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Fragment-Based Drug Discovery of 2-Thiazolidinones as BRD4 inhibitors: 2. Structure-based Optimization

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Abstract

The signal transduction of acetylated histone can be processed through a recognition module—bromodomain. Several inhibitors targeting BRD4, one of the bromodomain members, are in clinical trials as anticancer drugs. Hereby, we report our efforts on discovery, optimization of a new series of 2-thiazolidinones as BRD4 inhibitors along our previous study. In this work, guided by crystal structure analysis, we reversed the sulfonamide group and identified a new binding mode. SAR study on this new series led to several potent BRD4 inhibitors with IC_{50} about 0.05-0.1 μM in FP binding assay and GI_{50} 0.1-0.3 μM in cell based assays. To complete the lead-like assessment of this series, we further checked its effects on BRD4 downstream protein c-Myc, investigated its selectivity among five different bromodomain proteins, as well as the metabolic stability test, and reinforced the utility of 2-thiazolidinone scaffold as BET bromodomain inhibitors in novel anti-cancer drug development.

Keywords: SBDD; fragment; crystallography; 2-thiazolidinone; BRD4; bromodomain

Introduction

Lysine acetylation has been recognized as an important step of post-translational modifications in epigenetics.[1-3] Studies have shown that histone acetyltransferases (HATs) can add an acetyl group to the ε-nitrogen atom of lysine residues in histones to exert its "writer" function in histone modification.[4] This important mark can be erased by another type of enzymes called histone deactylases (HDACs, or the "eraser").[5, 6] For signal transduction involving acetylated histones, a "reader", as a recognition module binding to the acetylated histones is required. Currently, bromodomain was known as an important module to recognize and bind to acetylated histones.[7, 8]

The bromodomain was identified to function as lysine actylation reader in the early 1990s in the *brahma* gene from *Drosophila Melanogaster*.[9] After analysis of human genome, it was found that 46 different nuclear and cytoplasmic proteins contain 61 bromodomains.[10] These proteins include HATs and HAT-associated proteins, histone methyltransferases, helicases, ATP-dependent chromatin remodeling complexes, transcriptional co-activators, TBP-associated factors, and nuclear scaffolding proteins. Among them, BET (bromodomain and extra-terminal) proteins have received increasing interest as it was associated with the cancer development in multiple myeloma and medulloblastoma.[11] Besides, BRD4, an important member of BET subfamily, has recently been identified as a component of a recurrent chromosomeal translocation in a very aggressive form of human squamous carcinoma.[12] This

translocation expresses the tandom bromodomains of BRD4 combining with the NUT (nuclear protein in testis) protein to form NUT midline carcinoma (NMC). Previous in vivo studies[13] showed that the bromodomain inhibitors suppressed tumor growth in several mouse models, such as acute myeloid leukaemia (AML) and melanoma, and underscored the broad utility and therapeutic potential of the inhibitors of human bromodomain proteins.

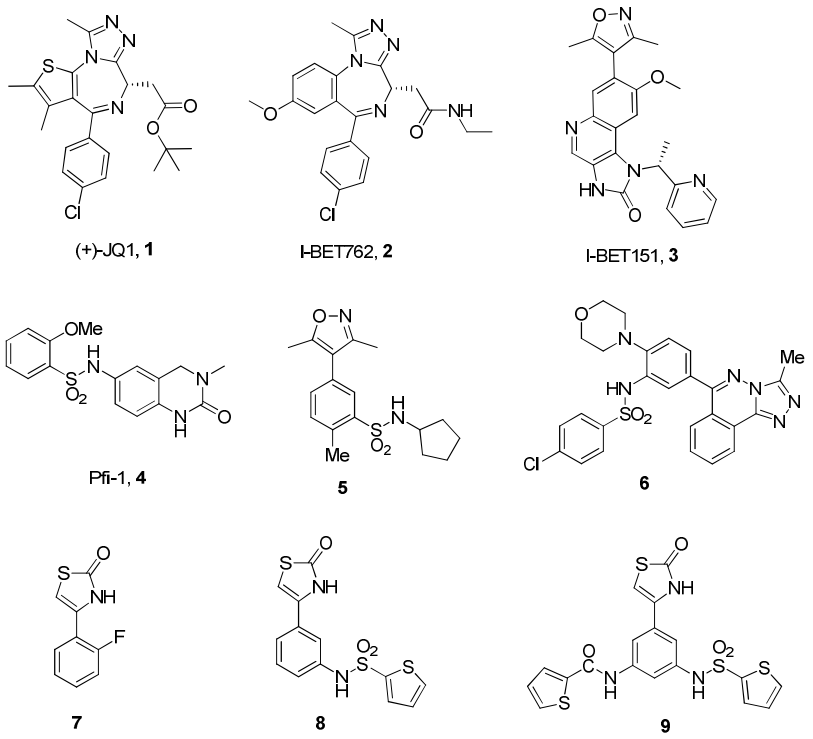


Figure 1. Structures of representative inhibitors of BET bromodomains: (+)-JQ1 (**1**)[12], I-BET762 (**2**)[14], I-BET151[15] (**3**), Pfi-1 (**4**)[16], **5**[17], **6**[18] and our previously reported BET bromodomain ligands **7**, **8**, and **9**[19].

With the importance of BRD4 in cancer development, many approaches were applied to discover novel inhibitors against BET family. Despite many fragment hits

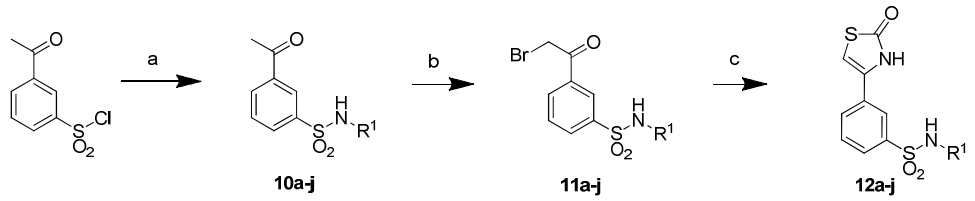
were disclosed in recent years,[7] there are only a few chemical scaffolds (Figure 1) became later potent inhibitors against bromodomain of BET subfamily, especially BRD4.[20-22] A molecule, (+)-JQ1, containing triazolothienodiazepine core scaffold, developed by Qi et al., was identified as a selective inhibitor of BET subfamily, and demonstrated its antitumor efficacy in the xenograft model of NMC.[12] Specificity of inhibitor JQ1 against other bromodomain proteins was also investigated in thermal shift assay, a method using the change in protein melting temperature to account for the inhibitor binding affinity. From the assay, it was found that (+)-JQ1 only showed the affinity to BET subfamily of bromodomains as it increased the melting temperature of bromodomains of BRD2, BRD3 and BRD4 more than 6 ℃. Meanwhile, Nicodeme et al. described that a similar molecule I-BET762 could suppress the inflammatory gene expression by inhibiting the BET family of proteins.[14, 23] Later, this compound was further extended to antitumor therapy and successfully entered into clinical trials in 2012. Besides these similar scaffolds, several novel series of potent compounds were developed in pharmaceutical and academic research group. 3,5-Dimethyl-isoxazole-containing I-BET151 was first discovered in GSK through fragment-based approach, then optimized to a potent and selective BET inhibitor, and demonstrated its in vivo efficacy murine model of MLL-AF9 leukaemia.[15, 24] In 2012, Pfizer identified a novel scaffold 3,4-dihydro-3-methyl-2(1H)-quinazolinone through fragment-based drug discovery. This scaffold was further optimized to sulfonamide derivatives with IC_{50} around 0.1-0.5 μM in a peptide displacement biochemical assay.[16] The selectivity of IBET-762 and IBET-151 were reported by Dawson et al., also

showing they are selective BET subfamily bormodomain inhibitors in a fluorescence anisotropy (FP) assay: IBET-762 has IC_{50} values of 0.79 μM, 0.4μM and 0.63μM to BRD2, BRD3 and BRD4 respectively; and IBET-151 has IC_{50} of 0.5, 0.25 and 0.79 μM to BRD2, BRD3 and BRD4. Other interesting scaffolds, including 3,5-Dimethylisoxazole and [1,2,4]triazolo[4,3-a]phthalazine, were also developed as potent BET bromodomain inhibitors in Structural Genomics Consortium. Similar to I-BET151, Compound **5** utilizes the isoxazole group to form hydrogen bonding interaction with the conserved residue Asparagine (N140 in BRD4 protein). Compound **6** containing a novel [1,2,4]triazolo[4,3-a]phthalazine scaffold was developed by Brennan et al. as a BRD4 inhibitor, but also shows weak binding to other bromodomains, including ATAD2, TIF1α and PCAF.

Previously, our BRD4 inhibitor discovery by computational docking and X-ray crystallography study revealed that 2-thiazolidinones **7**, **8** and **9** could mimic the acetylated lysine moiety to bind to the first bromodomain of BRD4 protein (BRD4(I)).[19] Initial optimization and lead-like assessment demonstrated the potential of this new scaffold. Herein, further SAR study of this new series of compounds will be reported. Guided by co-crystal structures, we identified a new binding mode of these 2-thiazolidinone derivatives. With this interesting finding, we further explored the SAR and obtained several potent BRD4 inhibitors, which could serve as a base for further drug development.

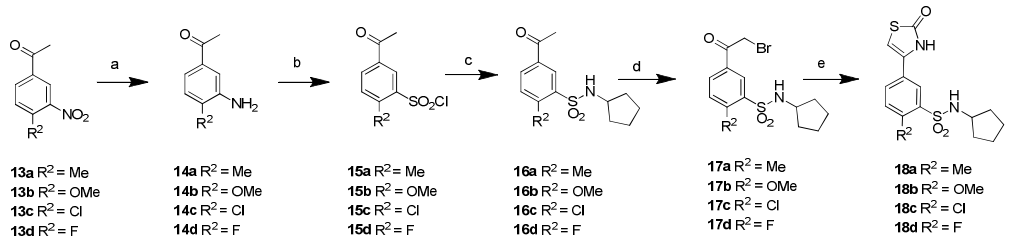
Chemistry

The syntheses of novel 2-thiazolidinone derivatives were illustrated in schemes 1-4. 3'-Sulfonamide derivatives **12a-j** were prepared using the synthetic route in Scheme 1. Benzenesulfonamide derivatives **10a-j** were obtained from commercially available sulfonyl chloride and different amines. Treatment of **10a-j** with $CuBr_2$ gave the desired 2-bromoacetophenones **11a-j**. KSCN was added to obtain the crude 2-thiocyanatoethanone products which were used without further purification. The thiazol-2(3H)-one products **12a-j** were obtained by reaction with 50% H_2SO_4 in acetic acid (v/v) at 100 °C for one hour.



Scheme 1**.** Synthesis of the 3'-Sulfoamide Substituted 2-Thiazolidinone Derivatives **12a-j**. Reagents and conditions: a) R^1NH_2, pyridine, CH_2Cl_2, rt; b) $CuBr_2$, EtOAc, 80 °C; c) KSCN, acetone, rt , then 50% H_2SO_4 solution (v/v) in acetic acid, 100 °C.

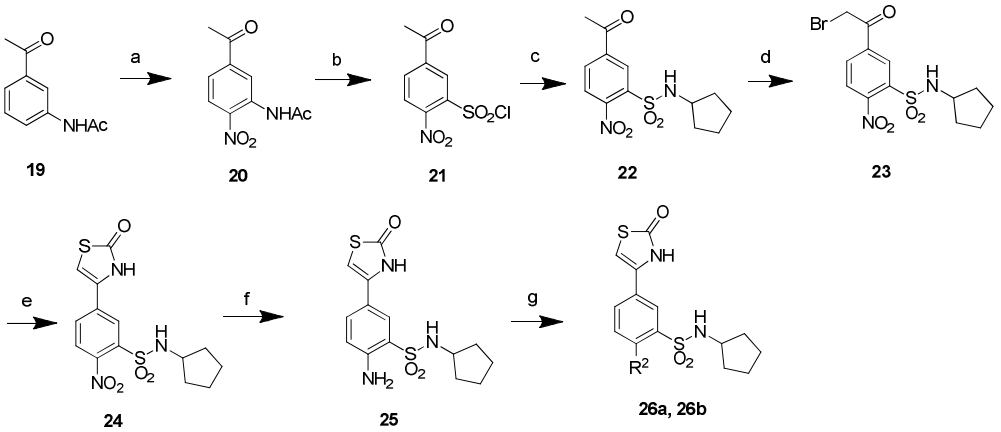
In Scheme 2, 4'-substituted-3'-nitroacetophenones **13a-d** were reduced with iron power to afford amine derivatives **14a-d**. Conversion of **14a-d** to sulfonyl chlorides **15a-d** followed the procedure described by R. V. Hoffman.[25] Subsequent reactions which were similar to scheme 1 furnished compounds **18a-d**.



Scheme 2. Synthesis of the 3',4'- Substituted 2-Thiazolidinones Derivatives **18a-d**. Reagents and

conditions: a) Fe, NH_4Cl, EtOH, 80 °C; b) $NaNO_2$, HCl (conc.), acetic acid, H_2O, -10 °C , then SO_2, CuCl, acetic acid, 10 °C; c) Cyclopentylamine, pyridine, CH_2Cl_2, rt; d) $CuBr_2$, EtOAc, 80 °C; e) KSCN, acetone, rt , then 50% H_2SO_4 solution (v/v), acetic acid, 100 °C.

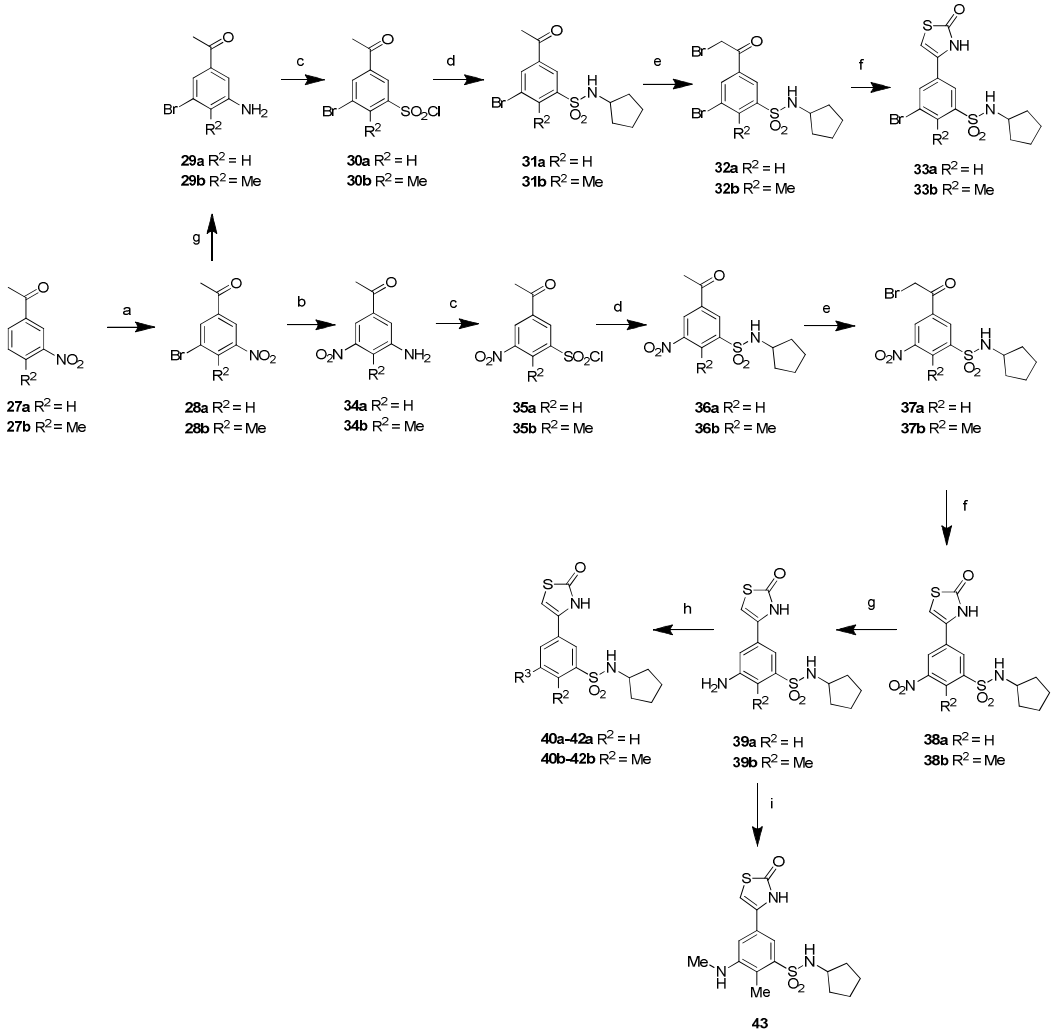
In Scheme 3, treatment of **19** with fuming nitric acid afforded template **20**, which was used directly without deacetylation. The 2-thiazolidinone scaffold **25** was obtained through similar procedures described in scheme 1 and 2. The 4'-amine derivative **25** was then reacted with different acyl chlorides to afford 4'-amide products **26a** and **26b**.



Scheme 3. Synthesis of the 3',4'- Substituted 2-Thiazolidinones Derivatives **25**, **26a** and **26b**. Reagents and conditions: a) Fuming nitric acid, acetic acid, 0 °C; b) $NaNO_2$, HCl (conc.), acetic acid, H_2O, -10 °C , then SO_2, CuCl, acetic acid, 10 °C; c) Cyclopentylamine, pyridine, CH_2Cl_2, rt; d) $CuBr_2$, EtOAc, 80 °C; e) KSCN, acetone, rt , then 50% H_2SO_4 solution (v/v), 100 °C; f) Fe, NH_4Cl, EtOH, 80 °C; g) R^2'COCl, pyridine, THF, rt.

In Scheme 4, **28a** and **28b** were synthesized with good overall yields using a reported sequence[26] starting from **27a** and **27b** and dibromoisocyanuric acid. Using Cu-catalyzed C-N coupling reaction, we converted bromo-substituted **28a** and **28b** to

corresponding amine-substituted **34a** and **34b**. Sulfonamide and thiazol-2(3H)-one ring construction using previously described conditions led to target compounds **33a**, **33b**, **39a-42a** and **39b-42b**.[27] Compound **43** was obtained by reductive amination from **39b**.



Scheme 4. Synthesis of the 3',5'- or 3',4',5'- Substituted 2-Thiazolidinones Derivatives **33a**, **33b**, **39a-42a**, **39b-42b** and **43**. Reagents and conditions: a) Dibromoisocyanuric acid, H_2SO_4 (conc.), rt, then NaI, Na_2SO_3, acetic acid, rt; b) tert-butyl carbamate, CuI, K_2CO_3, N,N'-dimethylethylenediamine, toluene, 110 °C, then TFA, CH_2Cl_2, 0 °C; c) $NaNO_2$, HCl (conc.), acetic acid, H_2O, -10 °C , then SO_2, CuCl, acetic acid, 10 °C; d) Cyclopentylamine, pyridine,

CH_2Cl_2, rt; e) $CuBr_2$, EtOAc, 80 °C; f) KSCN, acetone, rt , then 50% H_2SO_4 solution (v/v), acetic acid, 100 °C; g) Fe, NH_4Cl, EtOH, 80 °C; h) R^3'COCl, pyridine, THF, rt; i) 37% formaldehyde solution, $NaBH_3CN$, acetic acid, THF, rt.

Results and Discussion

Initial Optimization.

In our previous paper, 2-thiazolidinone scaffold with sulfonylaminophenyl substitution was reported.[19] These compounds displayed encouraging potency in the fluorescence anisotropy binding assay, with IC_{50} around 1 μM, but showed weak proliferation inhibition activity in human colon cancer HT-29 cell line (the most potent compound with GI_{50}=37.3±15.5 μM in HT-29 cells). Therefore, it would be worthwhile to find new 2-thiazolidinone bromodomain inhibitors with potency for both BRD4(I) binding and cellular based assays.

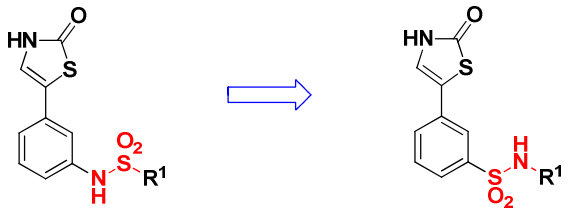
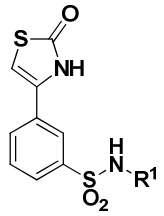


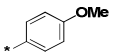
Figure 2. Initial optimization of 2-thiazolidinone BRD4 inhibitors with reversing the sulfonamide substituents

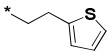
Lately, we continued the structural modification on 2-thiazolidinone by replacing the sulfonylaminophenyl substitution with amino sulfonyl phenyl group (Figure 2).

Various types of R^1-substituents were tested (Table 1, **12a-12j**) and significantly improved BRD4(I) activity was obtained when being compared with the fragment hit **7**. The cyclopropyl derivative **12b** had an encouraging ligand efficiency of 0.404 μM, while cyclopentyl derivative **12a** and cyclohexyl-containing **12c** showed marginally increased activity but with a trend of decreasing ligand efficiency. For R^1, introduction of aromatic rings including phenyl and isoxazole groups decreased the BRD4(I) potency dramatically (compounds **12d**, **12e** and **12f)**. Larger R^1 groups (**12g-12i)** were not tolerated at all. This was different from the SAR study of the previous work, implying that the reversed sulfonamide substituents may interact with distinct binding modes.

Table 1. Effects of R^1-substituted 2-thiazolidinones on inhibition of BRD4(I) by fluorescence anisotropy assay [a]



Compound number	R^1	BRD4 (I) IC_{50} (μM)	Ligand efficiency	Compound number	R^1	BRD4 (I) IC_{50} (μM)	Ligand efficiency
(+)-JQ1	-	0.058±0.01	0.319	**12d**		>10	-
I-BET 151	-	0.10±0.01	0.309	**12e**		8.33±0.71	0.300
7	-	>100	-	**12f**		7.34±1.65	0.332
8	-	4.1±0.5	0.352	**12g**		>10	0.299

12a		1.40±0.08	0.379[b]	**12h**		>10	0.304
12b		2.26±0.08	0.404	**12i**		9.31±0.20	0.311
12c		2.06±0.02	0.351	**12j**		5.82±1.12	0.339

[a] The IC_{50} in the table was calculated from two independent experiments. The fluorescent compound used in the assay was JQ1-FITC. (The synthesis route was provided in our previous work[19]). [b] Ligand efficiency (LE) was calculated as 1.37 (pIC50/number of heavy atoms).[28]

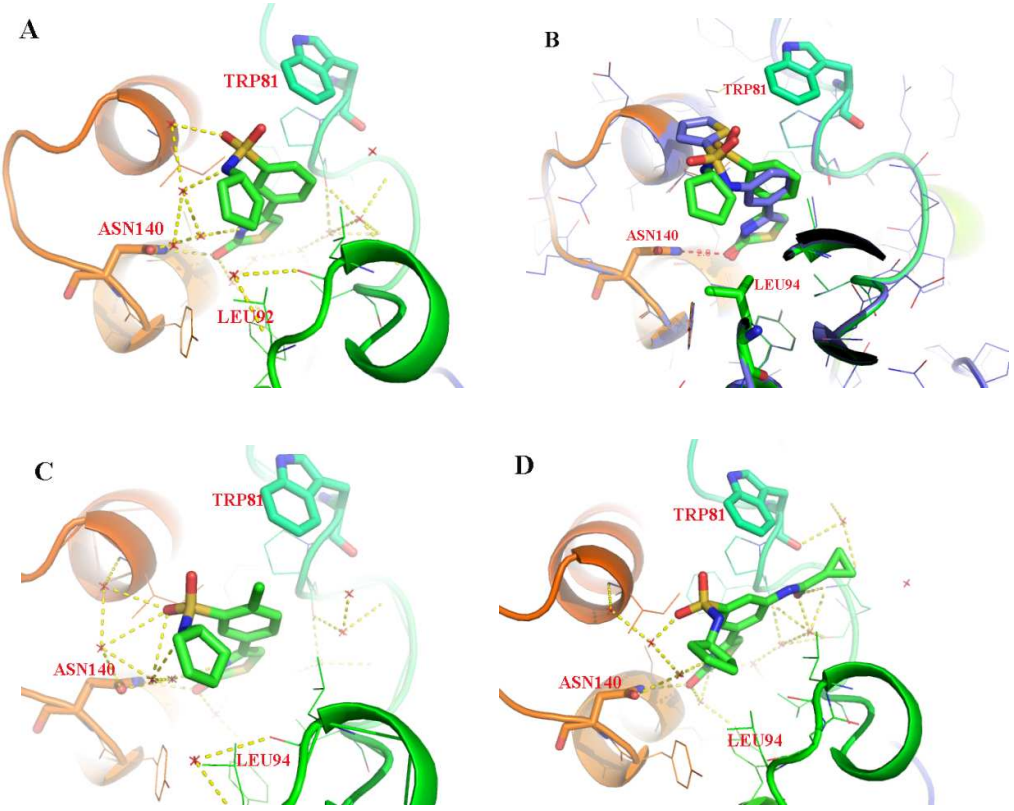


Figure 3. Co-crystal structures of BRD4(I) bound with 2-thiazolidinone derivatives. The ligands

and interacted important residues were shown in stick model, while the protein was shown in ribbon model. **(A) 12a-BRD4 (I) (**pdb entry: **4QR3)**; (B) superimposed the complexes of **2-BRD4 (I) (**pdb entry: **4HXR)** and **12a-BRD4 (I)**; (C) **18c-BRD4 (I) (**pdb entry: **4QR4)**; (D) **40a-BRD4 (I) (**pdb entry: **4QR5)**.

To decipher the interaction of this series of 2-thiazolidinone derivatives, we performed crystallization experiment and solved the co-crystal structure of **12a** bound to BRD4(I). As shown in Figure 3A-B, compound **12a** had a different binding mode compared to the previous N-phenyl sulfonamide **8**, as the cyclopentyl of **12a** pointed to LEU94 and ASN140 residues, which were on the opposite side of WPF shelf. The sulfonamide group formed a hydrogen bonding network through three water molecules, which may account for the increase of the binding affinity. From previous SAR studies, it was found that, for many known BRD4 inhibitors,[29] the WPF shelf was considered to be the major binding subsite of bromodomain module, however, this was not the case for this series of BRD4 inhibitors.

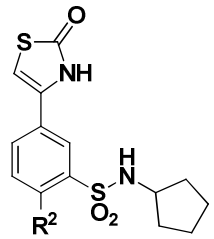
Structure-activity Relationships.

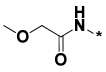
Introducing different substituents (Table 2) in R^2 position generally gave 2-10 folds improved activity compared with compound **12a**, and increased cellular activity.[18, 30] The electronic nature of the substituents on this position was not critical, for either electron donating (**18a**, **18b** and **25)** or electron withdrawing (**18c**) groups displayed similar BRD4(I) affinity. Larger amide substituents such as **26a** and **26b** were tolerable but not optimal, as indicated by their decreased activity and ligand efficiency when being compared with **25**. As studied in several cancer cells, including acute

myloid leukemia and multiple myeloma, BRD4-dependent transcriptional regulation was related to the MYC pathway activation, and upregulated MYC is a critical driver for many types of cancers. We also investigated their cellular activities in three different cell lines. In our previous study, we found that human colon cancer HT-29 cell, harboring evaluated expression of c-Myc protein, was the sensitive cell line among a panel of screened solid-tumor cancer cells. The reported sensitive cell lines MV4;11 and MM.1S were also utilized to test the cellular proliferation inhibition effects.[13] In general, our compounds exhibited encouraging potency in all of the three tested cell lines, especially in MV4;11 cell line. The results from cellular anti-proliferation assay together with the protein binding activity suggested that further exploration could be conducted to find useful BRD4 inhibitors.

To support further optimizations, we solved the complex structure of **18c** with BRD4(I) (Figure 3C). Similar to compound **12a**, the N-cyclopentyl group of compound **18c** situated at the same area as that of compound **12a**, and this further confirmed the unusual binding mode of this series of compounds. By detailed analysis of the orientation of phenyl ring in the binding site, we speculated that the substitution at another meta position will extend to the subpocket behind the WPF shelf, which was also a unique subpocket revealed in our previous study.

Table 2. Effects of R^2-substituted 2-thiazolidinones on inhibition of BRD4(I) in FA assay, and antiproliferation effects against cell lines HT-29, MV4;11 and MM.1S.



Compound number	R^2	BRD4 (I) IC_{50} (μM) [a]	HT-29 IC_{50} (μM) [a]	MV4;11 IC_{50} (μM) [b]	MM.1S IC_{50} (μM) [b]	Ligand efficiency
(+)-JQ1	-	0.058±0.01	0.104	0.023	0.109	0.319
I-BET 151	-	0.10±0.01	0.945	0.119	0.299	0.309
18a	Me	0.22±0.01	3.95±2.71	0.459	1.899	0.411[c]
18b	OMe	0.76±0.08	3.93±0.19	0.547	2.415	0.362
18c	Cl	0.25±0.00	23.67±1.71	0.341	3.369	0.408
18d	F	0.50±0.01	35.16±4.25	NT [d]	NT	0.389
25	NH_2	0.12±0.01	3.28±2.12	0.289	0.793	0.428
26a		0.41±0.15	24.64±5.58	0.681	2.310	0.321
26b		0.80±0.05	12.99±5.62	NT	NT	0.307

[a] The IC_{50} in the table was calculated from two independent experimental measurements. [b] The IC_{50} in the table was calculated from one experimental measurement. [c] Ligand efficiency (LE) was

calculated as 1.37 (pIC50/number of heavy atoms).[d] NT = not tested.

Inspired by the crystal structure of **18c**-BRD4(I), we continued to explore the R^3 position "directing" toward the loop behind the WPF shelf. For comparison, we synthesized the unsubstituted and R^2-methyl substituted products, as listed in Table 3. Similar to the previous study, the substitution at this niche can significantly increase the binding activity towards BRD4(I).

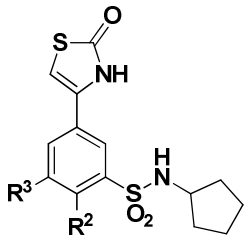
Significant BRD4(I) activity was observed if R^3 was Br and R^2 was kept as methyl (**33b**). This compound also showed excellent activity in MV4;11 cancer cell proliferation inhibition test, with an IC_{50} 0.14 μM and ligand efficiency of 0.427. Changing to amine (**39b)** or aminomethyl group (**43**) decreased the activity about 2-4 folds in BRD4(I) binding assay, while increasing the activity in cellular anti-proliferation assays. These three compounds displayed potencies comparable to I-BET151 both in FA and cell based assays. Comparing the methyl substituted and unsubstituted compounds **33a**, **33b** and **39a-39b**, it was found that when R^2 was methyl, the inhibitors have better cellular activities. This may be a result of better cell permeability of compounds **33b** and **39b,** as the methyl substitution has larger hydrophobic tendency.

To further explore the SAR of R^3 position, we expanded the small substitutions to larger amide groups (**40b-42b**). An interesting phenomenon was observed when comparing R^2-methyl substituted **40b** and **42b** with the unsubstituted analogs **40a** and **42a**: the unsubstituted analogs were 4-6 folds more active than the R^2-methyl

substituted compounds.

To investigate whether R^3 binds to the predicted subpocket, we solved the crystal structure of **40a** with BRD4(I) (Figure 3D). As speculated, the amide group of R^3 of **40a** forms a hydrogen bond with residue GLN85 and several indirect hydrogen bonds to residues PRO82 and GLN85 through one water molecule. Compound **40a** utilized the sulfonamide to form a hydrogen bonding network with water molecules around the ASN140 and ILE146. By comparing with the **18c**, it was found that the N-cyclopentane group of sulfonamide rotated about 180 degree, which may explain why the R^2-methyl substituted analogs were less active because the hydrogen atoms of methyl group may have a close contact with the hydrogen atom at nitrogen of sulfonamide group, therefore disturbing the binding conformation.

Table 3. Effects of R^2, R^3-substituted 2-thiazolidinones on inhibition of BRD4(I) in FA assay, and antiproliferation effects against cell lines HT-29, MV4;11 and MM.1S.



Compound number	R^2	R^3	BRD4 (I) IC_{50} (μM) [a]	HT-29 IC_{50} (μM) [a]	MV4;11 IC_{50}(μM) [b]	MM.1S IC_{50} (μM) [b]	Ligand efficiency
(+)-JQ1	-	-	0.058±0.01	0.104	0.023	0.109	0.319

I-BET 151	-	-	0.10±0.01	0.945	0.119	0.299	0.309
33a	H	Br	0.30±0.01	57.10±12.69	NT[d]	NT	0.403[c]
33b	Me	Br	0.06±0.01	5.46±1.84	0.141	0.999	0.427
39a	H	NH_2	0.46±0.01	6.15±0.90	0.689	3.580	0.392
39b	Me	NH_2	0.14±0.02	0.86±0.42	0.184	0.570	0.405
40a	H		0.06±0.01	5.69±0.48	0.228	1.055	0.364
40b	Me		0.22±0.08	28.48±2.02	0.450	1.755	0.323
41a	H		0.17±0.02	13.86±1.15	0.791	2.533	0.341
41b	Me		0.93±0.07	23.48±10.38	NT	NT	0.293
42a	H		0.11±0.01	25.22±6.08	0.272	3.305	0.326
42b	Me		0.79±0.00	20.09±4.15	0.602	4.095	0.276
43	Me	NHMe	0.25±0.01	0.85±.855	0.198	0.505	0.374

[a] The IC_{50} in the table was calculated from two independent experimental measurements. [b] The IC_{50} in the table was calculated from one experimental measurement. [c] Ligand efficiency (LE) was calculated as 1.37 (pIC50/number of heavy atoms). [d] NT = not tested.

Bromodomain selectivity profile

Bromodomain is the conserved module in evolution, and they share a common 3D structure pattern of one short helix (helix Z) and three long helices(Helix A-C). The acetylated lysine can bind to the top of the bromodomains by forming several hydrogen bonds with the conserved residue asparagine (ASN140 in BRD4). As selectivity is critical for the success of drug discovery, we selected six compounds from this series to profile the binding specificity in five representative bromodomain modules, including BRD4(I), BRD2(I), EP300, BRG1 and ATAD2 (detailed sequence information can be found in supporting materials). After cloned and expressed these modules, we utilized the thermal shift assay to test the binding tendency of inhibitors, which is to measure the thermal stability of a target protein and a subsequent increase in protein melting temperature upon binding of a ligand to the protein.[31] From the Table 4, it was clearly shown that compounds of this series are generally selective inhibitors for BET subfamily, showing good binding affinity to BRD2(I) and BRD4(I). And similar to (+)-JQ1, our compounds showed better binding affinity to BRD4(I) rather than BRD2(I). Interestingly, although compounds of this series showed little affinity for bromodomains of EP300 and BRG1, they displayed certain binding activity towards bromodomain of ATAD2, implying that it is a promising starting point for developing ATAD2 inhibitors.

Table 4. Thermal shift analysis of selected compounds against the bromodomains from BRD4(I), BRD2(I), EP300, BRG1 and ATAD2.

Compound number	BRD4(I) ΔT_m(°C)[a]	BRD2(I) ΔT_m (°C)	EP300 ΔT_m (°C)	BRG1 ΔT_m (°C)	ATAD2 ΔT_m (°C)
(+)-JQ1	9.58±0.20	5.47±0.19	-1.24±0.41	0.14±0.29	1.04±0.27
18a	6.30±0.15	3.47±0.25	-0.17±0.65	0.69±0.25	1.44±0.54
18b-	5.19±0.50	2.82±0.21	0.49±0.23	0.41±0.46	1.36±0.30
25	7.76±0.26	4.06±0.17	-0.08±0.28	0.39±0.25	3.28±0.62
33b	9.22±0.58	5.92±0.22	-1.01±0.38	0.94±0.26	3.42±0.08
39b	7.25±0.25	5.04±0.16	0.19±0.25	0.57±0.39	2.70±0.46
40a	8.48±0.20	5.63±0.20	-0.54±0.31	1.03±0.47	3.55±0.53

[a] Heat map shows relative ΔT_m: red indicates large ΔT_m, and green indicates small ΔT_m. The data are calculated from four time measurements.

Effects on c-Myc protein and mRNA Expression

Delmore et al. discovered the BET bromodomain proteins as regulatory factors for oncogene c-Myc.[13] And the BRD4 inhibitor (+)-JQ1 induces an antiproliferative effect associated with the down-regulation of c-Myc transcription. To assess the 2-thiazolidinone BRD4 inhibitors, we performed the Western blotting experiment and quantitative real-time PCR (RT-qPCR) to study the cellular effect related to c-Myc. Three potent inhibitors were selected (**33b, 39b** and **40a**) based on the protein binding and cellular antiproliferation assays. The results (Figure 4A) showed that at either 5 μM or 10 μM concentration, compound **39b** was more active than other two compounds (**33b** and **40a**), and could significantly inhibit the expression of c-Myc protein. As compound **39b** is most active in cellular antiproliferation assay, the

dose-dependent effect on c-Myc protein was also studied along with the effect of I-BET151 and (+)-JQ1. As indicated in Figure 4B and 4C, **39b** showed good inhibition to the expression of c-Myc, which is comparable to compounds (+)-JQ1 and I-BET151. Similarly, as indicated by RT-qPCR assays, compounds **33b, 39b** and **40a** strongly down-regulated the expression of *c-myc* mRNA (Figure 4D), three compounds of which revealed more than 60% inhibition at 5 μM, especially compound **39b** showing about 90% inhibition. From the dose-dependent study on expression of *c-myc* mRNA, **39b** showed clear effect even at concentration about 0.3μM, which is about 20% lower than (+)-JQ1 and I-BET151. Taking together the selectivity profiles, these data implied that the antiproliferation effects of 2-thiazolidinone series of compounds may go through the BRD4-dependent pathway, as confirmed from the study of (+)-JQ1.

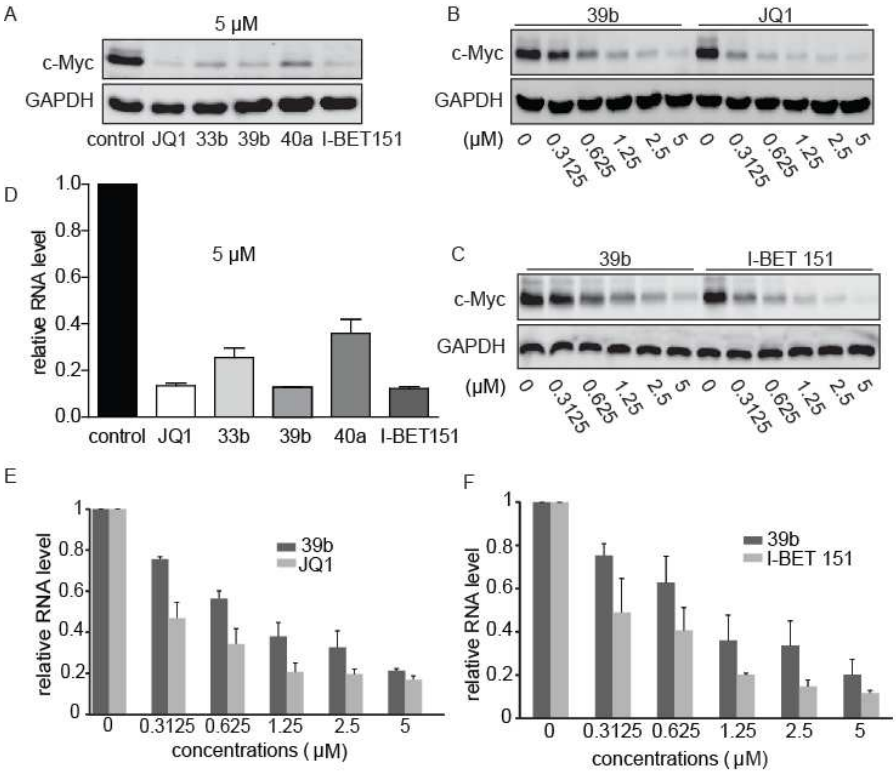


Figure 4. (A-C) Inhibition of the tested compounds on the expression of c-Myc protein. HT-29 cells were treated with different compounds (5 μM) (A), **39b** or JQ1 (0-5 μM) (B), and **39b** or I-BET 151 (0-5 μM) (C) for 24 h. Cells were collected and lyzed for Western blotting. The level of c-Myc protein was detected and the expression of GAPDH protein was chosen as the loading control. The experiments were repeated for three times. (D-F) Inhibition of the tested compounds on the expression of *c-myc* mRNA. HT-29 cells were treated with compounds (5 μM) (D), **39b** or JQ1 (0-5 μM) (E), and **39b** or I-BET 151 (0-5 μM) (F) for 24 h. Total RNA was isolated and reverse-transcribed for RT-qPCR analyses. The data were expressed as mean ± SD, representing the relative levels of *c-myc* mRNA from at least three independent experiments.

In Vitro metabolic stability Assessment

To complete the drug-likeness assessment of this series of BRD4 inhibitors, we conducted *in vitro* metabolic stability profile of eight potent inhibitors as the representatives of 2-thiazolidinone compounds. As shown in Table 5, the results provided that most compounds except **18a** and **43** have favorable metabolic stability profiles in the human liver microsome assay, as the clearance rates of six compounds are below than 100 μL/min/mg. **43** is the most metabolic unstable compound with the clearance rate about 174 μL/min/mg, indicating the aminomethyl group is sensitive in human liver. In addition to the liver microsome assay, five cytochrome P450 enzymes commonly metabolizing exogenous chemicals were used to test the direct inhibition of these eight compounds. The evaluation showed that compounds **18a**, **25** and **42a** can inhibit certain CYPs more than 50% ratios at the concentration of 10 μM; while other compounds showed satisfactory profiles in five CYPs inhibition assay. As CYP

enzymes usually have different structural preference when binding to small molecules, it is difficult to assess the metabolic stability simply from the functional group. From the time-dependent inhibition assay for five CYPs, the results showed that last four compounds in Table 5 have less possibilities of covalent binding to these CYPs. Nevertheless, from the metabolic stability profiling in vitro, it is promising that, with detailed exploration of chemical space of the 2-thiazolidinone series, useful inhibitors of BRD4 (I) such as **39b** can be identified as a good candidate for further drug development.

Table 5. In vitro liver microsome stability assay and cytochrome P450 enzymes inhibition assays

Cpd	HLM Stability (μl/min/mg protein)	CYP DI[a] ratio, % (compd concn of 10 μM)					TDI[b]
		3A4	2D6	2C9	1A2	2C19	
(+)-JQ1	78	30/28%	2%	18%	2%	18%	2C19
I-BET 151	0	21/22%	18%	33%	18%	19%	no inhibition
18a	128	62/68%	27%	no inhibition	52%	45%	2C9、2C19
18b	56	20/30%	no inhibition	10%	3%	26%	2D6、2C19
25	20	70/82%	18%	74%	22%	57%	3A4
33b	84	42/53%	39%	no inhibition	47%	28%	2C9
39b	66	42/46%	3%	no inhibition	17%	3%	no inhibiton

40a	74	14/21%	17%	14%	29%	32%	no inhibition
42a	64	17/34%	44%	57%	45%	46%	no inhibition
43	174	41/47%	36%	no inhibition	37%	17%	no inhibition

Footnote:

[a]CYPs direct inhibition, sometimes referred to reversible inhibition, is assessed by measurement of an enzyme (CYP) activity in the presence of increasing concentration of inhibitor without a pre-incubation step.

[b]TDI stands for time-dependent inhibition, which is referring to a change in enzyme inhibition during an in vitro incubation and means an irreversible inactivation of CYPs.

Conclusions

In summary, above studies disclosed the unexpected finding that reversing the sulfonamide moiety can significantly improve the binding activity of 2-thiazolidinone derivatives. Through the detailed analysis of a co-crystal structure of **12a**-BRD4(I), a new binding mode was revealed for this series of compounds. With the guidance of crystal structures, we elaborated the SAR of the newly designed 2-thiazolidinone analogs, optimized the structure to reach a high potency as indicated from protein binding assay and three cancer cell lines assays. From the selectivity profiling of eight compounds against five bromodomain proteins, it was found that this series of 2-thiazolidinones was selective BET subfamily inhibitors. Taking together with the cellular effect study on downstream protein c-Myc and metabolic stability test, we

regarded **39b** as a promising BRD4 inhibitor, and a useful lead for further anti-cancer drug development.

Experimental Section

Chemistry

General:

All solvents were dried and purified prior to use: Toluene was distilled from sodium, THF was distilled from potassium, and DCM was distilled from CaH_2. Compounds **13a-13d**, **19**, **27a**, **27b** and other commercially available reagents were used as received. ^{1}H NMR (400 MHz) spectra were recorded by using a Varian Mercury-400 High Performance Digital FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR (126 MHz) spectra were recorded by using a Varian Mercury-500 High Performance Digital FT-NMR spectrometer. Abbreviations for peak patterns in NMR spectra: br = broadened, s = singlet, d = doublet, t = triplet, dd = doublet of doublets and m = multiplet. Low-resolution mass spectra were obtained with a Finnigan LCQ Deca XP mass spectrometer using a CAPCELL PAK C18 (50 mm × 2.0 mm, 5 μM) or an Agilent ZORBAX Eclipse XDB C18 (50 mm × 2.1 m, 5 μM) in positive or negative electrospray mode. High-resolution mass spectra were recorded by using a Finnigan MAT-95 mass spectrometer. Purity of all compounds was determined by analytical Gilson high-performance liquid chromatography (HPLC) using an YMC ODS3 column (50 mm × 4.6 mm, 5 μM). Conditions were as follows: CH_3CN/H_2O eluent at 2.5 $mLmin^{-1}$ flow [containing 0.1% trifluoroacetic acid (TFA)]

at 35 °C, 8 min, gradient 5% CH_3CN to 95% CH_3CN, monitored by UV absorption at 214 nm and 254 nm. TLC analysis was carried out with glass precoated silica gel GF254 plates. TLC spots were visualized under UV light. Flash column chromatography was performed with a Teledyne ISCO CombiFlash R_f system. All solvents and reagents were used directly as obtained commercially unless otherwise noted. All air and moisture sensitive reactions were carried out under an atmosphere of dry argon with heat-dried glassware and standard syringe techniques. Melting points were determined using a SGW X-4 hot stage microscope and are uncorrected. (spectra data of the synthesized compounds were provided as supporting material)

Synthetic Procedures:

General procedure A for the reactions in Scheme 1, entries 10a-12a:

3-Acetyl-N-cyclopentylbenzenesulfonamide (**10a**). To a solution of cyclopentanamine (0.675 mL, 6.86 mmol) and pyridine (0.74 mL, 9.15 mmol) in CH_2Cl_2 (20 mL) was added commercially available 3-acetylbenzene-1-sulfonyl chloride (1 g, 4.57 mmol) and the mixture was stirred at room temperature for 1 hour. The reaction was monitored by TLC. Upon completion, 1 N HCl (10 mL) was added and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried with Na_2SO_4, and then removed in vacuo. The residue was purified by flash column chromatography (gradient elution, gradient 5 to 30% EtOAc/60-90 °C petroleum ether) to give the title compound **10a** as a pale yellow solid (1.07 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.16 (d, J = 7.8 Hz, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 4.84 (d, J = 6.2 Hz, 1H),

3.64 (q, J = 6.9 Hz, 1H), 2.67 (s, 3H), 1.85-1.75 (m, 2H), 1.67-1.57 (m, 2H), 1.55-1.46 (m, 2H), 1.41-1.31 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 266.15; retention time 2.99 min, > 99% pure.

3-(2-Bromoacetyl)-N-cyclopentylbenzenesulfonamide (**11a**). To a solution of **10a** (2 g, 7.48 mmol) in EtOAc (50 mL) was added $CuBr_2$ (2 g, 8.98 mmol) and the mixture was heated to reflux overnight. The mixture was cooled to room temperature and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product. The residue was purified by silica gel column chromatography (gradient elution, gradient 5 to 20% EtOAc/ 60-90 °C petroleum ether) to give **11a** as a pale yellow solid (1.84 g, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (t, J = 1.8 Hz, 1H), 8.20 (dd, J = 7.8, 1.8 Hz, 1H), 8.13 (dd, J = 7.8, 1.9 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 4.71 (d, J = 7.4 Hz, 1H), 4.47 (s, 2H), 3.72-3.61 (m, 1H), 1.84-1.77 (m, 2H), 1.68-1.61 (m, 2H), 1.56-1.46 (m, 2H), 1.41-1.32 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 345.91; retention time 3.26 min, > 98% pure.

N-Cyclopentyl-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**12a**). To a suspension of KSCN (702 mg, 7.22 mmol) in acetone (20 mL) was added **11a** (500 mg, 1.44 mmol), and the mixture was stirred at room temperature for 10 minutes. The mixture was concentrated in vacuo, extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The crude white solid was dissolved in acetic acid (10 mL) and 2 mL 50% H_2SO_4 solution (v/v), and the mixture was heated to 100 °C for 1 hour. Upon

completion, the mixture was cooled to room temperature. EtOAc (100 mL) was added, the organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL), water (100 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product. Purification by silica gel column chromatography (gradient elution, gradient 0 to 5% MeOH/CH_2Cl_2) to give **12a** as a white solid (0.32 g, 69% yield). Mp 190-191 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 8.08 (d, J = 1.8 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.71-7.63 (m, 2H), 6.97 (s, 1H), 3.47 (p, J = 6.9 Hz, 1H), 2.09 (s, 2H), 1.66-1.49 (m, 4H), 1.43-1.35 (m, 2H), 1.33-1.23 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 142.91, 133.07, 130.86, 130.36, 128.88, 126.63, 123.36, 100.78, 54.89, 32.92 (2 × C), 23.30 (2 × C); HRMS (EI) *m/z* calcd for $C_{14}H_{16}N_2S_2O_3$: 324.0602, found 324.0603; retention time 2.91 min, > 99% pure.

Following procedure A as for compound **12a** gave compounds **12b**-**12j**.

N-Cyclopropyl-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**12b**): White solid, 73% yield; mp 204-205 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 1H), 8.08 (s, 1H), 7.97 (d, J = 2.6 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 6.98 (s, 1H), 2.16-2.12 (m, 1H), 0.54-0.44 (m, 2H), 0.41-0.31 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 141.56, 133.04, 130.96, 130.40, 129.19, 126.94, 123.60, 100.88, 24.66, 5.64 (2 × C); HRMS (EI) *m/z* calcd for $C_{12}H_{12}N_2S_2O_3$: 296.0289, found 296.0287; retention time 2.65 min, > 99% pure.

N-Cyclohexyl-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**12c**): White

solid, 70% yield; mp 207-209 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.09 (t, J = 1.6 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.69 (d, J = 7.5 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 6.97 (d, J = 1.9 Hz, 1H), 3.04-2.98 (m, 1H), 1.59-1.53 (m, 4H), 1.46-1.42 (m, 1H), 1.20-1.07 (m, 4H), 1.19-1.00 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 143.62, 133.08, 130.83, 130.37, 128.79, 126.42, 123.08, 100.78, 52.47, 33.71 (2 × C), 25.29 (2 × C), 24.79 (2 × C); HRMS (EI) *m/z* calcd for $C_{15}H_{18}N_2S_2O_3$: 338.0759, found 338.0767; retention time 3.19 min, > 99% pure.

N-(4-Methoxyphenyl)-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**12d**): White solid, 80% yield; mp 221-222 °C (from CH_2Cl_2 and MeOH) ;^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 9.98 (s, 1H), 8.00 (s, 1H), 7.84 (d, J = 7.3 Hz, 1H), 7.66-7.55 (m, 2H), 6.99 (d, J = 8.0 Hz, 2H), 6.89 (s, 1H), 6.81 (d, J = 8.0 Hz, 2H), 3.66 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.21, 157.04, 140.79, 132.89, 130.89, 130.32, 130.30, 129.35, 126.79, 123.94 (2 × C), 123.55, 114.79 (2 × C), 100.90, 55.60; HRMS (EI) *m/z* calcd for $C_{16}H_{14}N_2S_2O_4$: 362.0395, found 362.0387; retention time 2.98 min, > 99% pure.

N-(4-Aminophenyl)-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamid (**12e**): White solid, 50% yield; mp 206-207 °C (from CH_2Cl_2 and MeOH) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (brs, 1H), 9.53 (s, 1H), 7.93 (s, 1H), 7.84-7.80 (m, 1H), 7.61-7.56 (m, 2H), 6.85 (s, 1H), 6.69 (d, J = 8.4 Hz, 2H), 6.39 (d, J = 8.4 Hz, 2H), 4.99 (brs, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.22, 147.14, 141.04, 133.01, 130.75, 130.10, 129.11, 126.92, 125.43, 125.18 (2 × C), 123.62, 114.43 (2 × C),

100.70; HRMS (EI) *m/z* calcd for $C_{15}H_{13}N_3S_2O_3$: 347.0398, found 347.0404; retention time 2.06 min, > 99% pure.

N-(Isoxazol-3-yl)-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**12f**): Offwhite solid (67% yield); mp 236-237 °C (from CH_2Cl_2 and MeOH) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 11.74 (s, 1H), 8.75 (s, 1H), 8.15 (s, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 7.7 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.01 (s, 1H), 6.53 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.21, 161.54, 157.33, 140.57, 132.68, 131.08, 130.72, 130.07, 126.85, 123.51, 101.33, 98.75; HRMS (EI) *m/z* calcd for $C_{12}H_9N_3S_2O_4$: 323.0034, found 323.0043; retention time 2.61 min, > 97% pure.

N-Benzyl-3-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**12g**): White solid (65% yield); mp 181-183 °C (from CH_2Cl_2 and MeOH) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.20 (t, J = 6.4 Hz, 1H), 8.05 (t, J = 1.7 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.63 (t, J = 7.8 Hz, 1H), 7.30-7.18 (m, 5H), 6.96 (d, J = 1.8 Hz, 1H), 4.04 (d, J = 6.3 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 142.07, 138.05, 133.03, 130.91, 130.40, 128.98, 128.68 (2 × C), 128.03 (2 × C), 127.61, 126.60, 123.36, 100.83, 46.57; HRMS (EI) *m/z* calcd for $C_{16}H_{14}N_2S_2O_3$: 346.0446, found 346.0449; retention time 2.96 min, > 99% pure.

3-(2-Oxo-2,3-dihydrothiazol-4-yl)-N-(2-(thiophen-2-yl)ethyl)benzenesulfonamide (**12h**): Offwhite solid (71% yield); mp 158-159°C (from CH_2Cl_2 and MeOH) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.06 (s, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.85 (t, J = 5.8 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.66 (t, J = 7.8 Hz, 1H), 7.32 (dd, J = 5.1, 1.2 Hz, 1H), 6.99 (d, J = 1.8 Hz, 1H), 6.93 (dd, J = 5.1, 3.4 Hz, 1H), 6.86 (d, J

= 2.5 Hz, 1H), 3.03 (dd, *J* = 13.3, 6.7 Hz, 2H), 2.91 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 141.70, 141.07, 133.01, 130.99, 130.46, 129.08, 127.38, 126.63, 125.95, 124.66, 123.29, 100.90, 44.56, 30.10; HRMS (EI) *m/z* calcd for $C_{15}H_{14}N_2S_3O_3$: 366.0167, found 365.9777; retention time 3.16 min, > 97% pure.

3-(2-Oxo-2,3-dihydrothiazol-4-yl)-N-((tetrahydrofuran-2-yl)methyl)benzenesulfonamide (**12i**): White solid (66% yield); mp 162-163 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.06 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.79-7.75 (m, 2H), 7.66 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 1.8 Hz, 1H), 3.82-3.77 (m, 1H), 3.69-3.64 (m, 1H), 3.59-3.53 (m, 1H), 2.82 (t, *J* = 6.0 Hz, 2H), 1.90-1.68 (m, 3H), 1.58-1.45 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 142.06, 133.06, 130.89, 130.36, 128.94, 126.62, 123.29, 100.80, 77.50, 67.74, 46.96, 28.79, 25.53; HRMS(EI) *m/z* calcd for $C_{14}H_{16}N_2S_2O_4$: 340.0551, found 340.0548; retention time 2.63 min, > 99% pure.

3-(2-Oxo-2,3-dihydrothiazol-4-yl)-N-(tetrahydrofuran-3-yl)benzenesulfonamide (**12j**): White solid (71% yield); mp 173-174 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.07 (s, 1H), 7.98 (d, *J* = 6.8 Hz, 1H), 7.90 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 6.98 (s, 1H), 3.80-3.75 (m, 1H), 3.71-3.54 (m, 3H), 3.35-3.31 (m, 1H), 1.93-1.82 (m, 1H), 1.61-1.54 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 142.26, 132.99, 130.98, 130.53, 129.17, 126.67, 123.39, 100.95, 72.48, 66.61, 53.53, 32.58; HRMS(EI) *m/z* calcd for $C_{13}H_{14}N_2S_2O_4$: 326.0395, found 326.0391; retention time 2.46 min, > 98% pure.

General procedure B for the reactions in Scheme 2, entries 14a-18a:

1-(3-Amino-4-methylphenyl)ethanone (**14a**). To a solution of **13a** (20 g, 112 mmol) in EtOH (200 mL) and fine iron powder (25 g, 446 mmol) was added at 50-55 °C followed by NH_4Cl solution (24 g, 446 mmol in 50 mL water). The reaction mixture was refluxed for 1 hour, then cooled to room temperature and filtered through Celite. The filtrate was basified with $NaHCO_3$ solution (pH 7-8) and extracted with EtOAc (3 × 250 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 20% EtOAc/ 60-90 °C petroleum ether) gave **14a** as a pale yellow solid (13 g, 78% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.25 (m, 2H), 7.12 (d, J = 7.7Hz, 1H), 3.77 (brs, 2H), 2.55 (s, 3H), 2.22 (s, 3H); LCMS *m/z* (ESI) found $[M+H]^+$ 150.1; retention time 1.49 min, > 99% pure.

5-Acetyl-2-methylbenzene-1-sulfonyl chloride (**15a**). **14a** (13 g, 87 mmol) was added in one portion to a mixture of con. HCl (40 mL) and acetic acid (30 mL). The white hydrochloride salt precipitated. The beaker was placed in a dry ice-ethanol bath and when the temperature of the stirred mixture had reached -10 °C, a solution of $NaNO_2$ (9.02 g, 131 mmol) in water (20 mL) was added dropwise at such a rate that the temperature did not exceed -5 °C. After all the solution had been added, the mixture was stirred for 45 minutes while the temperature was maintained between -10 °C and -5 °C. Meanwhile, CuCl (2.59 g, 26.1 mmol) was added to acetic acid (80 mL) which was saturated with SO_2 gas. The solution turned to green during 20-30 minutes. The mixture was then placed in an ice bath and cooled with stirring. When

the temperature approached 10 °C, the diazotization reaction mixture was added in portions over a 30-min period to the sulfur dioxide solution. After all the diazonium salt mixture had been added, the mixture was stirred at room temperature for another 1 hour, poured into ice water (500 mL). The aqueous layer was extracted with EtOAc (3 × 250 mL). The organic layers were combined, washed with brine, and dried with Na_2SO_4. Purification by silica gel column chromatography (gradient elution, gradient 0 to 15% EtOAc/ 60-90 °C petroleum ether) gave **15a** as a pale yellow oil (8.3 g, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.5(d, J = 7.8 Hz, 1H), 7.33 (d, J = 7.9 Hz, 1H), 2.58 (s, 3H), 2.44 (s, 3H); retention time 3.28 min, > 99% pure.

16a, **17a** and **18a** were prepared similar to procedure A.

5-Acetyl-N-cyclopentyl-2-methylbenzenesulfonamide (**16a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, J = 1.9 Hz, 1H), 8.05 (dd, J = 7.9, 1.9 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 4.69 (d, J = 7.5 Hz, 1H), 3.62 (q, J = 6.9 Hz, 1H), 2.72 (s, 3H), 2.64 (s, 3H), 1.85-1.77 (m, 2H), 1.65-1.58 (m, 2H), 1.57-1.46 (m, 2H), 1.44-1.33 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 280.18; retention time 3.08 min, > 99% pure.

5-(2-Bromoacetyl)-N-cyclopentyl-2-methylbenzenesulfonamide (**17a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.08 (d, J = 7.9 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 4.60 (d, J = 7.4 Hz, 1H), 4.46 (s, 2H), 3.64 (q, J = 6.9 Hz, 1H), 2.73 (s, 3H), 1.87-1.79 (m, 2H), 1.66-1.59(m, 2H), 1.56-1.49 (m, 2H), 1.44-1.35 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 359.97; retention time 3.33 min, > 77% pure.

N-Cyclopentyl-2-methyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**18a**): White solid, mp 151-153 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ

11.97 (s, 1H), 8.09 (s, 1H), 7.79-7.70 (m, 2H), 7.45 (d, J = 7.8 Hz, 1H), 6.86 (s, 1H), 3.46-3.41 (m, 1H), 2.58 (s, 3H), 1.60-1.50 (m, 4H), 1.39-1.22 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.34, 140.44, 137.18, 133.47, 133.20, 128.93, 128.27, 125.65, 99.65, 54.61, 32.77 (2 × C), 23.26 (2 × C), 20.05; HRMS (EI) *m/z* calcd for $C_{15}H_{18}N_2S_2O_3$: 338.0759, found 338.0758; retention time 2.97 min, > 97% pure.

Following procedure B as for compound **18a** gave products **18b**-**18j**.

N-Cyclopentyl-2-methoxy-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**18b**): White solid, mp 128-129°C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.00 (d, J = 2.4 Hz, 1H), 7.85 (dd, J = 8.7, 2.4 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.29 (d, J = 8.7 Hz, 1H), 6.76 (s, 1H), 3.94 (s, 3H), 3.44-3.39 (m, 1H), 1.60-1.46 (m, 4H), 1.38-1.30 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.37, 156.71, 133.08, 131.16, 129.63, 126.62, 122.44, 113.67, 98.10, 56.72, 54.99, 32.53 (2 × C), 23.16 (2 × C); HRMS (EI) *m/z* calcd for $C_{15}H_{18}N_2S_2O_4$: 354.0708, found 354.0704; retention time 2.88 min, > 99% pure.

2-Chloro-N-cyclopentyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**18c**): White solid, mp 205-206 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 8.21 (s, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.00 (s, 1H), 3.48 (q, J = 7.0 Hz, 1H), 1.58-1.51(m, 4H), 1.40-1.30 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.18, 139.53, 132.69, 132.28, 130.85, 130.30, 129.45, 127.46, 101.43, 54.87, 32.61 (2 × C), 23.25 (2 × C); HRMS (EI) *m/z* calcd for $C_{14}H_{15}ClN_2S_2O_3$: 358.0213, found 358.0214; retention time 3.07 min, > 99% pure.

N-Cyclopentyl-2-fluoro-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**18d**): White solid, mp 212-213 °C (from CH_2Cl_2 and MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 8.07-8.03 (m, 2H), 7.96-7.90 (m, 1H), 7.54 (t, J = 9.3 Hz, 1H), 6.93 (s, 1H), 3.56-3.51 (m, 1H), 1.62-1.54 (m, 4H), 1.38-1.30 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.24 , 159.31 , 157.27 , 132.27 , 131.84, 130.27, 127.01, 118.36, 100.43 , 54.86 , 32.73 (2 × C), 23.27 (2 × C); HRMS (EI) *m/z* calcd for $C_{14}H_{15}FN_2S_2O_3$: 342.0508, found 342.0511; retention time 2.97 min, > 97% pure.

General procedure C for the reactions in Scheme 3, entries 20-25 and 26a:

N-(5-Acetyl-2-nitrophenyl)acetamide (**20**): To a solution of **19** (10 g, 56.4 mmol) in acetic acid (50 mL) was added fuming nitric acid (10 mL, 238 mmol) slowly at 0 °C. The reaction mixture was stirred for 1 hour while the temperature was maintained at 0 °C. The mixture was poured into ice water (300 mL). The aqueous layer was extracted with EtOAc (3 × 150 mL). The organic layers were combined, washed with water (3 × 150 mL), then brine, and dried with Na_2SO_4. Purification by silica gel column chromatography (gradient elution, gradient 0 to 20% EtOAc/ 60-90 °C petroleum ether) gave **20** as a yellow solid (6.9 g, 55% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.28 (brs, 1H), 9.37 (s, 1H), 8.28 (d, J = 8.6 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 2.67 (s, 3H), 2.34 (s, 3H); LCMS *m/z* (ESI) found $[M+H]^+$ 222.9; retention time 2.33 min, > 99% pure.

5-Acetyl-2-nitrobenzene-1-sulfonyl chloride (**21**): Compound **20** was used directly without deacetylation and followed the similar procedure in scheme 2-b afforded **21** as a pale yellow solid (39% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.99-7.90 (m,

2H), 2.66 (s, 3H); retention time 3.05 min, > 76% pure.

22, **23** and **24** were prepared similar to procedure A.

5-Acetyl-N-cyclopentyl-2-nitrobenzenesulfonamide (**22**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 1.9 Hz, 1H), 8.27 (dd, J = 8.3, 1.9 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 5.26 (d, J = 7.7 Hz, 1H), 3.87-3.75 (m, 1H), 2.72 (s, 3H), 1.89-1.79 (m, 2H), 1.70-1.62 (m, 2H), 1.59-1.50 (m, 2H), 1.48-1.38 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 310.98; retention time 3.21 min, > 99% pure.

5-(2-Bromoacetyl)-N-cyclopentyl-2-nitrobenzenesulfonamide (**23**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, J = 1.9 Hz, 1H), 8.32 (dd, J = 8.3, 1.9 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 5.23 (d, J = 7.7 Hz, 1H), 4.48 (s, 2H), 3.82 (q, J = 6.9 Hz, 1H), 1.90-1.83 (m, 2H), 1.72-1.64 (m, 2H), 1.60-1.52 (m, 2H), 1.49-1.39 (m, 2H); retention time 3.42 min, > 70% pure.

N-Cyclopentyl-2-nitro-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**24**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 8.26 (s, 1H), 8.05 (m, 2H), 8.00 (d, J = 7.9 Hz, 1H), 7.16 (s, 1H), 3.65 (q, J = 7.1 Hz, 1H), 1.71-1.64 (m, 2H), 1.58-1.52 (m, 2H), 1.44-1.33 (m, 4H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 368.10; retention time 3.09 min, > 99% pure.

2-Amino-N-cyclopentyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**25**). To a solution of **24** (1 g, 2.71 mmol) in EtOH (20 mL) and fine iron powder (0.605 g, 10.83 mmol) was added at 50-55 °C followed by NH_4Cl solution (0.579 g, 10.83 mmol in 5 mL water). The reaction mixture was refluxed for 1 hour, then cooled to room temperature and filtered through Celite. The filtrate was basified with $NaHCO_3$ solution (pH 7-8) and

extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 30% EtOAc/ 60-90 °C petroleum ether) gave **25** as a pale yellow solid (0.69 g, 75% yield). Mp 199-200 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 7.78 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.55-7.50 (m, 1H), 6.82 (d, J = 8.6 Hz, 1H), 6.47 (s, 1H), 6.16 (brs, 2H), 3.45-3.37 (m, 1H), 1.63-1.48 (m, 4H), 1.42-1.23 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.51, 146.54, 134.00, 130.60, 126.54, 121.07, 117.52, 117.45, 95.20, 54.43, 32.82 (2 × C), 23.34 (2 × C); HRMS (EI) *m/z* calcd for $C_{14}H_{17}N_3S_2O_3$: 339.0711, found 339.0704; retention time 2.93 min, > 98% pure.

N-(2-(N-Cyclopentylsulfamoyl)-4-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)-2-methoxyacetamide (**26a**). To a solution of **25** (100 mg, 0.295 mmol) in anhydrous THF (20 mL) were added 2-methoxyacetyl chloride (40 μL, 0.442 mmol) and pyridine (48 μL, 0.589 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 hours. The mixture was diluted with EtOAc, washed with 1 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (gradient elution, gradient 0 to 30% EtOAc/ 60-90 °C petroleum ether) to give **26a** (0.107 g, 88%) as a white solid. Mp 200-202 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 10.27 (s, 1H), 8.49 (d, J = 8.7 Hz, 1H), 8.09 (s, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.88 (dd, J = 9.1, 2.3 Hz, 1H), 6.84 (s, 1H), 4.07 (s, 2H), 3.63-3.51 (m, 1H), 3.43 (s, 3H), 1.65-1.58

(m, 2H), 1.53-1.49 (m, 2H), 1.42-1.33 (m, 2H), 1.30-1.23 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.33, 169.09, 135.30, 132.80, 130.20, 129.49, 125.79, 125.66, 122.28, 99.61, 72.09, 59.53, 54.88, 32.83 (2 × C), 23.41 (2 × C); HRMS (EI) *m/z* calcd for $C_{17}H_{21}N_3S_2O_5$: 411.0923, found 411.0929; retention time 3.09 min, > 98% pure.

Following procedure C as for compound **26a** gave products **26b**.

N-(2-(N-Cyclopentylsulfamoyl)-4-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)cyclopropaneca rboxamide (**26b**): White solid, mp 196-198 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.55 (s, 1H), 8.30 (d, J = 8.8 Hz, 1H), 8.16-8.10 (d, J = 7.8 Hz, 1H), 8.08(s, 1H), 7.87-7.81 (d, J = 7.8 Hz, 1H), 6.84 (s, 1H), 3.58-3.53 (m, 1H), 1.88-1.80 (m, 1H), 1.64-1.51 (m, 4H), 1.42-1.34 (m, 2H), 1.30-1.23 (m, 2H), 0.93-0.84 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.33, 172.43, 135.83, 132.85, 130.07, 129.62, 125.88, 125.37, 123.45, 99.50, 54.71, 32.92 (2 × C), 23.37 (2 × C), 16.20, 8.63 (2 × C); HRMS (EI) *m/z* calcd for $C_{18}H_{21}N_3S_2O_4$: 407.0973, found 407.0979; retention time 3.25 min, > 98% pure.

General procedure D for the reactions in Scheme 4, entries 28b, 34b-40b and 43:

1-(3-Bromo-4-methyl-5-nitrophenyl)ethanone (**28b**). In a 250 mL round-bottom flask, 1-(4-methyl-3-nitrophenyl)ethanone (**27b**, 13.2 g, 73.7 mmol) was dissolved in 80 mL of concentrated H_2SO_4. A solution of dibromoisocyanuric acid (23.2 g, 81 mmol) in 40 mL of concentrated H_2SO_4 was slowly added. After the reaction mixture had been stirred for 16 hours at room temperature, it was carefully poured into 200

mL ice water and extracted with ethyl acetate. The insoluble cyanuric acid was filtered off, and the organic layer was washed with saturated $NaHCO_3$ solution. After the mixture was dried over Na_2SO_4, the solvent was removed under reduced pressure. The intermediate compound was dissolved in 70 mL of glacial acetic acid, and NaI (2.19 g, 14.73 mmol) followed by Na_2SO_3 (37.1 g, 295 mmol) was added. After 1 hour, the mixture was diluted with 100 mL of water and extracted with ethyl acetate. The organic layer was washed several times with saturated Na_2CO_3 solution and dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash column chromatography on silica gel (gradient elution, gradient 0 to 30% EtOAc/ 60-90 °C petroleum ether) gave **28b** as a bright yellow solid (11.03 g, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 2.0 Hz, 1H), 8.26 (d, J = 2.1 Hz, 1H), 2.63 (m, 6H); retention time 3.33 min, > 99% pure.

1-(3-Amino-4-methyl-5-nitrophenyl)ethanone (**34b**). To the mixture of **28b** (10 g, 38.7 mmol), tert-butyl carbamate (6.81 g, 58.1 mmol), CuI (0.74 g, 3.87 mmol) and K_2CO_3 (10.71 g, 77.5 mmol) was added N,N'-dimethylethylenediamine (0.55 mL, 3.87 mmol) in toluene (100 mL). The mixture was stirred under nitrogen at 110 °C for 24 hours. After being cooled to room temperature, the reaction mixture was filtered and washed with EtOAc (200 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was dissolved in 20% TFA in DCM (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 3 hours and concentrated under vacuum. The residue was dissolved in DCM (100 mL) and washed with saturated Na_2CO_3 aqueous solution and brine. The organic phase was dried over Na_2SO_4 and concentrated. Flash column chromatography on

silica gel (gradient elution, gradient 0 to 30% EtOAc/ 60-90 °C petroleum ether) gave **34b** as a pale yellow solid (8.07 g, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 1.7 Hz, 1H), 7.45 (d, J = 1.8 Hz, 1H), 4.10 (brs, 2H), 2.59 (s, 3H), 2.30 (s, 3H); LCMS *m/z* (ESI) found $[M+H]^+$ 195.08; retention time 2.58 min, > 96% pure.

35b, **36b**, **37b** and **38b** were prepared similar to procedure B.

5-Acetyl-N-cyclopentyl-2-methyl-3-nitrobenzenesulfonamide (**36b**) : ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.42 (s, 1H), 4.74 (d, J = 7.5 Hz, 1H), 3.68 (q, J = 7.0 Hz, 1H), 2.78 (s, 3H), 2.69 (s, 3H), 1.91-1.84 (m, 2H), 1.72-1.63 (m, 2H), 1.58-1.48 (m, 2H), 1.47-1.38 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 325.09; retention time 3.29 min, > 99% pure.

5-(2-Bromoacetyl)-N-cyclopentyl-2-methyl-3-nitrobenzenesulfonamide (**37b**) : ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.45 (s, 1H), 4.83 (d, J = 7.5 Hz, 1H), 4.70 (s, 2H), 3.68 (q, J = 7.0 Hz, 1H), 2.79 (s, 3H), 1.93-1.85 (m, 2H), 1.73-1.63 (m, 2H), 1.58-1.51 (m, 2H), 1.48-1.37 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 404.56.

N-Cyclopentyl-2-methyl-3-nitro-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**38b**) : ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.11 (brs, 1H), 8.43 (s, 1H), 8.08 (s, 1H), 6.60 (s, 1H), 5.00 (d, J = 7.4 Hz, 1H), 3.69 (q, J = 6.9 Hz, 1H), 2.74 (s, 3H), 1.94-1.85 (m, 2H), 1.73-1.65 (m, 2H), 1.58-1.51 (m, 2H), 1.48-1.38 (m, 2H); LCMS *m/z* (ESI, negative) found $[M-H]^-$ 382.11; retention time 3.17 min, > 99% pure.

3-Amino-N-cyclopentyl-2-methyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**39b**). To a solution of **38b** (0.9 g, 2.347 mmol) in EtOH (20 mL) and fine iron powder (0.52 g, 9.39 mmol) was added at 50-55 °C followed by NH_4Cl solution (0.502 g, 9.39

mmol in 10 mL water). The reaction mixture was refluxed for 1 hour, then cooled to room temperature and filtered through Celite. The filtrate was basified with $NaHCO_3$ solution (pH 7-8) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (gradient elution, gradient 0 to 40% EtOAc/ 60-90 °C petroleum ether) gave **39b** as a white solid (0.73 g, 88% yield). Mp 215-216 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 7.52 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 1.8 Hz, 1H), 7.03 (d, J = 1.9 Hz, 1H), 6.54 (d, J = 1.0 Hz, 1H), 5.38 (s, 2H), 3.40 (q, J = 6.8 Hz, 1H), 2.29 (s, 3H), 1.62-1.46 (m, 4H), 1.42-1.26 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.40, 149.22, 140.95, 134.36, 127.99, 119.96, 114.54, 114.21, 98.39, 54.65, 32.80 (2 × C), 23.25 (2 × C), 13.98; HRMS (EI) *m/z* calcd for $C_{15}H_{19}N_3S_2O_3$: 353.0868, found 353.0871; retention time 2.75 min , > 99% pure.

N-(3-(N-Cyclopentylsulfamoyl)-2-methyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)cyclopropanecarboxamide (**40b**). To a solution of **39b** (40 mg, 0.113 mmol) in anhydrous THF (20 mL) were added cyclopropanecarbonyl chloride (12 μL, 0.136 mmol) and pyridine (18 μL, 0.226 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 hours. The mixture was diluted with EtOAc, washed with 1 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (gradient elution, gradient 0 to 40% EtOAc/ 60-90 °C petroleum ether) to give **40b** (0.03 g, 63%) as a white solid. Mp 287-288 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s,

1H), 9.92 (s, 1H), 7.97 (s, 1H), 7.84 (s, 1H), 7.80 (d, J = 7.8 Hz, 1H), 6.85 (s, 1H), 3.47-3.42 (m, 1H), 2.45 (s, 3H), 1.92-1.85 (m, 1H), 1.62-1.48 (m, 4H), 1.45-1.23 (m, 4H), 0.85-0.76 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.26, 172.51, 141.47, 139.18, 132.97, 132.01, 127.65, 126.73, 122.95, 99.98, 54.67, 32.79 (2 × C), 23.26 (2 × C), 14.76, 14.41, 7.65 (2 × C); HRMS (EI) *m/z* calcd for $C_{19}H_{23}N_3S_2O_4$: 421.1130, found 421.1129; retention time 2.95 min, > 97% pure.

N-Cyclopentyl-2-methyl-3-(methylamino)-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**43**). To a solution of **39b** (80 mg, 0.226 mmol) in THF (20 mL) was added 37% formaldehyde solution (67 μL, 0.905 mmol) and the mixture was stirred at room temperature for 2 hours. $NaBH_3CN$ (57 mg, 0.905 mmol) and acetic acid (52 μL, 0.905 mmol) were then added and the reaction mixture was stirred overnight. The reaction was diluted with EtOAc (50 mL). The layers were separated and extracted twice with EtOAc (40 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (gradient elution, gradient 0 to 40% EtOAc/ 60-90 °C petroleum ether) to give **43** (0.045 g, 55%) as a white solid. Mp 204-205 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.38 (d, J = 1.8 Hz, 1H), 6.91 (d, J = 1.8 Hz, 1H), 6.74 (s, 1H), 5.60 (d, J = 5.0 Hz, 1H), 3.42-3.36 (m, 1H), 2.81 (d, J = 4.7 Hz, 3H), 2.30 (s, 3H), 1.59-1.52 (m, 4H), 1.41-1.25 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.40, 149.80, 140.71, 134.38, 128.11, 120.37, 112.71, 109.04, 98.57, 54.70, 32.82 (2 × C), 30.90, 23.24 (2 × C), 13.93; HRMS (EI) *m/z* calcd for $C_{16}H_{21}N_3S_2O_3$: 367.1024, found 367.1006; retention time 2.95 min, > 99% pure.

Following procedure D as for compound **40b** gave products **33a**, **33b**, **39a- 42a**, **41b** and **42b**.

3-Bromo-N-cyclopentyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**33a**): White solid, mp 230-231 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 8.15 (s, 1H), 8.08 (s, 1H), 7.90 (s, 1H), 7.80 (d, J = 7.3 Hz, 1H), 7.16 (s, 1H), 3.55-3.46 (m, 1H), 1.66-1.57 (m, 2H), 1.57-1.48 (m, 2H), 1.44-1.35 (m, 2H), 1.33-1.24 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.96, 144.76, 132.80, 131.62, 131.32, 128.73, 123.20, 122.23, 102.66, 54.97, 32.92 (2 × C), 23.32 (2 × C); HRMS (EI) *m/z* calcd for $C_{14}H_{15}BrN_2S_2O_3$: 401.9707, found 401.9712; retention time 3.38 min, > 96% pure.

3-Bromo-N-cyclopentyl-2-methyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**33b**) : Offwhite solid, mp 218-220 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.18 (d, J = 1.7 Hz, 1H), 8.14 (d, J = 1.7 Hz, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.02 (s, 1H), 3.50-3.42 (m, 1H), 2.65 (s, 3H), 1.64-1.49 (m, 4H), 1.41-1.25 (m,, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.08, 142.41, 135.96, 132.56, 131.68, 129.31, 128.15, 125.19, 101.43, 54.72, 32.81 (2 × C), 23.25 (2 × C), 20.08. HRMS (EI) *m/z* calcd for $C_{15}H_{17}BrN_2S_2O_3$: 415.9864, found 415.9827; retention time 3.42 min, > 99% pure.

3-Amino-N-cyclopentyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)benzenesulfonamide (**39a**) : Offwhite solid, mp 229-230 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (brs, 1H), 7.46 (d, J = 7.1 Hz, 1H), 7.15 (t, J = 1.5 Hz, 1H), 6.98 (t, J = 1.8 Hz, 1H), 6.91 (t, J = 1.5 Hz, 1H), 6.64 (s, 1H), 5.72 (s, 2H), 3.46-3.41 (m, 1H),

1.66-1.50 (m, 4H), 1.42-1.26 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.32, 150.06, 143.30, 134.22, 131.61, 113.65, 111.73, 110.89, 99.30, 54.87, 32.93 (2 × C), 23.35 (2 × C). HRMS (EI) *m/z* calcd for $C_{14}H_{17}N_3S_2O_3$: 339.0711, found 339.0715; retention time 2.69 min, > 99% pure.

N-(3-(N-Cyclopentylsulfamoyl)-5-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)cyclopropanecarboxamide (**40a**): White solid, mp 172-174 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 10.63 (s, 1H), 8.07 (t, J = 1.8 Hz, 1H), 7.98 (t, J = 1.8 Hz, 1H), 7.69 (t, J = 1.6 Hz, 1H), 7.67 (d, J = 7.2 Hz, 1H), 6.76 (s, 1H), 3.51-3.41 (m, 1H), 1.84-1.77 (m, 1H), 1.67-1.57 (m, 2H), 1.56-1.49 (m, 2H), 1.43-1.35 (m, 2H), 1.32-1.25 (m, 2H), 0.85-0.79 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.28, 172.71, 143.36, 140.65, 133.38, 131.58, 119.23, 118.18, 117.33, 100.67, 54.89, 32.95 (2 × C), 23.36 (2 × C), 15.07, 8.06 (2 × C); HRMS(EI) *m/z* calcd for $C_{18}H_{21}N_3S_2O_4$: 407.0973, found 407.0974; retention time 2.96 min, > 95% pure.

N-(3-(N-Cyclopentylsulfamoyl)-5-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)butyramide (**41a**): White solid, mp 244-245 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 10.30 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.73-7.64 (m, 2H), 6.76 (s, 1H), 3.49 (q, J = 7.0 Hz, 1H), 2.33 (t, J = 7.3 Hz, 2H), 1.67-1.58 (m, 4H), 1.55-1.48 (m, 2H), 1.42-1.34 (m, 2H), 1.33-1.23 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.28, 172.18, 143.35, 140.67, 133.38, 131.54, 119.30, 118.22, 117.36, 100.66, 54.89, 38.72, 32.96 (2 × C), 23.37 (2 × C), 18.87, 14.08; HRMS (EI) *m/z* calcd for $C_{18}H_{23}N_3S_2O_4$: 409.1130, found 409.1129; retention time 3.06 min, > 97% pure.

N-(3-(N-Cyclopentylsulfamoyl)-2-methyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)butyramide (**41b**): White solid, mp 229-231 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 9.63 (s, 1H), 7.98 (s, 1H), 7.83-7.77 (m, 2H), 6.85 (s, 1H), 3.47-3.39 (m, 1H), 2.43 (s, 3H), 2.35 (t, J = 7.2 Hz, 2H), 1.67-1.60 (m, 2H), 1.61-1.49 (m, 4H), 1.40-1.26 (m, 4H), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.28, 171.86, 141.47, 139.21, 132.96, 132.31, 127.64, 126.98, 123.06, 99.97, 54.68, 38.03, 32.79 (2 × C), 23.25 (2 × C), 19.14, 14.77, 14.17; HRMS (EI) *m/z* calcd for $C_{19}H_{25}N_3S_2O_4$: 423.1286, found 423.1282; retention time 3.00 min, > 98% pure.

N-(3-(N-Cyclopentylsulfamoyl)-5-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)thiophene-2-carboxamide (**42a**): White solid, mp 287-289°C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 1H), 10.63 (s, 1H), 8.22 (s, 1H), 8.19 (s, 1H), 8.07 (d, J = 3.9 Hz, 1H), 7.92 (d, J = 4.9 Hz, 1H), 7.78 (s, 1H), 7.70 (d, J = 7.3 Hz, 1H), 7.30-7.22 (m, 1H), 6.82 (s, 1H), 3.56-3.47 (m, 1H), 1.69-1.59 (m, 2H), 1.58-1.49 (m, 2H), 1.43-1.34 (m, 2H), 1.34-1.25 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.27, 160.66, 143.36, 140.22, 139.69, 133.30, 133.13, 131.53, 130.16, 128.77, 120.64, 118.84, 118.62, 100.84, 54.93, 32.98 (2 × C), 23.38 (2 × C); HRMS (EI) *m/z* calcd for $C_{19}H_{19}N_3S_3O_4$: 449.0538, found 449.0538; retention time 3.13 min, > 97% pure.

N-(3-(N-Cyclopentylsulfamoyl)-2-methyl-5-(2-oxo-2,3-dihydrothiazol-4-yl)phenyl)thiophene-2-carboxamide (**42b**): White solid, mp 215-217 °C (from 60-90 °C petroleum ether and EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, 1H), 10.27 (s, 1H), 8.14-8.01 (m, 2H), 7.92-7.84 (m, 3H), 7.29-7.20 (m, 1H), 6.93 (s, 1H), 3.41-3.35 (m, 1H), 2.48 (s, 3H), 1.65-1.50 (m, 4H), 1.42-1.28(m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.68, 141.40,

139.63, 138.60, 132.40, 129.85, 128.66, 128.02, 123.93, 99.67, 54.73, 32.81 (2 × C), 23.26 (2 × C), 14.89; HRMS (EI) *m/z* calcd for $C_{20}H_{21}N_3S_3O_4$: 463.0694, found 463.0701; retention time 3.17 min, > 98% pure.

Protein expression

The BRD4 (I) protein expression followed the protocol of Filippakopoulos et al.[12] Colonies from freshly transformed plasmid DNA in *E. coli* BL21(DE3)-condon plus-RIL cells, were grown overnight at 37 ºC in 50 mL of Terrific Broth medium with 50 μg/mL kanamycin and 34 μg/mL chloramphenicol (start-up culture). Then start-up culture was diluted 100 fold in 1 L of fresh TB medium and cell was growth at 37 ºC to an optical density of about 0.8 at OD600 before the temperature was decreased to 16 ºC. When the system equilibrated at 16 ºC the optical density was about 1.2 at OD600 and protein expression was induced over night at 16 ºC with 0.2 mmol isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were harvested by centrifugation (4000 × g for 20 min at 4 ºC) and were frozen at -80 ºC as pellets for storage. Cells expressing His6-tagged proteins were re-suspended in lysis buffer (50 mmol HEPES, pH 7.5 at 25 ºC, 500 mmol NaCl, 10 mmol imidazole, 5 % glycerol with freshly added 0.5 mmol TCEP (Tris(2-carboxyethyl)phosphine hydrochloride) and 1 mmol PMSF (phenylmethanesulfonyl fluoride) and lysed with an JN 3000 PLUS high pressure homogenizer (JNBIO - Guangzhou, China) at 4 ºC. The lysate was cleared by centrifugation (12,000 × g for 1 h at 4 ºC) and was applied to a nickel-nitrilotiacetic acid agarose column. The column was washed once with 50 mL

of wash buffer containing 30 mmol imidazole. The protein was eluted using a step elution of imidazole in elution buffer (100-250 mmol imidazole in 50 mmol HEPES, pH 7.5 at 25 ºC, 500 mmol NaCl, 5% glycerol). All fractions were collected and monitored by SDS-polyacrylamide gel electrophoresis (Bio-Rad Criterion™ Precast Gels, 4-12% Bis-Tris, 1.0 mm, from Bio-Rad, CA.). After the addition of 1 mmol dithiothreitol (DTT), the eluted protein was treated overnight at 4 ºC with Tobacco Etch Virus (TEV) protease to remove the His6 tag. The protein was concentrated and further purified with size exclusion chromatography on a Superdex 75 16/60 HiLoad gel filtration column. Samples were monitored by SDS-polyacrylamide gel electrophoresis and concentrated to 8-10 mg/mL in the gel filtration buffer, 10 mmol Hepes pH 7.5, 500 mmol NaCl, 1 mmol DTT and were used for protein binding assay and crystallization.

Other four bromodomain proteins (BRD2 (aa 67-200), EP300 (aa 1040-1161), BRG1 (aa 1448-1569), ATAD2 (aa 981-1108)) were prepared as BRD4 (I), with same protocol for expression and protein purification. These bromodomain proteins were used in thermal shift assay for ligand selectivity test.

Crystallization and Data collection

Aliquots of the purified proteins were set up for crystallization using the vapour diffusion method. BRD4 (I) Crystals were grown by mixing 1 μL of the protein (9 mg/mL) with an equal volume of reservoir solution containing 6 M sodium formate and 10 % glycerol. Its complex with 41 fragments was grown at 4 ºC in 1 μL protein (10 mg/mL + 5 mmol fragment) with an equal volume of reservoir solution containing

6 M sodium formate and 10 % glycerol. Crystals grew to diffracting quality within 1-3 weeks in all cases.

Data were collected at 100 K on beamLine BL17U (at wavelength 0.9793 Å) at the Shanghai Synchrotron Radiation Facility (SSRF) (Shanghai, China) for the co-crystallized structures. The data were processed with the HKL2000,[32] software packages, and the structures were then solved by molecular replacement, using the CCP4 program MOLREP.[33] The search model used for the crystals was the BRD4-JQ1 complex structure (PDB code 3mXF). The structures were refined using the CCP4 program REFMAC5 combined with the simulated-annealing protocol implemented in the program PHENIX.[34] With the aid of the program Coot,[35] compound, water molecules, and others were fitted into to the initial F_o-F_c maps.

Fluorescence anisotropy binding assay

The binding of compounds to BRD4 was assessed using a Fluorescence Anisotropy Binding Assay. The fluorescent ligand was prepared by attaching a fluorescent fragment (Fluorescein isothiocyanate isomer I, 5-FITC) to the (+)-JQ1. Generally the method involves incubating the Bromodomain protein BRD4, fluorescent ligand and a variable concentration of test compound together to reach thermodynamic equilibrium under conditions such that in the absence of test compound the fluorescent ligand is significantly (> 50%) bound and in the presence of a sufficient concentration of a potent inhibitor the anisotropy of the unbound fluorescent ligand is measurably different from the bound value.

Detailedly, all components were dissolved in buffer of composition 50 mmol HEPES pH 7.4, 150 mmol NaCl and 0.5 mmol CHAPS with final concentrations of BRD4 (I) 45 nM, fluorescent ligand 5 nM. This reaction mixture was added various concentrations of test compound or DMSO vehicle (5‰ final) in Corning 384 well Black low volume plate (CLS3575) and equilibrated in dark for 4 hours at room temperature. Fluorescence anisotropy was read on BioTek Synergy2 Multi-Mode Microplate Reader (ex= 485 nm, EM = 530 nm; Dichroic -505 nM).

In vitro metabolic stability study

Microsomes (Human microsome: Xenotech, Lot No.H0610; Rat microsome: Xenotech, Lot No. R1000) (0.5 mg/mL) were preincubated with 1 μM test compound for 5 min at 37 °C in 0.1 M phosphate buffer (pH 7.4) with 1 mmol EDTA, and 5 mmol $MgCl_2$. The reactions were initiated by adding prewarmed cofactors (1 mmol NADPH). After 0, 5, 10, and 30 min incubations at 37 ℃, the reactions were stopped by adding an equal volume of cold acetonitrile. The samples were vortexed for 10 min and then centrifuged at 10,000 × g for 10 min. Supernatants were analyzed by LC/MS/MS for the amount of parent compound remaining, and the corresponding loss of parent compound also determined by LC/MS/MS.

The CYP enzymatic activities were characterized based on their probe reactions: CYP3A4 (midazolam), CYP2D6 (dextromethorphan), CYP2C9 (Diclofenac), CYP1A2 (phenacetin) and CYP2C19 (Mephenytoin). Incubation mixtures were prepared in a total volume of 100 μL as follows: 0.2 mg/mL microsome (Human

microsome: Xenotech, Lot No.H0610), 1 mmol NADPH, 100 mmol phosphate buffer (pH 7.4), probe substrates cocktail (10 μM Midazolam, 100 μM Testosterone, 10 μM Dextromethophan, 20 μM Diclofenac, 100 μM Phenacetin, 100 μM Mephenytoin) and 10 μM tested compound or positive control cocktail (10μM ketoconazole，10 μM quinidine，100 μM Sulfaphenazole，10 μM Naphthoflavone, and 1000 μM Tranylcypromine) or negative control (PBS). The final concentration of organic reagent in incubation mixtures was less than 1% v/v. There was a 5 min preincubation period at 37 ℃ before the reaction was initiated by adding a NADPH-generating system. Reactions were conducted for 20 minutes for CYPs. For each probe drug, the percentage of metabolite conversion was less than 20% of substrate added. The inhibition rate was calculated as: (The formation of the metabolite of probe substrates with 10 μM tested compound)/ (The formation of the metabolite of probe substrates with PBS) × 100%.

Cellular assays

Cell culture and compounds

Human colon cancer HT-29 cells, leukemia MV4;11 and myeloma MM.1S cells were purchased from the American Type Culture Collection. The cells were cultured respectively in McCoy's 5A, IMDM and RPMI1640 medium modified supplemented with 10% FBS (Life Technologies) at 37 ℃ in a humidified atmosphere containing 5% CO_2. Compounds were dissolved in DMSO at a concentration of 0.01 mol/L as stock solutions, which were diluted to the desired concentrations with normal saline immediately before experiments.

Growth inhibition assays

For HT-29 cells, the proliferation inhibition of the tested compounds was examined by sulforhodamine B (SRB) assays: cells seeded in 96-well plates were treated in triplicate with gradient concentrations of tested compounds at 37 °C for 72 h, and then assessed with SRB (Sigma). The absorbance at 560 nm was detected with a plate reader (SpectraMax; Molecular Devices). The inhibition rate was calculated as (A560 treated/A560 control) × 100%. The proliferation inhibition of the compounds in MV4;11 and MM.1S cells was examined by the CellTiter-Glo Luminescent Cell Viability Assay (Promega, #G7572): cells seeded in 384-well plates were treated in 5-fold with gradient concentrations of tested compounds at 37 °C for 72 h, and then assessed with CellTiter-Glo Reagent. The luminescence signal was detected with a plate reader (Envision; PerkinElmer). The inhibition rate as (Max signal - Compound signal) / (Max signal - Min signal) × 100%.

Quantitative real time -PCR (RT-qPCR) analyses

HT-29 Cells were seeded into 6-well plates and incubated overnight. Cells were treated with compounds at various designed concentration for 24 h. Total RNA was isolated with the Trizol reagent (Invitrogen) and reverse-transcribed using PrimeScript®RTase (Takara). cDNA was used for RT-qPCR SYBR Green assays (Takara) with the following primers (synthesized by Sanggon Corporation): *β-actin* primer, 5′-GGATGCAGAAGGAGATCACTG-3′ (forward), 5′-CGATCCACACGGAGTACTTG-3′ (backward); *c-myc* primer, 5′-CGTCTCCACACATCAGCACAA-3′ (forward),

5′-TGTTGGCAGCAGGATAGTCCTT-3′ (backward). The relative levels of *c-myc* mRNA were calculated as: [(*c-myc* mRNA in the treatment group) /*β-actin* mRNA in the treatment group)] / [(*c-myc* mRNA in the control group) /*β-actin* mRNA in the control group)].

Western blotting

HT-29 cells were seeded into 6-well plates and incubated overnight. Cells were treated with compounds at various designed concentration for 24 h. Cells were lyzed in 1×SDS lysis buffer [50 mmol/L Tris-HCl (pH6.8), 100 mmol/L DTT, 2% SDS, 0.1% bromphenol blue, and 10% glycerol] and then boiled for 10 minutes. Western blotting analyses were conducted as previously described[36] using c-Myc (BD Bioscience) and GAPDH (Beyotime) antibodies and the levels of cellular proteins were visualized with peroxidase-coupled secondary antibodies (RD Systems and Jackson ImmunoResearch) using an ECL Kit from Thermo Scientific company.

Thermal Shift Assay

Thermal shift assay were carried out using Applied Biosystems 7500 Real-Time PCR system. Proteins were buffered in 10 mmol HEPES, pH 7.5, 500 mmol NaCl at a final concentration of 75 μg/mL. Compounds were added at a final concentration of 50 μmol. SYPRO Orange (ABI) was added as a fluorescence probe at a dilution of 1 in 1000. The 20 μL volume protein melt reaction mix was added to the wells of 96-well PCR plate, Sealed with MicroAmp® Optical Adhesive Film. Each protein reaction mixture was prepared to 4 replicates. For the “Experiment set”, selecting “Melt Curve” for the “Experiment type”, “ROX” for the “Reporter”, and “None” for

the "Quencher". The plate was heated from 25 °C to 99 °C with a heating rate of 1 °C /min and the fluorescence reading was taken with continues mode. Data analysis was proceeded by Protein Thermal Shift™ Software.

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Supporting Information Available: The spectra data and figures of synthesized compounds were provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Abbreviations Used

SBDD, structure-based drug discovery;

PTM, post-translational modification;

HAT, histone acetyltransferase;

HDAC, histone deacetylase;

KAc, acetylated lysine;

BET, Bromodomain and Extra-Terminal;

NUT, nuclear protein in testis;

BRD4 (I), first bromodomain of BRD4;

GSK, GlaxoSmithKline;

SSRF, Shanghai Synchrotron Radiation Facility;

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Graphic Abstract