control group and treated groups, containing five 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 (0.5 mL), whereas each treated group received orally the agents suspended in 5% CMC solution (500 mg/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.

Pharmacokinetic Profiles determination of compounds 2c and 4c. SPF female ICR mice weighing 18–22 g were used in the pharmacokinetic study. The ICR mice were fasted overnight before dosing. Every treatment group contain 3 rats. Mice were dosed with the tested compounds suspension at 50 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 ($t_{1/2}$), 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.

General Chemical Methods. All commercially available solvents and reagents were used without further purification. All moisture sensitive reactions were carried out under Argon atmosphere in commercially available anhydrous solvents. ^{1}H NMR spectra were determined on a Varian Mercury-400 or Bruker 500 M spectrometer in MeOD, $CDCl_3$, or DMSO-d_6 using tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra was obtained on an Agilent 1260-6420 Mass spectrum instruments. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F254).

Purity was determined by HPLC, and all target compounds were confirmed to have >95% purity.

Purity determination. All samples were performed on an Agilent 1260 HPLC-UV system. Conditions (solvent A = methanol, solvent B = 0.1% TFA + H2O): Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm, PN: 883975-902). Injection volumn: 10 μL. Flow: 1.3 mL/min. Gradient elution: 0.00 min, 10% A; 3 min, 50% A; 15 min, 100% A; 16 min, 10% A; 18 min 10%A. UV at 254 nm.

General synthesis procedure of compounds 1b-s. To a stirring solution of **8** (0.3 mmol) in MeOH (5 mL) was added the corresponding aldehyde (0.4 mmol) and $NaCNBH_3$ (0.5 mmol) at room temperature. The mixture was adjusted to pH 6-7 by acetic acid, stirred overnight at room temperature, and quenched by 1 M NaOH solution (5 mL). The mixture was diluted by H_2O (15 mL), and extracted by DCM (10 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 = 20 : 1) to yield oils **9b-s** (70-93%).

To a stirred solution of **9b-s** (0.2 mmol) in DCM (5 mL) was added TFA (1 mL) at room temperature. The mixture was stirred for 2 hours and concentrated to afford the crude product **12b-s** which was used directly in the next step without further purification. To a stirred solution of above crude **12b-s** in anhydrous MeOH (10 mL) was added BTZ core compound **15** (0.2 mmol) and Et_3N (0.6 mmol) at room temperature. The mixture was stirred overnight at 40 ^oC, and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH = 20 : 1) to yield the yellow solids **1b-s.**

2-(2-(2-fluorobenzyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1b) According to above general procedure, employing 2-flurobenzaldehyde afforded compound **1b** as a yellow solid (30% for two steps), mp: 115-117 ^oC; HPLC purity: 95.3%, retention time 9.02 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.78 (s, 1H), 7.43-7.40 (m, 1H), 7.30-7.28 (m, 1H), 7.17-7.15 (m, 1H), 7.10-7.06 (m, 1H), 4.22-3.70 (m, 6H), 2.74 (brs, 2H), 2.54 (brs, 2H), 1.79 (brs, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.54, 161.28, 160.93 (d, J = 244.2 Hz), 144.75, 134.80, 131.73 (q, J = 3.5 Hz), 131.50 (d, J = 4.5 Hz), 129.30 (d, J = 8.1 Hz), 127.75 (q, J = 35.5

Hz), 126.56, 126.50, 125.83 (d, J = 15.1 Hz), 124.66 (d, J = 2.8 Hz), 123.01 (q, J = 274.1 Hz), 115.60 (d, J = 21.8 Hz), 65.35, 53.12, 52.24, 45.13, 40.84, 37.37, 36.12; HRMS-ESI (m/z): Calcd. For $C_{24}H_{23}F_4N_4O_3S$ $(M+H)^+$: 523.1422; Found: 523.1422.

8-nitro-2-(2-(4-(trifluoromethoxy)benzyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1c) According to above general procedure, employing 3-(trifluromethoxy)benzaldehyde afforded compound **1c** as a yellow solid (35% for two steps), mp: 119-121 ^oC; HPLC purity: 97.5%, retention time 10.51 min; ^1H NMR (500 MHz, $CDCl_3$) δ9.14 (s, 1H), 8.79 (s, 1H), 7.39 (brs, 2H), 7.22 (d, J = 7.70 Hz, 2H), 4.30-3.85 (m, 4H), 3.64 (s, 2H), 2.70 (brs, 2H), 2.49 (brs, 2H), 1.80 (s, 6H);^{13}C NMR (125 MHz, DMSO-d_6) δ165.66, 161.36, 147.62, 144.83, 139.16, 134.84, 131.73 (q, J = 3.5 Hz), 130.44, 127.75 (q, J = 35.5 Hz), 126.56, 123.01 (q, J = 274.1 Hz), 121.12, 120.57 (q, J = 252.4 Hz), 65.40, 58.94, 53.25, 45.10, 40.84, 36.12; HRMS-ESI (m/z): Calcd. For $C_{25}H_{23}F_6N_4O_4S$ $(M+H)^+$: 589.1339; Found: 589.1328.

8-nitro-2-(2-(4-nitrobenzyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1d) According to above general procedure, employing 4-nitrobenzaldehyde afforded compound **1d** as a yellow solid (42% for two steps), mp: 136-138 ^oC; HPLC purity: 99.8%, retention time 9.01 min; ^1H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.81 (s, 1H), 8.22 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 4.07 (brs, 4H), 3.74 (s, 2H), 2.62 (t, J = 6.3 Hz, 2H), 2.48 (s, 2H), 1.74 (brs, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.70, 161.39, 148.05, 146.92, 144.89, 134.86, 131.75 (q, J = 3.5 Hz), 129.72, 127.84 (q, J = 35.1 Hz), 126.64, 126.59, 123.88, 122.53 (q, J = 272.3 Hz), 65.35, 59.02, 53.30, 45.13, 37.15, 36.21; HRMS-ESI (m/z): Calcd. For $C_{24}H_{23}F_3N_5O_5S$ $(M+H)^+$: 550.1367; Found: 550.1369.

8-nitro-2-(2-(4-nitrilebenzyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1e) According to above general procedure, employing 4-formylbenzonitrile afforded compound **1e** as a yellow solid (33% for two steps), mp: 161-162 ^oC; HPLC purity: 96.4%, retention time 8.90 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.79 (s, 1H), 7.64 (d, J = 7.60 Hz, 2H), 7.48 (d, J = 7.60 Hz, 2H), 4.30-3.90 (m, 4H), 3.70 (s, 2H), 2.69 (brs, 2H), 2.50 (brs, 2H), 1.80 (brs, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.61, 161.33, 145.67, 144.81, 134.82, 132.65, 131.73 (q, J = 3.5

Hz), 129.57, 127.84 (q, J = 35.5 Hz), 126.59, 126.54, 123.11 (q, J = 274.1 Hz), 119.37, 65.33, 59.27, 53.27, 45.13, 40.90, 36.99, 36.13; HRMS-ESI (m/z): Calcd. For $C_{25}H_{23}F_3N_5O_3S$ $(M+H)^+$: 530.1468; Found: 530.1465.

2-(2-(2,3-difluorobenzyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1f) According to above general procedure, employing 2,3-difluorobenzaldehyde afforded compound **1f** as a yellow solid (41% for two steps), mp: 99-101 ^oC; HPLC purity: 99.6%, retention time 9.15 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.20-7.10 (m, 3H), 4.24-3.88 (m, 4H), 3.76 (s, 2H), 2.75 (brs, 2H), 2.56 (brs, 2H), 1.83 (brs, 6H);^{13}C NMR (125 MHz, DMSO-d_6) δ 165.61, 161.33, 150.12 (dd, J = 150.1 Hz, 13.1 Hz), 148.46 (dd, J = 251.1 Hz, 12.8 Hz), 144.83, 134.82, 132.65, 131.73 (q, J = 3.5 Hz), 128.68 (d, J = 10.7 Hz), 127.84 (q, J = 35.5 Hz), 126.59, 126.53, 124.93, 123.13 (q, J = 274.1 Hz), 116.34 (d, J = 16.8 Hz), 65.26, 53.07, 52.00, 45.05, 40.84, 37.06, 36.10; HRMS-ESI (m/z): Calcd. For $C_{24}H_{22}F_5N_4O_3S$ $(M+H)^+$: 541.1327; Found: 541.1326.

2-(2-(2,4-bis(trifluoromethyl)benzyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1g) According to above general procedure, employing 2,4-bis(trifluoromethyl)benzaldehyde afforded compound **1c** as a yellow solid (45% for two steps), mp: 137-138 ^oC; HPLC purity: 95.8%, retention time 10.95 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.98 (d, J = 7.2 Hz, 1H), 7.92 (s, 1H), 7.83 (d, J = 7.2 Hz, 1H), 4.24-3.88 (m, 4H), 3.88 (s, 2H), 2.75 (brs, 2H), 2.56 (brs, 2H), 1.83 (brs, 6H);^{13}C NMR (100 MHz, $CDCl_3$) δ 166.56, 161.75, 143.94, 142.66, 134.21, 133.40 (q, J = 3.5 Hz), 130.68, 129.62 (q, J = 35.3 Hz), 128.66, 126.73, 126.05 (q, J = 3.5 Hz), 123.76, 123.09, 121.05, 65.34, 55.28, 53.25, 44.67, 40.92, 37.24, 36.24; HRMS-ESI (m/z): Calcd. For $C_{26}H_{22}F_9N_4O_3S$ $(M+H)^+$: 641.1263; Found: 641.1250.

2-(2-(3,5-bis(trifluoromethyl)benzyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1h) According to above general procedure, employing 3,5-bis(trifluoromethyl)benzaldehydeafforded compound **1h** as a yellow solid (37% for two steps), mp: 148-149 ^oC; HPLC purity: 97.3%, retention time 11.25 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.85-7.80 (brs, 3H),

4.24-3.76 (m, 6H), 2.72 (brs, 2H), 2.52 (brs, 2H), 1.82 (brs, 6H); HRMS-ESI (m/z): Calcd. For $C_{26}H_{22}F_9N_4O_3S$ $(M+H)^+$: 641.1263; Found: 641.1234.

2-(2-(3-bromo-4-nitrobenzyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1i) According to above general procedure, employing 3-bromo-4-nitrobenzaldehyde afforded compound **1i** as a yellow solid (41% for two steps), mp: 150-152 ^oC; HPLC purity: 98.9%, retention time 9.71 min; ^1H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.80 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.86 (s, 1H), 7.59 (d, J = 8.2 Hz, 1H), 4.07-3.86 (m, 4H), 3.71 (s, 2H), 2.63 (t, J = 6.3 Hz, 2H), 2.48 (s, 2H), 1.74 (brs, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.68, 161.38, 148.60, 146.96, 144.88, 134.86, 134.30, 131.75 (q, J = 3.5 Hz), 128.98, 127.84 (q, J = 35.5 Hz), 126.59, 126.15, 123.11 (q, J = 274.1 Hz), 113.56, 65.22, 58.24, 53.21, 45.18, 40.96, 36.96, 36.17; HRMS-ESI (m/z): Calcd. For $C_{24}H_{22}BrF_3N_5O_5S$ $(M+H)^+$: 628.0472; Found: 628.0443.

2-(2-(naphthalen-1-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1j) According to above general procedure, employing 1-naphthaldehyde afforded compound **1j** as a yellow solid (44% for two steps), mp: 195-197 ^oC; HPLC purity: 95.6%, retention time 10.33 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.77 (s, 1H), 8.33 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 7.6 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.57-7.52 (m, 2H), 7.47-7.43 (m, 2H), 4.08-3.82 (m, 6H), 2.76 (brs, 2H), 2.54 (brs, 2H), 1.79 (brs, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ166.48, 161.58, 134.25, 133.77, 133.29 (q, J = 3.5 Hz), 132.22, 129.49 (q, J = 35.5 Hz), 128.41, 127.92, 126.70, 126.47, 125.90 (q, J = 3.5 Hz), 125.72, 125.67, 125.19, 124.46, 122.40 (q, J =272.4 Hz), 65.24, 58.33, 53.27, 44.96, 40.72, 37.49, 36.19; HRMS-ESI (m/z): Calcd. For $C_{28}H_{26}F_3N_4O_3S$ $(M+H)^+$: 555.1672; Found: 555.1665.

2-(2-(naphthalen-2-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1k) According to above general procedure, employing 2-naphthaldehyde afforded compound **1k** as a yellow solid (53% for two steps), mp: 170-172 ^oC; HPLC purity: 98.6%, retention time 10.46 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.78 (s, 1H), 7.86 (brs, 4H), 7.52 (brs, 3H), 4.01-3.78 (m, 6H), 2.78 (brs, 2H), 2.55 (brs, 2H), 1.82 (brs, 6H). ^{13}C NMR (125 MHz, $CDCl_3$)

δ166.48, 161.63, 143.88, 136.58, 134.24, 133.34, 133.26 (q, J = 3.5 Hz), 132.72, 129.56 (q, J = 35.5 Hz), 127.96, 127.66, 127.64, 126.95, 126.72, 126.01, 125.92 (q, J = 4.0 Hz), 125.62, 122.40 (q, J =272.4 Hz), 65.24, 60.35, 53.37, 44.99, 40.72, 37.30, 36.40; HRMS-ESI (m/z): Calcd. For $C_{28}H_{26}F_3N_4O_3S$ $(M+H)^+$: 555.1672; Found: 555.1654.

2-(2-((5-methoxy-1H-indol-2-yl)methyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1l) According to above general procedure, employing 5-methoxy-1H-indole-3-carbaldehyde afforded compound **1l** as a yellow solid (50% for two steps), mp: 199-201 ^oC; HPLC purity: 95.1%, retention time 9.51 min; ^1H NMR (500 MHz, DMSO-d_6) δ 10.72 (s, 1H), 8.86 (s, 1H), 8.81 (s, 1H), 7.24 (d, J = 8.5 Hz, 1H), 7.19 (s, 1H), 7.14 (s, 1H), 6.76 (d, J = 8.2 Hz, 1H), 4.08-3.72 (m, 4H), 3.78 (s, 3H), 3.71 (s, 2H), 2.60 (brs, 2H), 2.46 (brs, 2H), 1.69 (brs, 6H); HRMS-ESI (m/z): Calcd. For $C_{27}H_{27}F_3N_5O_4S$ $(M+H)^+$: 574.1730; Found: 574.1728.

8-nitro-2-(2-(thiophen-2-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1m) According to above general procedure, employing thiophene-2-carbaldehyde afforded compound **1m** as a yellow solid (46% for two steps), mp: 143-145 ^oC; HPLC purity: 96.9%, retention time 8.75 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.80 (s, 1H), 7.28-7.26 (m, 1H), 6.99-6.96 (m, 2H), 4.10-3.88 (m, 6H), 2.77 (brs, 2H), 2.55 (brs, 2H), 1.82 (brs, 6H);^{13}C NMR (125 MHz, DMSO-d_6) δ 165.67, 161.37, 144.88, 143.23, 134.86, 131.72 (q, J = 3.5 Hz), 127.80 (q, J = 35.5 Hz), 126.95, 126.60, 126.56, 123.15 (q, J = 274.1 Hz), 65.03, 54.19, 53.02, 45.15, 40.85, 36.97, 36.12; HRMS-ESI (m/z): Calcd. For $C_{22}H_{22}F_3N_4O_3S_2$ $(M+H)^+$: 511.1080; Found: 511.1058.

2-(2-((1-methyl-1H-pyrrol-2-yl)methyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1n) According to above general procedure, employing 1-methyl-1H-pyrrole-2-carbaldehyde afforded compound **1n** as a yellow solid (38% for two steps), mp: 160-162 ^oC; HPLC purity: 95.5%, retention time 8.84 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.78 (s, 1H), 6.62 (s, 1H), 6.06 (brs, 1H), 6.02 (brs, 1H), 4.23-3.82 (m, 4H), 3.69 (s, 3H), 3.59 (brs, 2H), 2.65 (brs, 2H), 2.45 (brs, 2H), 1.76 (brs, 6H);^{13}C NMR (125 MHz, DMSO-d_6) δ 165.63, 161.34, 144.84, 134.85, 131.73 (q, J = 3.5 Hz), 129.95, 127.75 (q, J = 35.5 Hz), 126.57, 123.04 (q, J =

272.4 Hz), 122.59, 108.33, 106.22, 65.46, 53.10, 51.41, 45.13, 37.42, 35.99, 33.80; HRMS-ESI (m/z): Calcd. For $C_{23}H_{25}F_3N_5O_3S$ $(M+H)^+$: 508.1625; Found: 508.1649.

8-nitro-2-(2-(pyridin-2-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1o) According to above general procedure, employing picolinaldehyde afforded compound **1o** as a yellow solid (37% for two steps), mp: 120-122 ^oC; HPLC purity: 98.8%, retention time 8.37 min; ^1H NMR (500 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.77 (s, 1H), 8.47 (d, J = 4.3 Hz, 1H), 7.76 (t, J = 7.5 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.25-7.23 (m, 1H), 3.83 (brs, 4H), 3.69 (s, 2H), 2.62 (t, J = 6.7 Hz, 2H), 2.48 (brs, 2H), 1.80-1.68 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.67, 161.36, 159.46, 149.17, 144.88, 137.00, 134.86, 131.74 (q, J = 3.5 Hz), 127.75 (q, J = 35.5 Hz), 126.59, 123.04 (q, J = 272.4 Hz), 122.80, 122.50, 108.33, 106.22, 65.54, 61.68, 53.40, 45.20, 40.90, 37.42, 37.05, 36.27; HRMS-ESI (m/z): Calcd. For $C_{23}H_{23}F_3N_5O_3S$ $(M+H)^+$: 506.1468; Found: 506.1451.

8-nitro-2-(2-(pyridin-3-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1p) According to above general procedure, employing nicotinaldehyde afforded compound **1p** as a yellow solid (39% for two steps), mp: 154-156 ^oC; HPLC purity: 97.5%, retention time 7.53 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.78 (s, 1H), 8.59-8.56 (m, 2H), 7.73 (s, 1H), 7.30 (brs, 1H), 4.19-3.88 (m, 4H), 3.69 (brs, 2H), 2.72 (brs, 2H), 2.52 (brs, 2H), 1.81 (brs, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.56, 161.74, 150.01, 148.79, 143.94, 136.38, 134.23, 133.33 (q, J = 3.0 Hz), 129.63 (q, J = 35.5 Hz), 126.73, 125.96 (q, 3.6 Hz), 123.48, 122.52 (q, J = 273.2 Hz), 65.14, 57.37, 53.23, 44.7, 40.77, 37.31, 36.17; HRMS-ESI (m/z): Calcd. For $C_{23}H_{23}F_3N_5O_3S$ $(M+H)^+$: 506.1468; Found: 506.1457.

8-nitro-2-(2-(pyridin-4-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1q) According to above general procedure, employing isonicotinaldehyde afforded compound **1q** as a yellow solid (48% for two steps), mp: 137-139 ^oC; HPLC purity: 99.0%, retention time 7.32 min; ^1H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.81 (s, 1H), 8.53 (d, J = 5.1 Hz, 2H), 7.36 (d, J = 5.1 Hz, 2H), 4.07-3.85 (m, 4H), 3.63 (s, 2H), 2.62 (t, J = 6.3 Hz, 2H), 2.46 (s, 2H), 1.72 (brs, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.65, 161.36, 150.00, 148.70, 144.86, 134.85, 131.73 (q,

J = 3.5 Hz), 127.75 (q, J = 35.5 Hz), 126.58, 123.78, 123.08 (q, J = 272.4 Hz), 65.36, 58.59, 53.34, 45.17, 37.01, 36.19; HRMS-ESI (m/z): Calcd. For $C_{23}H_{23}F_3N_5O_3S$ $(M+H)^+$: 506.1468; Found: 506.1486.

2-(2-((1l2-pyrrol-2-yl)methyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1r) According to above general procedure, employing 1H-pyrrole-2-carbaldehyde afforded compound **1r** as a yellow solid (52% for two steps), mp: 94-96 ^oC; HPLC purity: 95.1%, retention time 8.90 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 6.82 (s, 1H), 6.16 (s, 1H), 6.09 (s, 1H), 4.17-3.90 (m, 4H), 3.75 (brs, 2H), 2.80 (brs, 2H), 2.59 (brs, 2H), 1.84 (brs, 6H);^{13}C NMR (100 MHz, $CDCl_3$) δ 166.56, 161.84, 143.94, 134.16, 133.41 (q, J = 3.5 Hz), 129.65 (q, J = 35.5 Hz), 126.70, 126.03 (q, J = 3.2 Hz), 122.54 (q, J = 272.3 Hz), 118.42, 107.97, 64.46, 58.49, 52.89, 44.58, 40.81, 37.11, 35.98; HRMS-ESI (m/z): Calcd. For $C_{22}H_{23}F_3N_5O_3S$ $(M+H)^+$: 494.1468; Found: 494.1492.

8-nitro-2-(2-(oxazol-2-ylmethyl)-2,8-diazaspiro[4.5]decan-8-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (1s) According to above general procedure, employing oxazole-5-carbaldehyde afforded compound **1s** as a yellow solid (42% for two steps), HPLC purity: 95.5%, retention time 7.90 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.75 (s, 1H), 7.19 (s, 1H), 4.17-3.90 (m, 4H), 3.12-2.96 (m, 4H), 1.84 (brs, 6H);); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.67, 161.84, 161.37, 144.87, 140.26, 134.85, 131.73 (q, J = 3.5 Hz), 127.80 (q, J = 35.5 Hz), 127.31, 126.60, 126.56, 123.15 (q, J = 274.1 Hz), 64.91, 52.81, 51.17, 44.41, 40.92, 37.14, 36.24; HRMS-ESI (m/z): Calcd. For $C_{21}H_{21}F_3N_5O_4S$ $(M+H)^+$: 496.1261; Found: 496.1266.

General synthesis procedure of compounds 2a-b. To a stirring solution of **8** (0.5 mmol) in CH_3CN (10 mL) was added 1-(2-Bromoethyl)-4-fluorobenzene or 1-(3-bromopropyl)-4-fluorobenzene (0.5 mmol) and K_2CO_3 (1.0 mmol) at room temperature. The mixture was stirred overnight at room temperature and filtered. The filtrate was diluted by H_2O (25 mL), and extracted by DCM (20 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 = 20 : 1) to yield oils **10a-b** (yield, 65-89%).

To a stirred solution of **10a-b** (0.2 mmol) in DCM (5 mL) was added TFA (1 mL) at room temperature. The mixture was stirred for 2 hours and concentrated to afford the crude product **13a-b** which was used directly in the next step without further purification. To a stirred solution of above crude **13a-b** in anhydrous MeOH (10 mL) was added BTZ core compound **15** (0.2 mmol) and Et_3N (0.6 mmol) at room temperature. The mixture was stirred overnight at 40 ^oC, and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH = 20 : 1) to yield the yellow solids, which were further treated by n-hexane to give compounds **2a-b.**

2-(2-(4-fluorophenethyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (2a) According to above general procedure, employing 1-(2-Bromoethyl)-4-fluorobenzene afforded compound **2a** as a yellow solid (38% for two steps), mp: 174-175 ^oC; HPLC purity: 96.8%, retention time 9.63 min; ^1H NMR (500 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.79 (s, 1H), 7.30 (brs, 2H), 7.15 (brs, 2H), 4.07-3.87 (m, 4H), 3.33 (s, 4H), 2.94 (brs, 4H), 1.76 (brs, 6H); HRMS-ESI (m/z): Calcd. For $C_{25}H_{25}F_4N_4O_3S$ $(M+H)^+$: 537.1578; Found: 537.1596.

2-(2-(3-(4-fluorophenyl)propyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (2b) According to above general procedure, employing 1-(3-bromopropyl)-4-fluorobenzene afforded compound **2b** as a yellow solid (45% for two steps), HPLC purity: 95.4%, retention time 9.95 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.79 (s, 1H), 7.18 (brs, 2H), 7.03-7.01 (m, 2H), 4.20-3.84 (m, 6H), 3.07-2.65 (m, 4H), 2.34-2.10 (m, 4H), 1.81 (brs, 6H); HRMS-ESI (m/z): Calcd. For $C_{26}H_{27}F_4N_4O_3S$ $(M+H)^+$: 551.1735; Found: 551.1739.

2-(2-(4-fluorophenyl)-2,8-diazaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (2c) To a stirring solution of **8** (60 mg, 0.3 mmol) in anhydrous toluene (5 mL) was added 1-bromo-4-fluorobenzene (45 μL, 0.4 mmol), BINAP (19 mg, 0.03 mmol), t-BuONa (58 mg, 0.6 mmol), and $Pd(OAc)_2$ (7 mg, 0.03 mmol) at room temperature. The mixture was stirred overnight at room temperature and filtered. The filtrate was diluted by H_2O (15 mL), and extracted by DCM (10 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 (EtOAc: n-hexane = 5 : 1) to yield oils **10c** (58 mg, 58%).

To a stirred solution of **10c** (50 mg, 0.15 mmol) in DCM (5 mL) was added TFA (2 mL) at room temperature. The mixture was stirred for 2 hours and concentrated to afford the crude product **13c** which was used directly in the next step without further purification. To a stirred solution of above crude **13c** in anhydrous MeOH (10 mL) was added BTZ core compound **15** (48 mg, 0.15 mmol) and Et_3N (0.6 mmol) at room temperature. The mixture was stirred overnight at 40 ^oC, and concentrated. The residue purified by column chromatography over silica gel (DCM : MeOH = 20 : 1) to yield the yellow solids, which were further treated by n-hexane to give **2c** (32 mg, 43%), mp: 192-194 ^oC; HPLC purity: 96.3%, retention time 14.07 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.80 (s, 1H), 7.00 (t, J = 8.5 Hz, 2H), 6.53-6.50 (m, 2H), 4.20-3.84 (m, 4H), 3.45 (t, J = 6.7 Hz, 2H), 3.26 (brs, 2H), 2.03 (t, J = 6.7 Hz, 2H), 1.86 (brs, 4H); HRMS-ESI (m/z): Calcd. For $C_{23}H_{21}F_4N_4O_3S$ $(M+H)^+$: 509.1265; Found: 509.1245.

General synthesis procedure of compounds 3c-g. A mixture of compound **8** (0.3 mmol) and corresponding ketones (0.4 mmol) in $Ti(OPr)_4$ was stirred at 70 ^oC for 8 hours and cooled to room temperature. MeOH (5 mL) and $NaCNBH_3$ (1.6 mmol) was added to the mixture, and stirred for 5 hours at 40 ^oC. The mixture was quenched by 1 N NaOH (10 mL), filtered by celite, and washed by MeOH. The MeOH was evaporated under vacuo. The residue was diluted by H_2O, and extracted by Et_2O. 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 = 30 : 1) to yield oils **11c-g** (yield, 40-70%).

To a stirred solution of **11c-g** in DCM (5 mL) was added TFA (1 mL) at room temperature. The mixture was stirred for 2 hours and concentrated to afford the crude product **14c-g** which was used directly in the next step without further purification. To a stirred solution of above crude **14c-g** in anhydrous MeOH (10 mL) was added BTZ core compound **15** (0.3 mmol) and Et_3N (0.6 mmol) at room temperature. The mixture was stirred overnight at 40 ^oC, and concentrated. The residue was purified by column

chromatography over silica gel (DCM : MeOH = 20 : 1) to yield the yellow solids, which were further treated by n-hexane to give **3c-g.**

2-**(9-(1-(4-bromophenyl)ethyl)-3,9-diazaspiro[5.5]undecan-3-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3c)** According to above general procedure, employing 1-(4-bromophenyl)ethan-1-one as ketone afforded compound **3c** as a yellow solid (38% for two steps), mp: 171-172 ^{o}C; HPLC purity: 97.7%, retention time 10.67 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (s, 1H), 7.53 (d, J = 7.9 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 3.96 (brs, 2H), 3.79 (brs, 2H), 3.48 (q, J = 6.8 Hz, 1H), 2.39 (brs, 2H), 2.32 (brs, 2H), 1.52 (brs, 8H), 1.29 (d, J = 6.5 Hz, 3H);^{13}C NMR (100 MHz, DMSO-d_6) δ 165.69, 161.37, 144.90, 143.81, 134.90, 131.71, 131.4, 130.05, 126.63, 122.53 (q, J = 272.5 Hz), 120.04, 63.44, 45.76, 35.55, 30.41, 19.33; HRMS-ESI (m/z): Calcd. For $C_{26}H_{27}BrF_3N_4O_3S$ $(M+H)^+$: 611.0934; Found: 611.0936.

2-(9-(1-(4-fluorophenyl)ethyl)-3,9-diazaspiro[5.5]undecan-3-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3d) According to above general procedure, employing 1-(4-fluorophenyl)ethan-1-one as ketone afforded compound **3d** as a yellow solid (35% for two steps), mp: 166-167 ^{o}C; HPLC purity: 97.5%, retention time 9.74 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.30 (brs, 2H), 7.06-7.03 (m, 2H), 4.11 (brs, 2H), 3.83 (brs, 2H), 3.46 (q, J = 6.4 Hz, 1H), 2.50 (brs, 2H), 2.42 (brs, 2H), 1.82 (brs, 8H), 1.41 (d, J = 6.4 Hz, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 166.55, 162.35 (d, J = 247.5 Hz), 161.69, 143.93, 139.66 (d, J = 3.0 Hz), 134.29, 133.35 (q, J = 3.5 Hz), 129.54 (q, J = 35.5 Hz), 128.98, 126.79, 125.96 (q, J = 3.7 Hz), 122.54 (q, J = 272.5 Hz), 115.12, 114.91, 64.17, 45.99, 43.06, 35.95, 30.25, 19.69; HRMS-ESI (m/z): Calcd. For $C_{26}H_{27}F_4N_4O_3S$ $(M+H)^+$: 551.1735; Found: 551.1721.

2-(9-(1-(4-chlorophenyl)ethyl)-3,9-diazaspiro[5.5]undecan-3-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3e) According to above general procedure, employing 1-(4-chlorophenyl)ethan-1-one as ketone afforded compound **3e** as a yellow solid (46% for two steps), mp: 178-179 ^{o}C; HPLC purity: 98.9%, retention time 10.42 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.30-7.28 (m, 4H), 4.11 (brs, 2H), 3.83 (brs, 2H), 3.45 (q, J = 6.4 Hz, 1H), 2.50 (brs, 2H), 2.41 (brs, 2H), 1.82 (brs, 8H), 1.38 (d, J = 6.4 Hz, 3H);^{13}C NMR (125 MHz, DMSO-d_6)165.69, 161.38,

144.91, 143.35, 134.89, 131.74, 131.56, 129.66, 128.52, 127.88, 126.65, 123.10 (q, J = 272.5 Hz), 63.38, 45.79, 35.55, 30.40, 19.32; HRMS-ESI (m/z): Calcd. For $C_{26}H_{27}ClF_3N_4O_3S$ $(M+H)^+$: 567.1439; Found: 567.1427.

8-nitro-6-(trifluoromethyl)-2-(9-(1-(4-(trifluoromethyl)phenyl)ethyl)-3,9-diazaspiro[5.5]undecan-3-yl)-4H-benzo[e][1,3]thiazin-4-one (3f) According to above general procedure, employing 1-(4-(trifluoromethyl)phenyl)ethan-1-one as ketone afforded compound **3f** as a yellow solid (51% for two steps), mp: 141-142 ^oC; HPLC purity: 97.3%, retention time 10.64 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.79 (s, 1H), 7.61 (d, J = 7.8 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 4.12 (brs, 2H), 3.83 (brs, 2H), 3.50 (q, J = 6.4 Hz, 1H), 2.52 (brs, 2H), 2.41 (brs, 2H), 1.63 (brs, 8H), 1.40 (d, J = 6.4 Hz, 3H);^{13}C NMR (100 MHz, DMSO-d_6) δ 164.60, 160.30, 148.48, 143.83, 133.81, 130.67 (q, J = 3.6 Hz), 127.47, 126.71 (q, J = 35.5 Hz), 125.57, 125.51 (q, J = 3.5 Hz), 124.43, 124.41, 122.08 (q, J = 272.4 Hz), 62.68, 44.85, 42.13, 34.48, 29.31, 18.29; HRMS-ESI (m/z): Calcd. For $C_{27}H_{27}F_6N_4O_3S$ $(M+H)^+$: 601.1703; Found: 601.1695.

8-nitro-6-(trifluoromethyl)-2-(9-(1-(4-(trifluoromethyl)phenyl)propyl)-3,9-diazaspiro[5.5]undecan-3-yl)-4H-benzo[e][1,3]thiazin-4-one (3g) According to above general procedure, employing 1-(4-(trifluoromethyl)phenyl)propan-1-one as ketone afforded compound **3g** as a yellow solid (38% for two steps), mp: 231-233 ^oC; HPLC purity: 99.1%, retention time 10.89 min; ^1H NMR (500 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.81 (s, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.79 (d, J = 7.5 Hz, 2H), 3.98 (brs, 2H), 3.80 (brs, 2H), 3.69 (d, J = 10.5 Hz, 1H), 3.39 (brs, 2H), 3.02 (brs, 2H), 2.32 (brs, 1H), 2.08 (brs, 1H), 2.03-1.54 (m, 8H), 1.29 (t, J = 6.5 Hz, 3H); HRMS-ESI (m/z): Calcd. For $C_{28}H_{29}F_6N_4O_3S$ $(M+H)^+$: 615.1859; Found: 615.1846.

General synthesis procedure of compounds 4a-d. To a stirring solution of **16a-d** (0.3 mmol) in MeOH (5 mL) was added the f 4-fluorobenzaldehyde (0.4 mmol) and $NaCNBH_3$ (0.5 mmol) at room temperature. The mixture was adjusted to pH 6-7 by acetic acid, stirred overnight at room temperature, and quenched by 1 M NaOH solution (5 mL). The mixture was diluted by H_2O (15 mL), and extracted by DCM (10 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 = 20 : 1) to yield oils **17a-d** (yields，65-83%).

To a stirred solution of **17a-d** (0.3 mmol) in DCM (10 mL) was added TFA (3 mL) at room temperature. The mixture was stirred for 2 hours and concentrated to afford the crude product **18a-d** which was used directly in the next step without further purification. To a stirred solution of above crude **18a-d** in anhydrous MeOH (10 mL) was added BTZ core compound **15** (0.3 mmol) and Et_3N (0.6 mmol) at room temperature. The mixture was stirred overnight at 40 ^oC, and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH = 20 : 1) to yield the yellow solids, which were further treated by n-hexane to give **4a-d.**

2-(5-(4-fluorobenzyl)hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4a) According to above general procedure, employing compound **16a** and 4-fluorobenzaldehyde afforded compound **4a** as a yellow solid (45% for two steps), mp: 175-176 ^oC; HPLC purity: 95.2%, retention time 8.96 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.81 (s, 1H), 7.30 (brs, 2H), 7.05-7.01 (m, , 2H), 4.23 (brs, 1H), 4.03-3.96 (m, 2H), 3.64 (m, 3H), 3.17 (brs, 1H), 3.04 (brs, 1H), 2.73-2.61 (m, 4H);^{13}C NMR (125 MHz, DMSO-d_6) δ165.03, 161.62 (d, J = 243.6 Hz), 159.43, 144.50, 135.53, 135.13, 132.10, 130.60, 130.54, 127.83 (q, J = 35.5 Hz), 126.64, 125.50 (q, J = 3.6 Hz), 123.14 (q, J = 272.3 Hz), 115.41, 115.27,59.94, 59.81, 58.03, 56.35, 53.48, 41.74; HRMS-ESI (m/z): Calcd. For $C_{22}H_{19}F_4N_4O_3S$ $(M+H)^+$: 495.1109; Found: 495.1109.

2-(5-(4-fluorobenzyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4b) According to above general procedure, employing compound **16b** (tert-butyl 2,5-diazabicyclo[2.2.1]heptane-2-carboxylate) and 4-fluorobenzaldehyde afforded compound **4b** as a yellow solid (47% for two steps), mp: 240-241 ^oC; HPLC purity: 95.2%, retention time 8.59 min; ^1H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.81 (s, 1H), 5.49 (brs, 0.6H), 4.81 (brs, 0.4H), 4.22 (brs, 0.4H), 3.84-3.59 (m, 4.6H), 3.17-2.76 (m, 2H), 2.24-2.14 (m, 1H), 2.02-1.89 (m, 1H);^{13}C NMR (125 MHz, DMSO-d_6) δ 166.19, 166.00, 162.88, 161.26, 160.59, 159.33, 143.59, 143.54, 134.43, 134.31, 133.73, 133.68, 129.88, 129.85, 129.83, 129.49, 127.05, 126.98,

125.95 (q, J = 3.5 Hz), 125.86 (q, J = 3.5 Hz), 122.40 (q, J = 272.4 Hz), 115.40, 115.38, 115.26, 60.90, 60.24, 59.70, 59.12, 58.78, 58.74, 57.91, 57.15, 54.45, 52.56, 36.55, 34.83; HRMS-ESI (m/z): Calcd. For $C_{21}H_{17}F_4N_4O_3S$ $(M+H)^+$: 481.0952; Found: 481.0931.

2-(5-(4-fluorobenzyl)-2,5-diazabicyclo[2.2.2]octan-2-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4c) According to above general procedure, employing compound **16c** (tert-butyl 2,5-diazabicyclo[2.2.2]octane-2-carboxylate) and 4-fluorobenzaldehyde afforded compound **4c** as a yellow solid (49% for two steps), HPLC purity: 95.1%, retention time 8.20 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86-8.80 (m, 2H), 7.38-7.35 (m, 2H), 7.15-7.12 (m, 2H), 5.01 (brs, 0.6H), 4.39 (brs, 0.4H), 4.04-3.97 (m, 1H), 3.78-3.70 (m, 3H), 3.07-3.03 (m, 1H), 2.91-2.83 (m, 2H), 2.07 (brs, 1H), 1.96 (brs, 1H), 1.86 (brs, 1H), 1.68 (brs, 1H); HRMS-ESI (m/z): Calcd. For $C_{22}H_{19}F_4N_4O_3S$ $(M+H)^+$: 495.1109; Found: 495.1093.

2-(4-(1-(4-fluorobenzyl)piperidin-4-yl)piperazin-1-yl)-8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4d) According to above general procedure, employing compound **16d** and 4-fluorobenzaldehyde afforded compound **4d** as a yellow solid (55% for two steps), mp: 157-158 ^{o}C; HPLC purity: 95.0%, retention time 7.45 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.78 (s, 1H), 7.31 (dd, J = 7.2 Hz, 6.5 Hz, 2H), 7.12 (t, J = 8.0 Hz, 2H), 3.93 (brs, 2H), 3.83 (brs, 2H), 3.42 (s, 2H), 2.82 (d, J = 10.4 Hz, 2H), 2.63 (brs, 4H), 2.25 (t, J = 11.1 Hz, 1H), 1.92 (brs, 2H), 1.72 (d, J = 10.5 Hz), 1.41 (dd, J = 11.1 Hz, 10.4 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.64, 162.49, 161.67 (d, J =245.5 Hz), 144.87, 134.73, 131.79, 131.07, 127.84 (q, J = 34.5 Hz), 126.63, 126.60, 123.10 (q, J = 272.4 Hz), 125.37, 115.23, 61.54, 61.36, 52.78, 48.77, 40.53, 28.19; HRMS-ESI (m/z): Calcd. For $C_{25}H_{26}F_4N_5O_3S$ $(M+H)^+$: 552.1687; Found: 552.1668.

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Highlights

1. **2c** and **4c** showed lower hERG binding affinity (IR < 50% at 10 μM) than IMB1603.
2. **2c** and **4c** exhibited potent *in vitro* anti-TB activity (MIC < 0.035-0.078 μM).
3. **2c** and **4c** have acceptable safety and pharmacokinetic properties.