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Design, synthesis and biological evaluation of novel 6-phenyl-1,3a,4,10b-tetrahydro-2*H*-benzo[*c*]thiazolo[4,5-e]azepin-2-one derivatives as potential BRD4 inhibitors

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ABSTRACT

Bromodomain-containing protein 4 (BRD4) is a key epigenetic regulator in cancer, and inhibitors targeting BRD4 exhibit great anticancer activity. By replacing the methyltriazole ring of the BRD4 inhibitor I-BET-762 with an *N*-methylthiazolidone heterocyclic ring, fifteen novel BRD4 inhibitors were designed and synthesized. Compound **13f** had a hydrophobic acetylcyclopentanyl side chain, showing the most potent BRD4 inhibitory activity in the BRD4-BD1 inhibition assay (IC_{50} value of 110 nM), it also significantly suppressed the proliferation of MV-4-11 cells with high BRD4 level (IC_{50} value of 0.42 μM). Furthermore, the potent apoptosis-promoting and G0/G1 cycle-arresting activity of compound **13f** were indicated by flow cytometry. As the downstream-protein of BRD4, c-Myc was in significantly low expression by compound **13f** treatment in a dose-dependent manner. All the findings supported that this novel compound **13f** provided a perspective for developing effective BRD4 inhibitors.

1. Introduction

The bromodomain and extra-terminal domain (BET) protein family includes BRD2, BRD3, BRD4 and bromodomain testis-specific protein (BRDT). The BET protein consists of two *N*-terminal bromodomains and one C-terminal domain (ET), and their sequences are highly conserved.[1] Among them, BRD2 functions through the E2F transcription factor pathway;[2] BRD3 helps in recruiting hematopoietic transcription factor, GATA1, by regulating the maturation of red blood cells, megakaryocytes and mast cells.[3,4] BRD4 is currently the most widely studied protein of BET family, and it is involved in many human diseases, including cancer, inflammation, cardiovascular disease, central nervous system (CNS) disease and human immunodeficiency virus (HIV) infection.[5–8] BRD4 is a critical mediator of transcriptional elongation, and it influences mitotic progression.[9–11] The abnormal expression of BRD4 will cause the activation of the downstream gene c-Myc, which could lead to the occurrence of cancers such as acute leukemia, lymphoma, melanoma and so on.[12] Therefore, BRD4 has been developed as therapeutic target for research on cancer drug, and such research is applicable to the development of small molecule inhibitors.

A pair of bromodomains (BD1 and BD2) are contained in the BRD4 structure, each of which is consisted of a conserved fold and two loop regions.[1] Although BD1 and BD2 possess a high similarity in sequence of substrate-binding site, some functions of BD1 and BD2 are different. For example, BD2 have wide effects towards other acetylated substrates, whereas BD1 is inclined to only discerning H4 acetylation marks.[13] Therefore, BD1 is the main targeted binding site for BRD4 inhibitors.[14,15] BRD4 inhibitor (+)-JQ-1 and I-BET-762 are potent BRD4 inhibitors. Provided by PDB data, the core structures of the two potent inhibitors can form essential hydrogen bonds with the residue Tyr 97 in BRD4 in Fig. 1 (PDB ID: 4c66 and 3mxf). Additionally, the co-crystal structure of BRD4 bromodomain 1 (BRD4-BD1) and histone peptide H4K8acK12ac indicates that acetylated lysine is recognized by a central hydrophobic cavity, and hydrogen bond interaction is formed between the acetylcarbonyl oxygen atom and the conserved Tyr97 via water molecules.[16] Therefore, Tyr 97 residue is an important binding site for BRD4-BD1.

As shown in Fig. 2, the researchers have made some structural

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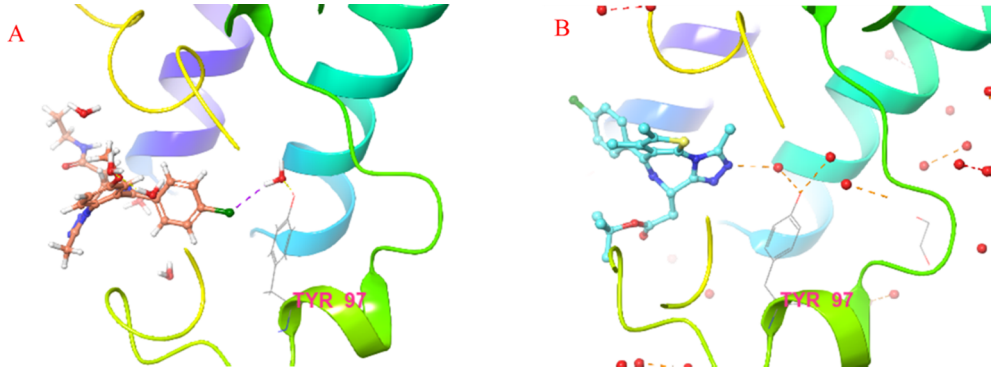


Fig. 1. (A) Hydrogen bonds formed between I-BET-762 inhibitor and residue Tyr 97 via water molecule; (B) hydrogen bonds formed between (+)-JQ-1 inhibitor and residue Tyr 97 via water molecule.

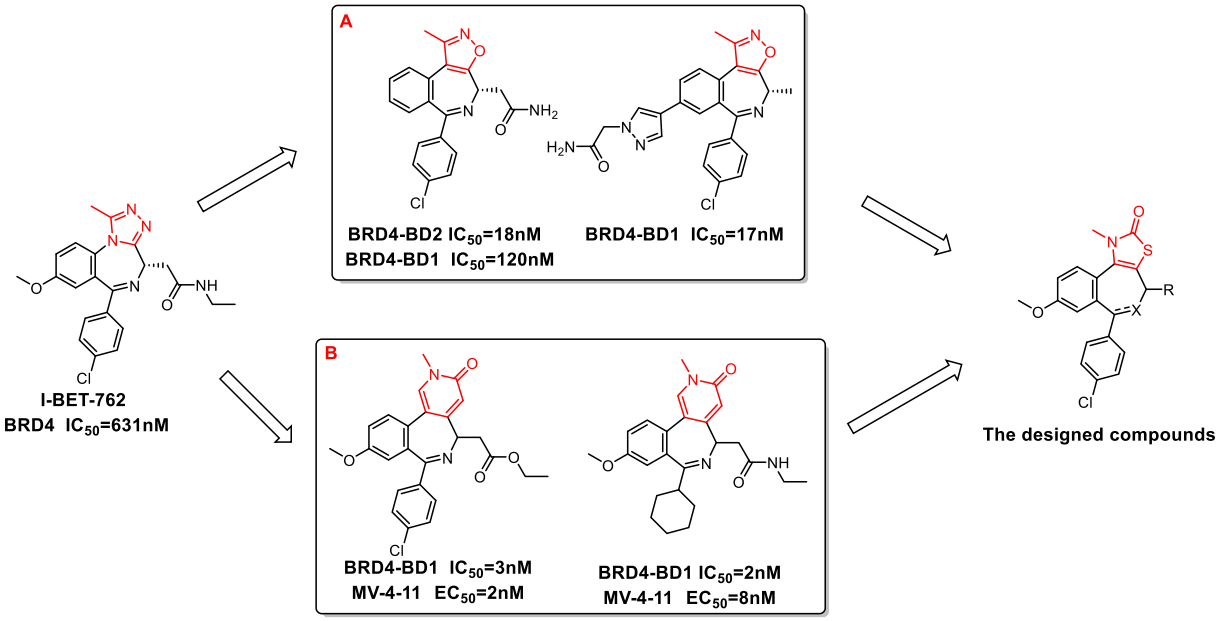


Fig. 2. Design of target compounds.

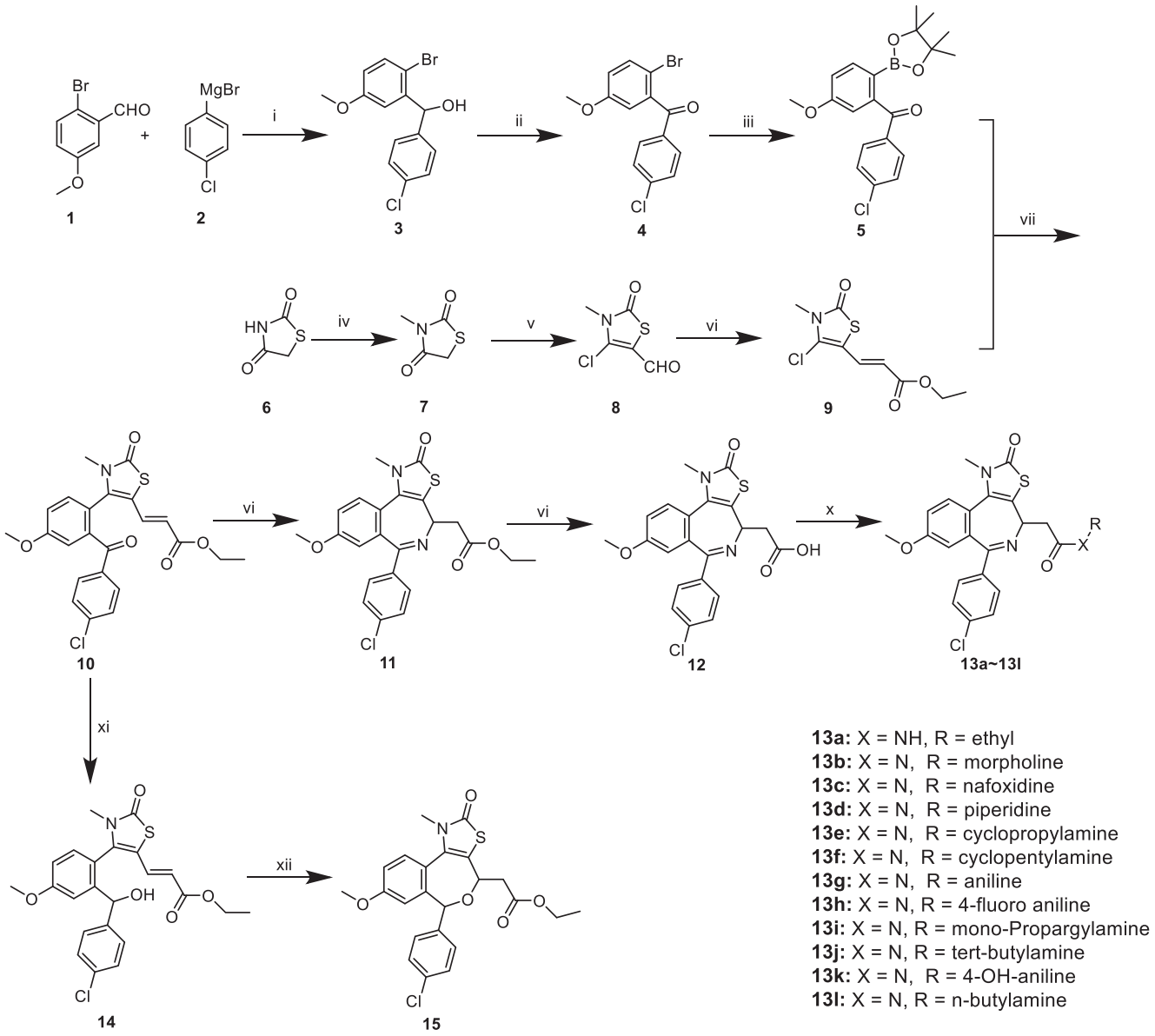
modifications to the BRD4 inhibitor I-BET-762. It has been reported by Hewitt, et al. that the BRD4 inhibitory activity was significantly improved when the methyltriazole ring of the lead compound I-BET-762 was substituted with methylisoxazole ring (Fig. 2A).[17,18] In addition, Li, et al. has reported that on the basis of retaining the predominant skeletal structure of benzoazepine and replacing methyltriazole with *N*-methylpyridone, compounds with more potent BRD4 inhibitory activity were obtained (Fig. 2B).[19] Therefore, the methyltriazole ring of compound I-BET-762 could be further modified, and these modifications had little impact on target- selectivity between BRD4 and other BRD proteins. Since the acetylated lysine (KAc) binding site was the most important part in the design of BRD4 inhibitors, interacting with the acetylated lysine binding site have been used for developing several kinds of specific small molecular BRD4 inhibitors.[20–24] It has been reported that the 2-thiazolidinone structure could well simulate the acetylated lysine structure, and it acted as an effective BRD4 mimic group.[25,26] Thus, the 2-thiazolidinone was a new strategy to replace the methyltriazole ring of compound I-BET-762, which was different from the previous studies to replace the methyltriazole ring of compound I-BET-762 with *N*-methylpyridone or methylisoxazole ring.

Here we synthesized a series of new scaffold compounds based on the structure of I-BET-762. The BRD proteins and their binding site were different, and a lot of research has been done on the BRD4 binding site. On the basis of this binding site, a reasonable compound design was carried out. Based on the structure-activity relationship of I-BET-762, the methyltriazole moiety was replaced with a methylthiazolidone moiety to maintain the interaction with the residue Tyr 97, which could keep selective inhibition of BRD4-BD1 instead of BRD4-BD2. Consequently, a series of biological evaluation demonstrated that compound **13f** did exhibit more powerful BRD4 inhibitory activity than I-BET-762, and its activity was comparable to (+)-JQ-1. Compound **13f** was shown to be potent BRD4 inhibitor with an IC_{50} value of 110 nM, and it had an IC_{50} value of 0.42 μM against MV-4-11 cell antiproliferation. It provided a structural perspective for developing novel effective BRD4 inhibitors.

2. Results and discussion

2.1. Synthesis

Synthesis of 6-phenyl-1,3a,4,10b-tetrahydro-2H-benzo[*c*]thiazolo[4,5-e]azepin-2-one derivatives was shown in Scheme 1. Compounds **11**, **12**, **13a-l** and **15** were synthesized using 2-bromo-5-methoxybenzaldehyde (**1**) and 2,4-thiazolidinedione (**6**) as starting materials, and the final product was obtained through ten steps of reaction. First, commercially available 2-bromo-5-methoxybenzaldehyde (**1**) reacted with 4-chlorophenyl magnesium bromide (**2**) to prepare **3**, and the intermediate **3** was oxidized to **4**. Alcohol borate reacted with **4** to



Scheme 1. Reagents and conditions: (i) anhydrous THF, 0℃→r.t, 2 h; (ii) dry DCM, pyridinium chlorochromate, r.t, 1.5 h; (iii) Bis(pinacolato)diborane, dioxane, KOAc, Pd(dppf)Cl_2, 95 ℃, 2.5 h; (iv) MeI, K_2CO_3, DMF, 75℃, 2 h; (v) $POCl_3$, DMF, 90-115℃, 4 h; (vi) NaH, Triethyl phosphonoacetate, THF, 0 ℃→r.t; (vii) **I-5**, Na_2CO_3, dioxane/H_2O, Pd(PPh_3)$_4$, 90 ℃, 20 h; (viii) Ammonium formate, EtOH, reflux; (ix) 1 N NaOH, THF, r.t; (x) ROH/RNH_2, EDC·HCl, DMAP, r.t; (xi) $NaBH_4$, MeOH, r.t; (xii) K_2CO_3, EtOH, r.t for 24 h.

produce intermediate **5**. Commercially available thiazolidine-2,4-dione (**6**) was methylated to obtain intermediate **7**, and then Vilsmeir formylation was used for **7** to get **8**. Compound **8** reacted with triethylphosphonoacetate to obtain **9**, and then **9** reacted with intermediate **5** through Suzuki coupling reaction to obtain **10**. Target compound **11** was synthesized via **10** reacting with ammonium formate to close the ring. Finally, **11** was hydrolyzed by NaOH, producing target compound **12** which was substituted with different types of amines to produce the target products, **13a** ~ **13 k**. In addition, compound **10** was reduced by sodium borohydride to obtain compound **14**, and then **14** was reacted under alkaline conditions to obtain target compound **15**.

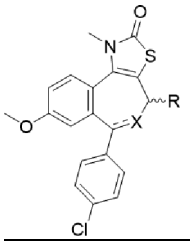
2.2. Biological evaluations

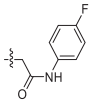
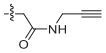
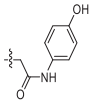
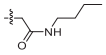
2.2.1. Inhibitory activity against BRD4

To probe the inhibition activities of the designed compounds targeting BRD4, time-resolved fluorescence resonance energy transfer (TR-FRET) binding assay was used, and (+)-JQ-1 and I-BET-762 were applied as positive controls. As shown by the IC_{50} values in Table 1, all compounds had relatively better inhibition activities than I-BET-762. Particularly, the IC_{50} value of compounds **13f** and **13j** was 0.11 μM and 0.16 μM, respectively. When X was nitrogen atom, the compounds' inhibitory activities were better than those when X was oxygen atom. In addition, polar groups, such as hydroxyl, were introduced into the side chain leading to decreased inhibitory activities for BRD4-BD1. The R side chain was substituted with acetylcyclopentylamino giving compound **13f** which had the strongest inhibitory activity for BRD4-BD1 among all the compounds. Acetyl *t*-butylamine side chain had significantly better inhibitory activity for BRD4-BD1 than acetobutylamine side chain comparing the results for compound **13j** (0.16 μM) with that for **13 l** (1.02 μM). This indicated that the introduction of long fatty side chains was not beneficial to improve the inhibitory activity. Furthermore, compound with acetylarylamine side chain (**13g**) had better activity than others with acetylheterocyclic aliphatic amine side chains (**13b**, **13c**, **13d**).

In summary, the inhibition activities of compounds towards BRD4

Table 1
Structures and inhibitory activities of compounds for BRD4-BD1.



Compound	R	X	BRD4-BD1 Inhibitory Activity IC_{50} (μM)[a]
11		N	0.42 ± 0.06
12		N	0.87 ± 0.08
13a		N	0.74 ± 0.05
13b		N	0.48 ± 0.02
13c		N	0.54 ± 0.06
13d		N	0.43 ± 0.04
13e		N	0.14 ± 0.02
13f		N	0.11 ± 0.01
13 g		N	0.20 ± 0.01
13 h		N	0.68 ± 0.03
13i		N	0.21 ± 0.02
13j		N	0.16 ± 0.01
13 k		N	0.51 ± 0.04
13 l		N	1.02 ± 0.1
15		O	0.48 ± 0.02
(+)-JQ-1	–	–	0.074 ± 0.002
I-BET-762	–	–	0.32 ± 0.07

[a] Each value was reproduced in three assays, the values were expressed as the means ± SD.

could be improved by introducing a hydrophobic R side chain with a small structure, or retaining an aromatic R side chain that did not have a hydrophilic group.

2.2.2. Inhibition of cell growth

It is well known that BRD4 is a recognized target for treating leukemia. Therefore, the anti-proliferative effects of the target compounds against MV-4-11 (biphenotypic B myelomonocytic leukemia) cell line and HL-60 (acute promyelocytic leukemia) cell line were evaluated *in vitro*. As shown in Table 2, most of the compounds showed potent inhibition effect against MV-4-11 cells. Half of the compounds had better inhibition effect against MV-4-11 cell line than the positive control, I-BET762. In the anti-proliferation against MV-4-11 cells, compound **13e**, **13f**, **13g**, **13i** and **13j** were a little inferior to (+)-JQ-1. These

Table 2
Anti-proliferation activity of target compounds against MV-4-11 and HL-60 cells.

Compound	HL-60 IC_{50} (μM)[a]	MV-4-11 IC_{50} (μM)[a]
11	6.21 ± 0.26	0.88 ± 0.13
12	NT[b]	2.86 ± 0.17
13a	12.85 ± 1.07	1.78 ± 0.08
13b	7.62 ± 0.63	0.94 ± 0.05
13c	10.37 ± 0.42	1.12 ± 0.14
13d	8.69 ± 0.36	0.91 ± 0.12
13e	4.56 ± 0.21	0.51 ± 0.08
13f	5.52 ± 0.39	0.42 ± 0.06
13g	7.74 ± 0.33	0.67 ± 0.03
13h	12.36 ± 0.48	1.44 ± 0.08
13i	8.46 ± 0.94	0.70 ± 0.07
13j	5.41 ± 0.22	0.64 ± 0.02
13k	9.05 ± 0.36	1.06 ± 0.11
13l	NT[b]	3.48 ± 0.12
15	5.44 ± 0.24	0.92 ± 0.04
(+)-JQ-1	3.46 ± 0.18	0.16 ± 0.05
I-BET-762	NT[b]	1.23 ± 0.06

[a] The values were reproduced in three assays, the values were expressed as the means ± SD.
[b] Not tested.

compounds could also suppress the proliferation of HL-60 cells at some extent, which was significantly weaker than that of MV-4-11 cells. BRD4 has higher expression in MV-4-11 cells than that in HL-60, so MV-4-11 cells were more sensitive to these designed BRD4 inhibitors. All in all, compound **13e** and **13f** had better anti-proliferation against MV-4-11 cells.

2.2.3. Cell cycle analysis

Flow cytometry analysis was utilized to explore the effect of compound **13f** on the cell cycle in MV-4-11 cells. As shown in Fig. 3, compound **13f** and (+)-JQ-1 could significantly arrest MV-4-11 cells in G1 phase in a dose-dependent manner. The cell-cycle arrest potency of compound **13f** was inferior to (+)-JQ-1 at the same concentration.

2.2.4. Cell apoptosis

BRD4 inhibitors could induce apoptosis.[27] Therefore, the apoptosis-promoting effect of compound **13f** in MV-4-11 cells were determined using flow cytometry. The results in Fig. 4 showed that compound **13f** and (+)-JQ-1 could lead to MV-4-11 cell apoptosis at different drug concentrations. The total number of cells undergoing apoptosis significantly increased with the increase of concentration, suggesting that compound **13f** could effectively induce apoptosis of MV-4-11 cells in a dose-dependent manner.

2.2.5. Western blot assay

It has been reported that BET bromodomain proteins can act as regulatory factors for c-Myc.[28,29] To further evaluate the as-synthesized compound, western blotting was carried out to determine c-Myc expression. Compound **13f** and the positive control (+)-JQ-1 were investigated. Fig. 5 showed that compound **13f** and (+)-JQ-1 could well inhibit the expression of c-Myc protein. The higher was concentration of the compound, the lower was expression. (+)-JQ-1 had better potent ability to inhibit c-Myc expression at the same concentration.

2.2.6. Molecular docking assay

To explore the interaction between compound **13f** and BRD4, Schrödinger software was employed to analyze the possible binding modes. Fig. 6 showed that Pi interactions were formed between the thiazolidinone ring of compound **13f** and Trp81. Meanwhile, compound **13f** had hydrogen-bond interactions with Asp 145 through water molecule. Moreover, the acetylcyclopentylamine side chain of

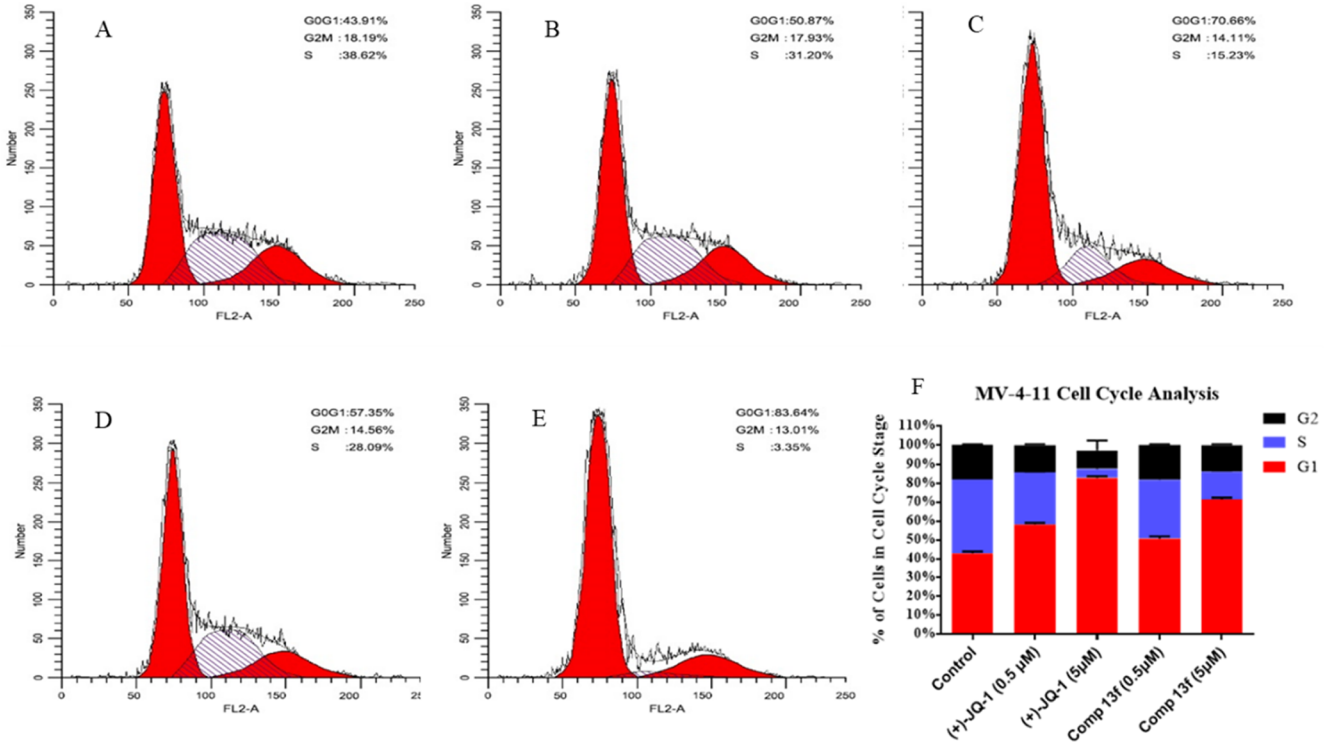


Fig. 3. Effects of compound **13f** and (+)-JQ-1 on MV-4-11 cell cycle analysis: (A) control; (B) compound **13f** (0.5 μM); (C) compound **13f** (5 μM); (D) (+)-JQ-1 (0.5 μM); (E) (+)-JQ-1 (5 μM); (F) cell cycle analysis, the values were expressed as the means ± SD.

compound **13f** was embed with cavity of BRD4-BD1 and formed hydrogen-bond interactions with Tyr 97. These results showed that the compounds had relatively good binding ability to BRD4-BD1 as designed.

3. Conclusions

In summary, the BRD4 inhibitor, I-BET-762, was chosen as the core structure to design and construct fifteen novel BRD4 inhibitors, and their biological activities were evaluated. The results indicated that these compounds had potent BRD4 inhibitory activity. Particularly, the BRD4 inhibitory activity of compound **13f** (with an IC_{50} value of 110 nM) was superior to that of I-BET-762. Furthermore, the as-synthesized compounds exhibited better anti-proliferation activity in MV-4-11 cells than HL-60 cell, suggesting that the designed compounds might be more sensitive to MV-4-11 than to HL-60 cells. Cell cycle analysis revealed that compound **13f** significantly arrested MV-4-11 cells in G1 phase in a dose-dependent manner. Apoptosis analysis and western blotting assay showed that compound **13f** induced cell apoptosis by down-regulating c-Myc expression. Moreover, the docking results showed that compound **13f** could form hydrogen-bond interactions with residue Tyr 97 of BRD4, further indicating that the designed compounds had better binding affinity to BRD4-BD1. In conclusion, the thiazolidinone structure could well simulated the structure of acetylated lysine[25,26] and improve BRD4 inhibitory activity. Compound **13f** could be considered as a promising BRD4 inhibitor candidate for further development.

4. Experimental section

4.1. Methods and materials

All materials, reagents and solvents were obtained from commercial sources. They were utilized without further purification. The ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Bruker AV300 (300 MHz). Chemical shifts were showed as ^{d} H part per million (ppm) relative to tetramethyl silane (TMS) as internal standard. Hertz (Hz) expressed coupling constants (J values). Liquid chromatography-mass spectrometer (LC-MS) spectra were operated on Waters ACQUITY UPLC-TQD in ESI mode. Column chromatography silica gel (200–300 mesh) was used to purify the compounds. The purification process was monitored by thin layer chromatography which was performed on GF/UV 254 plates and were visualized using UV light at 254 and 365 nm. The purity of compounds was assessed by HPLC (> 95%).

4.2. General procedure for the synthesis of 11,12 13a–13 l and 15

4.2.1. Preparation of *3*

Compound **1** (15.18 g, 70.59 mmol) was dissolved in anhydrous tetrahydrofuran (100 mL) followed by slowly adding compound **2** (1 M) in tetrahydrofuran (85 mL, 84.71 mmol) at 0 °C under nitrogen protection. The system was then stirred at room temperature for 2 h. Saturated ammonium chloride solution (70 mL) was added at low temperature with stirring for 30 min. The mixture was filtered by celite and washed with ethyl acetate. The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **3** (18.5 g) as a white solid (yield: 80%).

4.2.2. Preparation of *4*

Compound **3** (18.47 g, 56.38 mmol) was dissolved in dry dichloromethane (150 mL), and pyridinium chlorochromate (15.80 g, 73.29 mmol) was added under nitrogen protection. The system was stirred at room temperature for 1.5 h. The mixture was filtered by celite and washed with dichloromethane. The organic layers were combined, washed with saturated sodium bicarbonate and saturated brine, dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **4** (16.2 g) as a white solid (yield: 88%). ^{1}H NMR (300 MHz, $CDCl_3$)

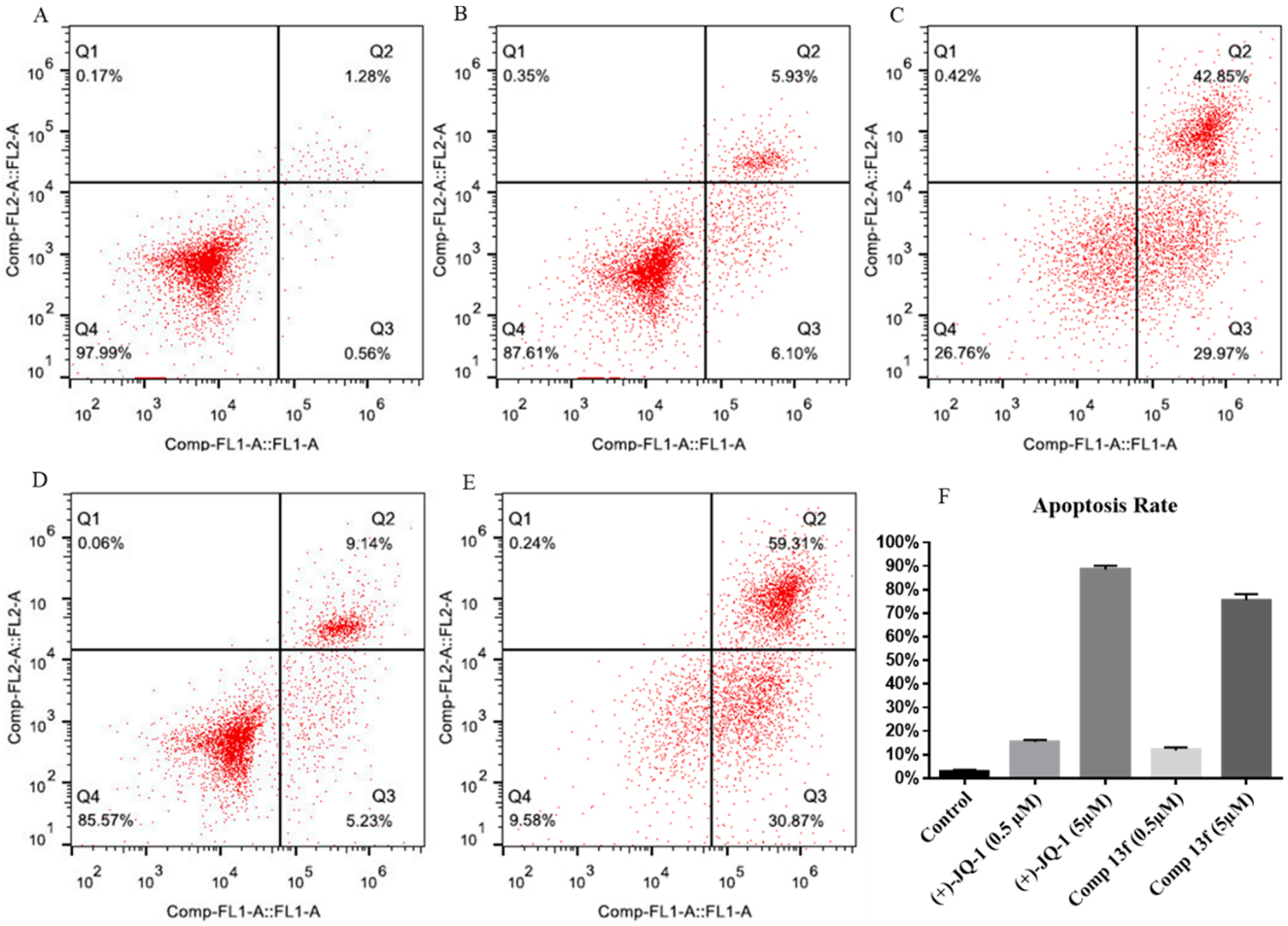


Fig. 4. Effects of compound **13f** and (+)-JQ-1 on MV-4-11 cell apoptosis: (A) control; (B)compound **13f** (0.5 μM); (C) compound **13f** (5 μM); (D) (+)-JQ-1 (0.5 μM); (E) (+)-JQ-1 (5 μM); (F) cell apoptosis analysis, the values were expressed as the means ± SD.

δ: 7.76 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.8 Hz, 1H), 7.49 – 7.39 (m, 2H), 6.91 (dd, J = 8.8, 3.0 Hz, 1H), 6.89 – 6.83 (m, 1H), 3.81 (s, 3H).

4.2.3. Preparation of *5*

Under nitrogen, compound **4** (5.08 g, 15.60 mmol), biboronic acid pinacol borate (5.94 g, 23.40 mmol), potassium acetate (6.13 g, 62.41 mmol), and [1,1′-bis (Diphenylphosphino) ferrocene] palladium dichloride (0.688 g, 0.94 mmol) were dissolved in 1,4-dioxane (85 mL). The system was stirred at 95 °C for 2.5 h, which was then diluted with water (60 mL) and extracted with ethyl acetate (3 × 60 mL). The organic layers were combined, washed with saturated brine, dried over anhydrous sodium sulfate and filtered with celite. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **5** (4.2 g) as a yellow solid (yield: 72%).[1]H NMR (300 MHz, $CDCl_3$) δ: 7.80 – 7.65 (m, 3H), 7.38 (d, J = 8.4 Hz, 2H), 7.06 – 6.91 (m, 2H), 3.83 (s, 3H), 1.08 (s, 12H).

4.2.4. The preparation of *7*

The 2,4-thiazolidinedione (8.4 g, 71.72 mmol) compound was dissolved in DMF (70 mL), anhydrous potassium carbonate (19.82 g,

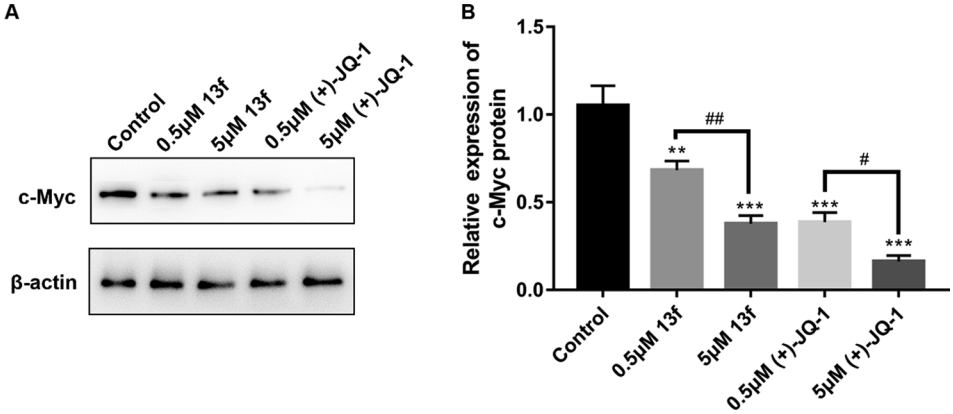


Fig. 5. Effect of compound **13f** on c-Myc expression; (A) representative western blotting band of c-Myc in MV-4-11cells; (B) band intensity of c-Myc expression analysis for three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs 0.5 μM group.

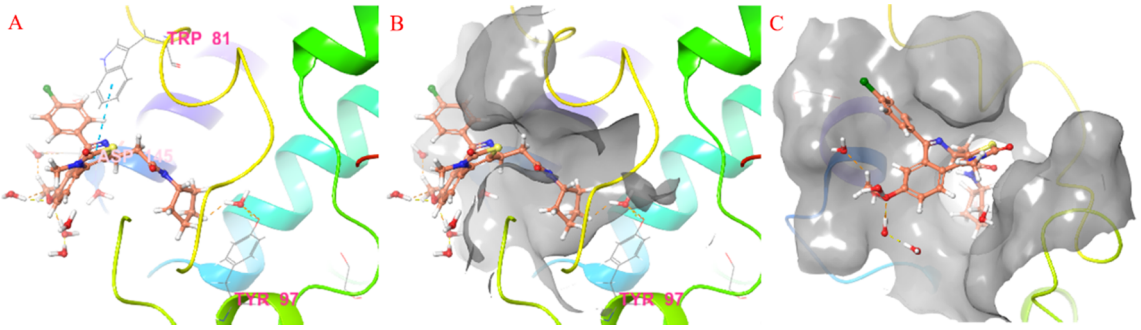


Fig. 6. Docking assay for compound **13f** with BRD4-BD1. (A) Pi interactions formed between the thiazolidinone ring of compound **13f** and Trp81, hydrogen-bond interactions with Asp 145 and Tyr 97. (B) The acetylcyclopentylamine side chain of compound **13f** was embed with cavity of BRD4-BD1 and formed hydrogen-bond interactions with Tyr 97. (C) Compound **13f** was bound with cavity of BRD4-BD1.

46.0 mmol) was added under nitrogen protection, and methyl iodide (6.64 mL, 0.11 mol) was then added. The system was stirred at 75 °C for 2 h. The mixture was filtered by celite and washed with ethyl acetate. The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **7** (6.5 g) as a white solid (yield: 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.97 (s, 2H), 3.13 (s, 3H).

4.2.5. Preparation of *8*

Compound **7** (4.5 g, 34.31 mmol) was dissolved in phosphorus oxychloride (9.57 mL, 0.10 mol) at 0 °C followed by slowly adding DMF (5.28 mL, 68.62 mmol). The system was stirred at 90 °C for 3 h, and then at 115° C for 1 h. The reaction solution was poured into an ice-water mixture, stirred, and extracted with ethyl acetate (3 × 60 mL). The organic layers were combined, washed with saturated brine, dried over anhydrous sodium sulfate and filtered with suction. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **8** (3.8 g) as a yellow solid (yield: 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.84 (s, 1H), 3.45 (s, 3H).

4.2.6. Preparation of *9*

Sodium hydride (1.62 g, 40.54 mmol, 60%) was added into anhydrous tetrahydrofuran (70 mL) at 0 °C followed by adding triethyl phosphorylacetate (8.04 mL, 40.54 mmol) under nitrogen protection. The mixture was stirred at low temperature for 30 min. Compound **8** (3.60 g, 20.27 mmol) was added into the system. The mixture was stirred at room temperature for 1 h. The system was quenched with saturated ammonium chloride, and the reaction solution was concentrated. The system was added with 40 mL water and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **9** (18.5 g) as a yellow solid (yield: 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.68 (d, J = 15.5 Hz, 1H), 5.83 (d, J = 15.5 Hz, 1H), 4.24 (q, J = 7.1 Hz, 2H), 3.41 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H).

4.2.7. Preparation of *10*

Compound **5** (5.40 g, 14.49 mmol) and compound **9** (3.59 g, 14.49 mmol) were added in a 250 mL double-necked round-bottomed flask, and dissolved into 1,4-dioxane (80 mL). Tetratriphenylphosphonium (0.67 g, 0.58 mmol) and aqueous solution of sodium carbonate (1.5 M, 20 mL) were added into the system under nitrogen protection. After the addition, the system was transferred to an oil bath and heated to reflux for about 20 h. The mixture was diluted with water and extracted with ethyl acetate (3 × 60 mL). The organic layer was evaporated, and the residue was separated by silica gel column chromatography to obtain **10** (4.3 g) as a yellow solid (yield: 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.66 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 1H), 7.19 (dd, J = 8.5, 2.5 Hz, 1H), 7.13 – 7.05 (m, 2H), 5.61 (d, J = 15.3 Hz, 1H), 4.17 – 4.10 (m, 2H), 3.91 (s, 3H), 3.12 (s, 3H), 1.26 – 1.22 (m, 3H).

4.2.8. Preparation of target compound *11*

Compound **10** (2.80 g, 6.11 mmol) was dissolved in ethanol (50 mL) followed by adding ammonium formate (3.86 g, 61.15 mmol) under nitrogen protection. The reaction was heated for 24 h. The mixture was filtered by celite and washed with ethyl acetate. The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **11** (0.9 g) as a white solid (yield: 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.92 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.3 Hz, 1H), 7.21 (s, 1H), 7.05 (dd, 1H), 5.36 (t, J = 6.8 Hz, 1H), 4.07 (q, J = 7.3 Hz, 2H), 3.90 (s, 3H), 3.00 (d, J = 6.8 Hz, 2H), 2.51 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.66, 169.37, 167.44, 160.97, 141.16, 138.50, 137.04, 132.36, 130.73, 129.22, 128.70, 123.92, 115.61, 114.97, 108.60, 95.77, 60.42, 55.40, 35.18, 26.91, 13.57; LC-MS (ESI) m/z: 457.5 [M + H] $^+$; Elemental Analysis for $C_{23}H_{22}ClN_2O_4S$: C, 60.32; H, 4.84; N, 6.12; Found: C, 60.36; H, 4.85; N, 6.09;

4.2.9. Preparation of target compound *12*

Compound **11** (3.40 g, 7.44 mmol) was dissolved in tetrahydrofuran (60 mL), and then 1 N aqueous solution of sodium hydroxide (0.89 g, 22.32 mL) was added dropwise. The reaction was stirred at room temperature for 4 h. The solution was neutralized with 1 N hydrochloric acid aqueous solution and extracted with dichloromethane (3 × 50 mL). The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain **12** (2.5 g) as a white solid (yield: 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.39 (s, 1H), 8.05 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.5 Hz, 1H), 7.36 (dd, J = 8.5, 2.5 Hz, 1H), 7.20 – 7.16 (m, 1H), 5.26 (m, 1H), 3.89 (s, 3H), 2.96– 2.94 (m, 2H), 2.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.86, 167.95, 161.06, 141.29, 138.62, 137.45, 132.90, 130.56, 129.18, 128.81, 123.81, 115.81, 108.78, 95.77,54.44, 37.78, 34.09, 29.19; LC-MS (ESI) m/z: 429.4 [M + H] $^+$; Elemental Analysis for $C_{21}H_{18}ClN_2O_4S$: C, 58.67; H, 4.22; N, 6.52; Found: C, 58.69; H, 4.26; N, 6.49;

4.2.10. Preparation of target compound *13a*

Compound **12** (0.3 g, 0.70 mmol), ethylamine (54.1 μL, 0.84 mmol), EDC · HCl (0.16 g, 0.84 mmol) and DMAP (85.46 mg, 0.70 mmol) were dissolved in dichloride (20 mL). The reaction was stirred at room temperature for 12 h. The reaction solution was diluted with water and extracted with dichloromethane (20 mL × 3). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate.

The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain target compound **13a** (0.2 g) as a white solid (yield: 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90 (dd, *J* = 8.5, 1.9 Hz, 2H), 7.53 (dd, *J* = 8.5, 2.0 Hz, 2H), 7.42 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.21 (s, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 5.50 (s, 1H), 5.37 (t, *J* = 5.9 Hz, 1H), 3.89 (s, 3H), 3.19 (t, *J* = 10.7 Hz, 2H), 2.87 (d, *J* = 8.6 Hz, 2H), 2.51 (s, 3H), 1.07 (q, *J* = 7.2 Hz, 3H).

4.2.11. Compound 13b–13l were synthesized via similar procedures as for compound 13a

4.2.11.1. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-ethylacetamide ***(13a)***. White solid, yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90 (dd, *J* = 8.5, 1.9 Hz, 2H), 7.53 (dd, *J* = 8.5, 2.0 Hz, 2H), 7.42 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.21 (s, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 5.50 (s, 1H), 5.37 (t, *J* = 5.9 Hz, 1H), 3.89 (s, 3H), 3.19 (t, *J* = 10.7 Hz, 2H), 2.87 (d, *J* = 8.6 Hz, 2H), 2.51 (s, 3H), 1.07 (q, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.90, 167.92, 161.06, 141.26, 138.56, 137.28, 132.90, 130.66, 129.18, 128.81, 123.81, 115.81, 108.78, 55.44, 37.72, 34.07, 29.19, 27.00, 14.17; LC-MS (ESI) *m/z*: 456.4 [M + H] $^+$; Elemental Analysis for $C_{23}H_{23}ClN_3O_3S$: C, 60.45; H, 5.07; N, 9.20; Found: C, 60.41; H, 5.03; N, 9.24;

4.2.11.2. 6-(4-chlorophenyl)-8-methoxy-1-methyl-4-(2-morpholino-2-oxoethyl)-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-2-one ***(13b)***. White solid, yield 67%, m.p. 145–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.03 – 7.86 (m, 2H), 7.60 – 7.49 (m, 2H), 7.48 – 7.38 (m, 1H), 7.22 (s, 1H), 7.10 – 6.98 (m, 1H), 5.47 (d, *J* = 2.7 Hz, 1H), 3.90 (d, *J* = 2.6 Hz, 3H), 3.61 (s, 4H), 3.53 (s, 2H), 3.40 (s, 2H), 3.09 – 2.94 (m, 2H), 2.52 (d, *J* = 2.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.68, 167.32, 161.02, 141.15, 138.62, 137.13, 131.57, 130.70, 129.19, 128.76, 123.85, 116.14, 115.65, 108.69, 95.86, 66.19, 66.05, 55.41, 45.52, 41.63, 34.59, 27.01; LC-MS (ESI) *m/z*: 498.5 [M + H] $^+$; Elemental Analysis for $C_{25}H_{25}ClN_3O_4S$: C, 60.18; H, 5.05; N, 8.42; Found: C, 60.23; H, 5.01; N, 8.46;

4.2.11.3. 6-(4-chlorophenyl)-8-methoxy-1-methyl-4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-2-one ***(13c)***. White solid, yield 64%, m.p. 152–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 1.8 Hz, 1H), 7.03 (dd, *J* = 8.2, 1.9 Hz, 1H), 5.52 (t, *J* = 6.5 Hz, 1H), 3.89 (s, 3H), 3.47 – 3.33 (m, 4H), 2.97 (d, *J* = 6.5 Hz, 2H), 2.51 (s, 3H), 2.00 – 1.89 (m, 2H), 1.86 – 1.74 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ:170.68, 167.71, 166.99, 160.98, 141.25, 138.62, 137.05, 130.91, 130.79, 129.22, 128.71, 123.94, 116.72, 115.64, 108.62, 95.93, 55.40, 46.13, 45.32, 36.05, 26.95, 25.53, 23.80; LC-MS (ESI) *m/z*: 482.5 [M + H] $^+$; Elemental Analysis for $C_{25}H_{25}ClN_3O_3S$: C, 62.17; H, 5.22; N, 8.70; Found: C, 62.15; H, 5.22; N, 8.74;

4.2.11.4. 6-(4-chlorophenyl)-8-methoxy-1-methyl-4-(2-oxo-2-(piperidin-1-yl)ethyl)-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-2-one ***(13d)***. White solid, yield 64%, m.p. 160–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 1.8 Hz, 1H), 7.15 (dd, *J* = 8.4, 1.8 Hz, 1H), 5.56 (t, *J* = 6.5 Hz, 1H), 3.81 (s, 3H), 3.55 – 3.36 (m, 4H), 2.94 (d, *J* = 6.5 Hz, 2H), 2.53 (s, 3H), 2.14 – 1.81 (m, 4H), 1.87 – 1.76 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.98, 167.32, 166.35, 161.02, 141.10, 138.68, 136.22, 131.15, 130.25, 129.06, 124.42, 117.74, 116.49, 108.75, 95.94, 55.77, 45.88, 42.06, 34.44, 27.20, 25.87, 25.16, 23.88; LC-MS (ESI) *m/z*: 496.5 [M + H] $^+$; Elemental Analysis for $C_{26}H_{27}ClN_3O_3S$: C, 62.83; H, 5.48; N, 8.45; Found: C, 62.79; H, 5.44; N, 8.46

4.2.11.5. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-cyclopropylacetamide ***(13e)***. White solid, yield 66%, m.p.164–166 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.19 (d, *J* = 1.8 Hz, 1H), 7.02 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.18 (s, 1H), 5.36 (t, *J* = 6.5 Hz, 1H), 3.86 (s, 3H), 2.80 (m, 2H), 2.46 (s, 3H), 0.64 – 0.37(m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.88, 169.66, 167.58, 161.03, 141.01, 138.53, 137.20, 132.27, 130.60, 130.43, 129.18, 128.77, 123.96, 123.89, 116.26, 116.22, 115.71, 108.76, 95.81, 55.43, 37.27, 26.99, 22.20, 5.86; LC-MS (ESI) *m/z*: 468.4 [M + H] $^+$; Elemental Analysis for $C_{24}H_{23}ClN_3O_3S$: C, 61.47; Cl, 7.56; N, 8.96; Found: C, 61.45; Cl, 7.52; N, 8.98;

4.2.11.6. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-cyclopentylacetamide ***(13f)***. White solid, yield 64%, m.p. 168–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.89(dd, *J* = 8.3, 2.2 Hz, 2H), 7.52 (dd, *J* = 8.3, 2.2 Hz, 2H), 7.41 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.20 (s, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 5.61 (s, 1H), 5.35 (d, *J* = 2.5 Hz, 1H), 4.07 (s, 1H), 3.88 (d, *J* = 2.4 Hz, 3H), 2.84 (d, *J* = 4.3 Hz, 2H), 2.50 (d, *J* = 2.4 Hz, 3H), 1.87 (s, 2H), 1.56 (s, 4H), 1.25 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ:171.27, 168.00, 161.54, 141.65, 139.08, 137.73, 133.42, 131.14, 129.67, 129.27, 124.29, 116.55, 116.15, 109.27, 96.31, 55.94, 51.31, 38.14, 32.98, 27.53, 23.63; LC-MS (ESI) *m/z*: 496.5 [M + H] $^+$; Elemental Analysis for $C_{26}H_{27}ClN_3O_3S$: C, 62.83; H, 5.48; N, 8.45; Found: C, 62.85; H, 5.52; N, 8.41;

4.2.11.7. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-phenylacetamide ***(13 g)***. White solid, yield 72%, m.p.182–184 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.05 – 7.76 (m, 3H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.33 – 6.95 (m, 4H), 6.65 (d, *J* = 6.8 Hz, 2H), 5.47 (s, 1H), 3.86 (s, 3H), 2.94 (s, 2H), 2.52 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 170.14, 167.11, 161.04, 140.97, 138.78, 138.70, 136.24, 131.09, 130.91, 130.28, 129.08, 128.64, 124.49, 123.22, 118.91, 117.16, 116.55, 108.80, 95.83, 55.75, 37.75, 27.22; LC-MS (ESI) *m/z*: 504.5 [M + H] $^+$; Elemental Analysis for $C_{27}H_{23}ClN_3O_3S$: C, 64.22; H, 4.59; N, 8.32; Found: C, 64.18; H, 4.55; N, 8.36;

4.2.11.8. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-(4-fluorophenyl)acetamide ***(13 h)***. White solid, yield 60%, m.p.174–176 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.04 – 7.76 (m, 3H), 7.52 (d, *J* = 7.3 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.31 – 6.97 (m, 3H), 6.61 (d, *J* = 6.8 Hz, 2H), 5.46 (s, 1H), 3.81 (s, 3H), 2.96 (s, 2H), 2.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.16, 167.10, 167.03, 161.04, 156.31, 140.97, 138.70, 136.27, 135.16, 131.09, 131.02, 130.26, 130.20, 129.06, 124.46, 120.73, 120.62, 117.07, 116.51, 115.34, 115.27, 115.04, 108.82, 95.84, 55.74, 37.65, 27.19; LC-MS (ESI) *m/z*: 522.5 [M + H] $^+$; Elemental Analysis for $C_{27}H_{22}ClFN_3O_3S$: C, 62.01; H, 4.24; N, 8.03; Found: C, 62.03; H, 4.26; N, 8.07;

4.2.11.9. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-(prop-2-yn-1-yl)acetamide ***(13i)***. White solid, yield 58%, m.p.142–144 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.25 (s, 1H), 7.06 (d, *J* = 6.3 Hz, 1H), 6.32 (s, 1H), 5.40 (t, *J* = 6.7 Hz, 1H), 4.01 – 3.92 (m, 2H), 3.91 (s, 3H), 2.90 (d, *J* = 6.7 Hz, 2H), 2.51 (s, 3H), 2.19 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.00, 168.01, 167.47, 161.08, 141.01, 138.56, 137.25, 132.89, 130.60, 129.24, 128.80, 123.94, 115.81, 115.70, 108.80, 95.81, 78.76, 71.16, 55.44, 37.13, 28.76, 27.03; LC-MS (ESI) *m/z*: 466.4 [M + H] $^+$; Elemental Analysis for $C_{24}H_{21}ClN_3O_3S$: C, 61.73; H, 4.53; N, 9.00; Found: C, 61.75; H, 4.56; N, 9.04;

4.2.11.10. N-(tert-butyl)-2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]-thiazolo[4,5-e]azepin-4-yl)acetamide ***(13j)***. White solid, yield 67%; m.p.152–154 °C; ^{1}H NMR (300 MHz,

$CDCl_3$) δ: 7.87 (dd, J = 8.4, 2.0 Hz, 2H), 7.48 (dd, J = 8.5, 2.1 Hz, 2H), 7.39 (dd, J = 8.2, 2.0 Hz, 1H), 7.18 (s, 1H), 7.00 (dd, J = 6.3, 2.0 Hz, 1H), 5.58 (s, 1H), 5.42 – 5.28 (m, 1H), 3.84 (d, J = 2.2 Hz, 3H), 2.78 (d, J = 5.9 Hz, 2H), 2.46 (d, J = 2.1 Hz, 3H), 1.21 (d, J = 2.2 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.70, 167.62, 167.29, 161.03, 141.11, 138.61, 137.13, 132.31, 130.69, 129.20, 128.73, 123.85, 116.45, 115.71, 108.73, 95.83, 55.41, 50.82, 38.19, 28.14, 26.98; LC-MS (ESI) m/z: 484.5 [M + H] $^+$; Elemental Analysis for $C_{25}H_{27}ClN_3O_3S$: C, 61.91; H, 5.61; N, 8.66; Found: C, 61.94; H, 5.65; N, 8.68;

4.2.11.11. 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo[4,5-e]azepin-4-yl)-N-(4-hydroxyphenyl)acetamide ***(13 k)***. White solid, yield 48%, m.p.169–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 – 7.77 (m, 3H), 7.48 (d, J = 7.3 Hz, 2H), 7.40 (d, J = 7.8 Hz, 1H), 7.31 – 6.97 (m, 4H), 6.61 (d, J = 6.8 Hz, 2H), 5.45 (s, 1H), 3.85 (s, 3H), 2.95 (s, 2H), 2.50 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.12, 167.17, 166.37, 161.03, 153.28, 140.99, 138.70, 136.26, 131.09, 130.70, 130.45, 130.27, 129.07, 124.47, 120.71, 117.47, 116.53, 115.47, 115.19, 114.97, 108.81, 95.85, 55.74, 37.65, 27.20; LC-MS (ESI) m/z: 520.4 [M + H] $^+$; Elemental Analysis for $C_{27}H_{23}ClN_3O_4S$: C, 62.24; H, 4.45; N, 8.07; Found: C, 62.28; H, 4.48; N, 8.12;

4.2.11.12. N-butyl-2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,4-dihydro-2H-benzo[c]thiazolo-[4,5-e]azepin-4-yl)acetamide ***(13 l)***. White solid, yield 68%; m.p.170–172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.91 (d, J = 8.1 Hz, 2H), 7.54 (d, J = 8.0 Hz, 2H), 7.43 (d, J = 8.1 Hz, 1H), 7.22 (s, 1H), 7.05 (d, J = 6.0 Hz, 1H), 5.77 (s, 1H), 5.39 (t, J = 5.8 Hz, 1H), 3.90 (s, 3H), 3.15 (d, J = 5.6 Hz, 2H), 2.88 (d, J = 6.7 Hz, 2H), 2.51 (s, 3H), 1.33 (m, 4H), 0.96 – 0.73 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.35, 168.55, 168.01, 161.53, 141.61, 139.04, 137.71, 133.12, 131.13, 129.68, 129.27, 124.34, 116.70, 116.18, 109.25, 96.30, 55.93, 39.37, 38.14, 31.42, 27.50, 19.96, 13.69; LC-MS (ESI) m/z: 520.4 [M + H] $^+$; Elemental Analysis for $C_{25}H_{27}ClN_3O_3S$: C, 61.91; H, 5.61; N, 8.66; Found: C, 61.93; H, 5.61; N, 8.69;

4.2.11.13. Ethyl (E)-3-(4-(2-((4-chlorophenyl)(hydroxy)methyl)-4-methoxyphenyl)-3-methyl-2-oxo-2,3-dihydrothiazol-5-yl)acrylate ***(14)***. Compound **10** (1.20 g, 2.62 mmol) was dissolved in methanol (40 mL) followed by adding sodium borohydride (0.20 g, 5.24 mmol) under nitrogen protection. The reaction was stirred at room temperature for 6 h. The reaction was quenched with saturated ammonium chloride and extracted with dichloromethane (3 × 30 mL). The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain target compound 14 (0.8 g) as a white solid (yield: 66%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.49–7.48 (d, J = 3 Hz, 1H), 7.26–7.23 (m, 3H), 7.05–7.02 (m, 3H), 6.76–6.71 (d, J = 15 Hz, 1H), 6.15–6.14 (d, J = 3 Hz, 1H), 5.68–5.63 (d, J = 15 Hz, 1H), 5.57–5.55 (d, J = 6 Hz, 1H), 4.12–4.06 (q, J = 6 Hz, 2H), 3.91 (s, 3H), 2.36 (s, 3H), 1.21–1.16 (t, J = 6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.35, 168.55, 168.01, 161.53, 141.61, 139.04, 137.71, 133.12, 131.13, 129.68, 129.27, 124.34, 116.70, 116.18, 109.25, 96.30, 55.93, 39.37, 38.14, 31.42, 27.50, 19.96, 13.69; LC-MS (ESI) m/z: 484.5 [M + H] $^+$;

4.2.11.14. Ethyl 2-(6-(4-chlorophenyl)-8-methoxy-1-methyl-2-oxo-1,2,4,6-tetrahydrobenzo[5,6]oxepino-[4,3-d]thiazol-4-yl)acetate ***(15)***. Compound **14** (0.8 g, 1.74 mmol) was dissolved in ethanol (30 mL) followed by adding potassium carbonate (0.48 g, 3.48 mmol) under nitrogen protection. The reaction was stirred at room temperature for 24 h. The mixture was filtered by celite and washed with ethyl acetate. The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate. The organic layer was evaporated and the residue was separated by silica gel column chromatography to obtain 3 (0.45 g) as a white solid (yield: 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (dd, J = 8.5, 1.9 Hz, 2H), 7.54 (dd, J = 8.5, 2.0 Hz, 2H), 7.49 (dd, J = 8.2, 1.9 Hz, 1H), 7.21 (s, 1H), 7.11 (d, J = 8.3 Hz, 1H), 5.50 (s, 1H), 5.36 (t, J = 5.9 Hz, 1H), 3.86 (s, 3H), 3.17 (t, J = 10.7 Hz, 2H), 2.87 (d, J = 8.6 Hz, 2H), 2.51 (s, 3H), 1.05 (q, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.93, 167.95, 161.08, 141.24, 138.58, 137.28, 132.92, 130.66, 129.18, 128.85, 123.87, 115.83, 108.78, 55.42, 37.72, 34.05, 29.19, 27.06, 14.15; LC-MS (ESI) m/z: 460.4 [M + H] $^+$; Elemental Analysis for $C_{23}H_{23}ClNO_5S$: C, 59.93; H, 5.03; N, 3.04; Found: C, 59.95; H, 5.05; N, 3.08;

4.3. Biological evaluations

4.3.1. BRD4 inhibition TR-FRET assay

The BRD4-BD1 TR-FRET Assay Kit (Item No. 600520) from Cayman (Ann Arbor, MI, USA) was used to perform the BRD4 inhibition assay. All assays were carried out in 384 well plates. Concentrations of compounds were set as 20 μM, 10 μM, 5 μM, 1 μM, 0.5 μM, 0.2 μM, 0.1 μM, 0.05 μM and 0.01 μM. First, compounds (5 μL) containing assay buffer was added into plate, BRD4-BD1 Europium Chelate in 10 μL of assay buffer was then added into 5 μL of compounds. After a 15-minute room temperature incubation, 5 μL of BRD4-BD1 Ligand/APC acceptor mixture was added. The plates were incubated at room temperature for 60 min plates. PerkinElmer multimode plate reader with TR-FRET technique was used to read the plates under 340 nm excitation and 670 nm emission/620 nm emission. The BRD4 inhibition rate was calculated by the absorbance between the negative control and the compound group. IC_{50} was calculated using the BRD4 inhibition rate data.

4.3.2. Cell growth inhibition assay

In all assays, MV-4-11 and HL-60 leukemia cells were cultured in IMDM modified with 10% FBS and 1% penicillin–streptomycin at 37 °C condition in Thermo CO_2 incubators. In all assays, cells were collected in the logarithmic growth phase, and 100 μL of medium containing 10,000 cells were seeded in 96-well cell culture plates per well. After 12 h of culture, 10 μL of compounds with different concentrations were added to each cell well. After 72 h of culture, 5 μL of CCK-8 was added into each well. After 1–4 h culture, PerkinElmer multimode plate reader was used to read the plate at 450 nm. IC_{50} value was calculated by the data using GraphPad Prism 7 software.

4.3.3. Apoptosis and cell cycle Analysis assay

In all assays, MV-4-11 leukemia cells were cultured in IMDM modified with 10% FBS and 1% penicillin–streptomycin at 37 °C condition in Thermo CO_2 incubators. Flow cytometry was used to analyze cycle and apoptosis of cell which was treated by compounds. In this assay, cells were collected in the logarithmic growth phase, and 2 mL of medium containing 1 × 10^6 cells were seeded in 6-well cell culture plates per well followed by adding the test compound. After 12 h of culture, flow cytometry was used to analyze the effects of cycle and apoptosis on cell. Annexin V-FITC Apoptosis Detection Kit and Cell Cycle Detection Kit were obtained from KeyGEN BioTECH.

4.3.4. Western blotting

In all assays, MV-4-11 leukemia cells were cultured in IMDM modified with 10% FBS and 1% penicillin–streptomycin at 37 °C condition in Thermo CO_2 incubators. Cells were collected in the logarithmic growth phase, which were treated with compounds for another 4 h. The cells were lysed at 4 °C and placed in 1.5 mL centrifuge tubes to further determine the corresponding protein concentration. A 10% separation adhesive and a 4% concentration adhesive were prepared, and the SDS-polyacrylamide gel was set in an electrophoresis tank for sample loading, electrophoresis, and protein transfer. Subsequently, the PVDF membrane was washed 3 times with TBST for 10 min each. The PVDF membrane was immersed in a 5% non-fat milk TBST solution, then placed on a shaker at room temperature, and shaken slowly for 2 h

to block non-specific protein binding sites. The PVDF membrane was transferred overnight with the primary antibody diluent at 4 °C. After the reaction, the PVDF membrane was washed 3 times with TBST for 10 min each. Although the secondary antibody was incubated only 2 h before development.

4.3.5. Molecular docking assay

Schrödinger 2017 was used for the molecular docking assay. The structure of BRD4 protein (PDB ID: 4O74) was obtained from the Protein Data Bank. It was prepared, optimized and minimized using relevant module by Schrodinger software. Receptor Grid Generation Module was generated to dock the compound which was prepared using the LigPrep module. Finally, the calculation was performed based on the Schrödinger XP precision, and the structure of lowest-energy was shown as the final result.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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