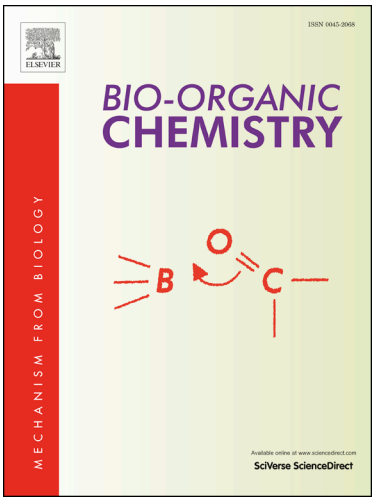


Exploiting the 7-methylimidazo[1,5-a]pyrazin-8(7H)-one Scaffold for the Development of Novel Chemical Inhibitors for Bromodomain and Extraterminal Domain (BET) Family

Yifei Yang, Pan Chen, Leilei Zhao, Fangqing Zhang, Bing Zhang, Changliang Xu, Huibin Zhang, Jinpei Zhou

PII: S0045-2068(19)30699-6
DOI: https://doi.org/10.1016/j.bioorg.2019.103044
Article Number: 103044
Reference: YBIOO 103044

To appear in: *Bioorganic Chemistry*

Received Date: 1 May 2019
Revised Date: 31 May 2019
Accepted Date: 4 June 2019

Please cite this article as: Y. Yang, P. Chen, L. Zhao, F. Zhang, B. Zhang, C. Xu, H. Zhang, J. Zhou, Exploiting the 7-methylimidazo[1,5-a]pyrazin-8(7H)-one Scaffold for the Development of Novel Chemical Inhibitors for Bromodomain and Extraterminal Domain (BET) Family, *Bioorganic Chemistry* (2019), doi: https://doi.org/10.1016/j.bioorg.2019.103044

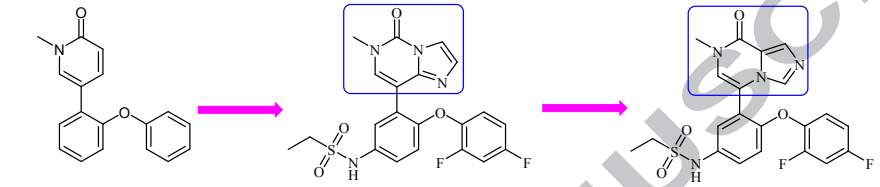
Exploiting the 7-methylimidazo[1,5-a]pyrazin-8(7H)-one Scaffold for the Development of Novel Chemical Inhibitors for Bromodomain and Extraterminal Domain (BET) Family

Yifei Yang[a], Pan Chen[a], Leilei Zhao [b], Fangqing Zhang [b], Bing Zhang [b], Changliang Xu[c,*], Huibin Zhang[b,*], Jinpei Zhou[a,*]

[a] *Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, PR China.*

[b] *Center of Drug Discovery, Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, China Pharmaceutical University, Nanjing 210009, PR China.*

[c] *Jiangsu Collaborative Innovation Center of Traditional Chinese Medicine Prevention and Treatment of Tumor, Nanjing University of Chinese Medicine, Nanjing, Jiangsu 210023, PR China.*



Fragment 9
BRD4 TR-FRET Ki=890nM
BRD4 Engagement EC_{50}=4100nM

Compound 17
BRD4 BD1 IC_{50}=270nM
BRD4 BD2 IC_{50}=310nM

Compound 28
BRD4 BD1 IC_{50}=30nM
BRD4 BD2 IC_{50}=29nM

Abstract

The bromodomain and extraterminal (BET) family of proteins play a crucial role in promoting gene expression of critical oncogenes. Novel BET bromodomain inhibitors with excellent potency, drug metabolism and pharmacokinetics (DMPK) properties were in strong need for development. We reported a series of potential BET inhibitors through incorporation of imidazole into pyridine scaffold. Among them, a novel BET inhibitor with 7-methylimidazo[1,5-a]pyrazin-8(7H)-one core, compound **28**, was considered to be the most promising for in-depth study. Compound **28** exhibited excellent BRD4-inhibitory activity with IC_{50} value of **33 nM** and anti-proliferation potency with IC_{50} value of **110 nM** in HL-60 (human promyelocytic leukemia) cancer cell lines. Western Blot indicated that compound **28** can effectively trigger apoptosis in BxPc3 cells by modulating the intrinsic apoptotic pathway. In conclusion, these results suggested that compound **28** has merely potential for leukemia treatment.

Keywords: BRD4 inhibitors, Molecular docking, Anticancer, BET family

Introduction

The acetylation of lysine residues were proposed to play crucial roles in post-translational modifications of histones[1]. Ever since 1992, when the bromodomains comprised of 110-amino acid were first identified as 'readers' of e-N-acetyl-lysine modifications in nucleosomal chromatin, there are a lot of researches focused on the mechanism of bromodomains in epigenetics [2-4]. The human genome codes 46 proteins containing 61 BRDs [5], including the bromodomain and extra-terminal (BET) protein family, which contains four members: BRD2, BRD3, BRD4 and BRDT [6]. These proteins comprise of conservative tandem bromodomains that recognize acetylated lysine residues on histone tails and recruit transcriptional factors to promote gene expression [3, 7, 8]. For example, BRD4 recruits positive transcription elongation factor (P-TEFb) to acetylated chromatin and plays a role in activating the cyclin dependent kinase (CDK9) subunit, whereby it acts as a vital transcription moderator [9].

BET proteins were confirmed as a promising therapeutic target for competitive inhibitors displacing bromodomains from chromatin to influence changes in gene expression. Therefore great interests and

* Corresponding authors. E-mail addresses: cl.xu81@gmail.com (C. Xu); zhanghb80@163.com (H. Zhang); jpzhou668@163.com (J. Zhou).

attention were attracted for the development of novel BET inhibitors for cancer treatment [10-12]. Several BET inhibitors with different scaffolds have been reported since the first small molecule BRD4 inhibitor JQ1 was disclosed [13]. There are some inhibitors undergoing clinical trials for the treatment of different diseases, such as **OTX015** (Merck & Co. Phase I/II) [14], **I-BET762** (GlaxoSmithKline. Phase II) [15], **BI-2536** (Boehringer Ingelheim. Phase II) [16], **ABBV-075** (AbbVie . Phase I) [17-19] (**Figure 1**). However, the major indications of these inhibitors were hematologic malignancies, such as multiple myeloma (MM) [20].

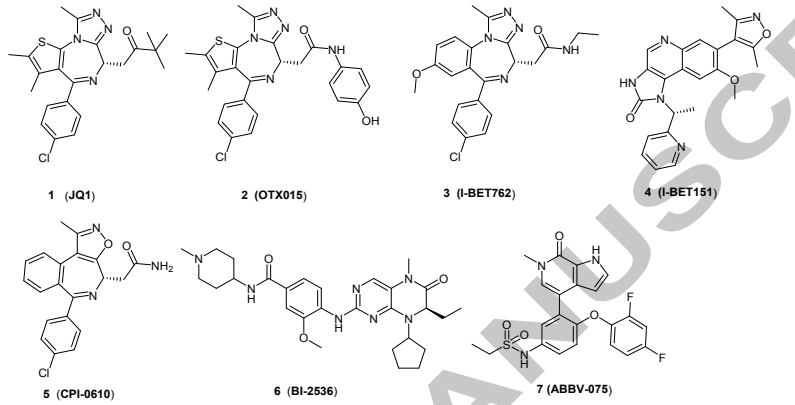
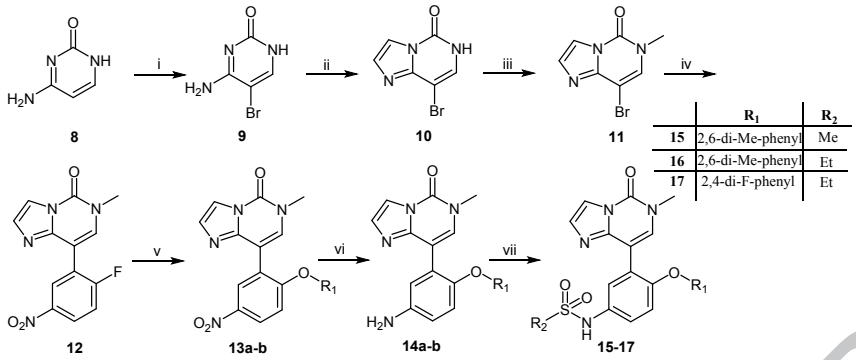


Figure 1. Structures of known BET bromodomain inhibitors

Recent study also revealed the potency of BET inhibitors in solid tumor, such as pancreatic cancer [21]. Pancreatic ductal adenocarcinoma (PDA) is one of the most aggressive cancers with a median survival time of 6 months, with less than 5% survival 5 years after diagnosis[22]. PDA surpassed only by lung cancer, will become the second leading cause of cancer-related deaths in the United States by 2030[23]. According to the important role that BET proteins play in the progression and evolution of pancreatic cancer, we designed and synthesized a series of imidazolopyridone derivatives containing the moiety 7-methylimidazo [1, 5-a]pyrazin-8(7H)-one via scaffold hopping as BET inhibitors. Then their activities in AlphaScreen binding assays and effects on cell viability were evaluated in vitro. Cell cycle analysis was performed to characterize the molecular mechanisms underlying the cytotoxic effect of compound **28** in BxPC-3 cell.

Synthesis

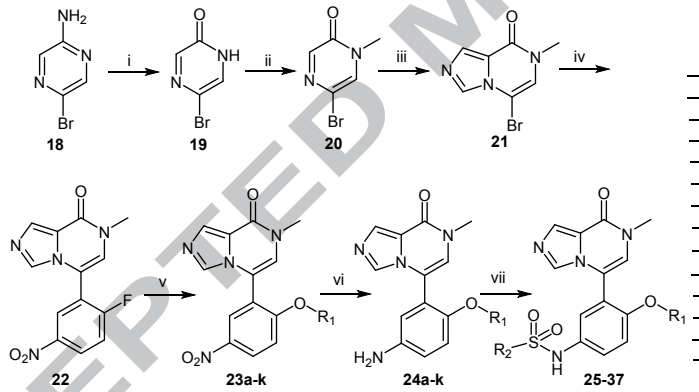
Target compounds (**15-17, 25-37**) were synthesized as outlined in **Schemes 1** and **2**. All compounds were purified by chromatography and purity was checked by HPLC before biological evaluation (purity was >98%). The structures were confirmed by ^{1}H NMR, ^{13}C NMR spectrum, and mass spectra. Some representative spectrometry was shown in Supporting Information. As depicted in **Scheme 1**, commercially available cytosine **8** reacted with bromine in acetic acid to give 6-amino-5-bromo-1H-pyrimidin-2-one (**9**). Cyclization of compound **9** with chloroacetaldehyde in dimethyl formamide was utilized to obtain intermediate **10**. The key intermediate **11** was prepared by methylation of intermediate **10** in the presence of dimethylsulfate. Then Suzuki-Miyaura cross coupling reaction was used to get compound **12**, followed by reacting with different phenol in the presence of potassium carbonate. Iron-catalyzed reduction of the nitro group of **13** in the presence of ammonium chloride followed by functionalization of the resulting aniline **14** provided facile access to sulfonamides **15-17**.



	R_1	R_2
15	2,6-di-Me-phenyl	Me
16	2,6-di-Me-phenyl	Et
17	2,4-di-F-phenyl	Et

Scheme 1. Reagents and conditions: (i) Br_2, CH_3COOH, 70 °C, 85%; (ii) chloroacetaldehyde, DMF, 100 °C, 65%; (iii) Dimethylsulfate, Cs_2CO_3, CH_3CN, 60 °C, 49%; (iv) $Pd(PPh_3)_4$, Na_2CO_3, toluol /water/MeOH, 110 °C, 69%; (v) phenol, K_2CO_3, DMF, 80 °C, 84%; (vi) Fe, NH_4Cl, H_2O/EtOH, 100 °C, 83%; (vii) RSO_2Cl, NEt_3, CH_2Cl_2, 0 °C, then 2N NaOH, 1,4-dioxane, 70 °C, 58-87%.

As shown in **Scheme 2**, 5-bromopyrazin-2(1H)-one (**19**) was got via 5-bromopyrazin-2-amine (**18**) reacting with sodium nitrite in concentrated sulfuric acid. Methylation reaction was utilized to prepare 5-bromo-1-methylpyrazin-2(1H)-one (**20**) followed by cyclization with tosylmethyl isocyanide to give 5-bromo-7-methylimidazo[1,5-a]pyrazin-8 (7H)-one (**21**). The bond between imidazolopyridone and biaryl ether was formed through Suzuki-Miyaura cross coupling reaction. Then an analogous approach depicted in **Scheme 1** provided access to the target compounds (**25-37**).



	R_1	R_2
25	2,4-di-F-phenyl	Me
26	3-pyridyl	Et
27	m-F-phenyl	Et
28	2,4-di-F-phenyl	Et
29	3-pyridyl	Me
30	3,4,5-tri-F-phenyl	Me
31	o-F- phenyl	Me
32	m-F-phenyl	Me
33	2-Me-5-F- phenyl	Me
34	2-F-5-Me- phenyl	Me
35	p-isopropylphenyl	Me
36	p-Br-phenyl	Et
37	m-Br-phenyl	Et

Scheme 2. Reagents and conditions: (i) $NaNO_2$, conc. H_2SO_4, 0 °C to 45 °C, 91%; (ii) dimethyl sulfate, Cs_2CO_3, CH_3CN, 60 °C, 81%; (iii) Tosylmethyl isocyanide, NaH, THF, 0 °C to rt; (iv) $Pd(PPh_3)_4$, Na_2CO_3, toluol/water/MeOH, 110 °C, 68%; (v) phenol, K_2CO_3, DMF, 80°C, 95%; (vi) Fe, NH_4Cl, H_2O/EtOH, 100 °C, 83%; (vii) RSO_2Cl, NEt_3, CH_2Cl_2, 0 °C, then 2 N NaOH, 1,4-dioxane, 70 °C, 58-87%.

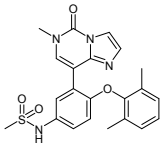
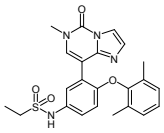
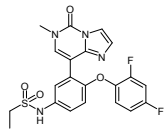
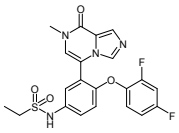
Results and Discussion

2.1 Design strategy

Firstly, to further improve the potency of pyridone-based BET bromodomain inhibitors such as **Fragment 9** [17], imidazole was introduced to afford target compounds (**15-17**) with 5,6-dihydroimidazo[1,2-c]pyrimidin scaffold. Biological activities were evaluated using an Amplified Luminescent Proximity Homogeneous (AlphaScreen) binding assay and three complementary cellular assays. The AlphaScreen binding assay was used to evaluate the efficacy of compounds for a construct containing BRD4(BD1) and BRD4(BD2). Growth of cancer cell lines are dependent on BET proteins [24], the effects of compounds on cancer cell proliferation were measured using the lymphoblast-like cell line(Raji), promyelocytic cell line (HL-60) and human pancreatic cancer cell line(BxPC-3). However,

potency of BET bromodomain inhibitors with 5,6-dihydroimidazo[1,2-c]pyrimidin scaffold such as **17** was relatively poor (**Table 1**). To further improve the biochemical efficiency, compound **17** was docked into BRD4 (BD1) and superimposed with **ABBV-075** via Glide docking (PDB id: 3P5O). Docking studies revealed that although the carbonyl moieties of these cores were situated at an ideal distance away from the Asn433 NH_2 group (2.95 Å), there was not an efficient interaction built between the Asn433 amide carbonyl and moieties of compound **17** (**Figure 2**). However, 7-methylimidazo[1,5-a]pyrazin-8(7H)-one-based compounds were designed and evaluated in order to provide a bidentate contact and improve the binding potency. Compared to compound **17**, novel imidazolopyridone of **28** improved both biochemical and cellular activity by 8-20-fold (**Table 1**).

Table 1. Biochemical and Cellular Potency of Compounds **15**, **16**, **17** and **28**

				
Compound ID	**15**	**16**	**17**	**28**
BRD4(BD1) Alpha Screen IC_{50}(μM)[a]	0.57±0.022	0.43±0.012	0.27±0.013	0.033±0.005
BRD4(BD2) Alpha Screen IC_{50}(μM)	0.48±0.031	0.39±0.024	0.31±0.011	0.025±0.009
HL-60 Proliferation IC_{50} (μM)[b]	4.3±0.025	3.9±0.019	2.2±0.028	0.11±0.012
Raji Proliferation IC_{50} (μM)[b]	3.8±0.021	5.1±0.016	1.8±0.024	NT
BxPC-3 Proliferation IC_{50} (μM)[b]	>10	>10	5.6	NT

[a] BRD4 AlphaScreen IC_{50} values are reported as the mean ± SD value of three independent determinations.

[b] IC_{50} values presented are the mean derived from three measurements.

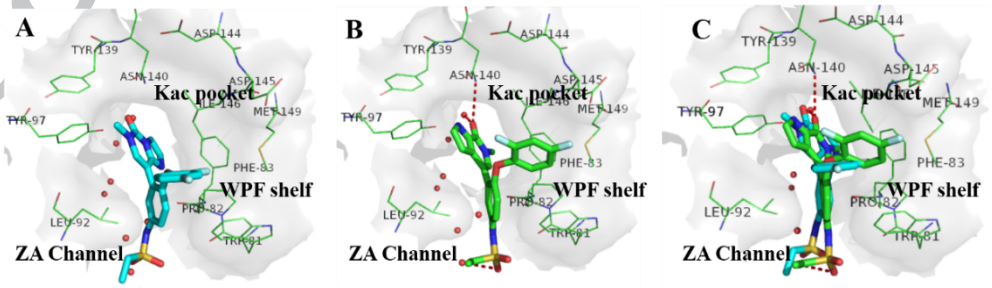


Figure 2. Molecular modeling of compound **17** with BRD4(1) (PDB id: 3P5O). (A) Docking conformation of compound **17** in BRD4(1) (PDB id: 3P5O). (B) Docking conformation of **ABBV-075** in BRD4(1) (PDB id: 3P5O). (C) Superimposition docking conformation of compound **17** (Blue) and **ABBV-075** (Green) in BRD4(1) (PDB id: 3P5O).

In order to elaborate the specific interaction between the imidazolopyridone core and BET bromodomain, we performed docking studies for compound **28** and **ABBV-075.** Docking studies indicated that the ligand-protein interactions of two molecules were similar. The 7-methylimidazo[1,5-

a]pyrazin-8(7H)-one acted as a KAc mimic, with the methyl group occupying the small hydrophobic pocket. The pyridone carbonyl formed a water mediated hydrogen bond with the NH_2 of TRY97, and accepted a hydrogen bond from the conserved Asn residue (ASN140) with an ideal 2.83 Å distance measured. In addition, the phenyl ether moiety of compound **28** offers valuable interactions by occupying a hydrophobic part of the WPF shelf and ethylsulfonyl moiety is directed into the ZA channel. The key hydrogen bond with Asn140 and the additional water mediated interactions would be important for improving affinity. Gratifyingly, the second imidazolopyridone core exhibited more remarkable binding effects than the first motif, as evaluated by an improvement in IC_{50} from **270** to **33** nM (**28** vs **17**).

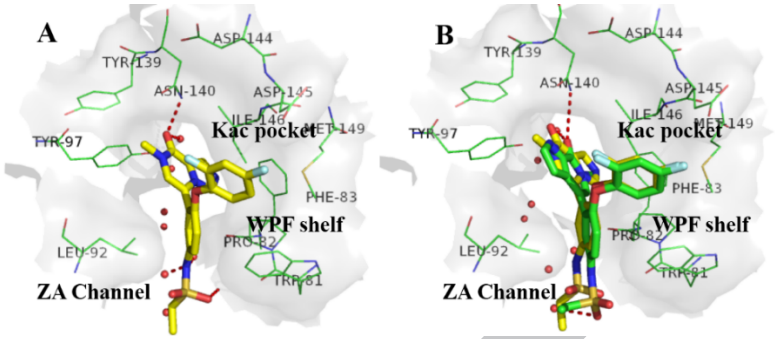


Figure 3. Molecular modeling of compound **28** with BRD4(1) (PDB id: 3P5O). (A) Docking conformation of compound **28** in BRD4(1) (PDB id: 3P5O). (B) Superimposition docking conformation of compound **28** (Yellow) and **Abbv-075** (Green) in BRD4(1) (PDB id: 3P5O).

The 7-methylimidazo[1,5-a]pyrazin-8(7H)-one core exhibited effective and potent inhibitory activity of BET protein, we evaluated the SAR at other sites of the compounds for further improving potency (**Table 2**). Compound **28** with the BRD4(BD1) IC_{50} of **33 nM** was selected as the lead compound. Different substituents (compound **26-37**) were introduced into the phenyl group which was located at the WPF shelf to further improve the binding affinity. Firstly, pyridines (compound **26**) was incorporated here to reduce lipophilicity and improve the developability profile, though substantial potency was significantly reduced compared to the phenyl ethers. The mono fluoro substituted compound **27** exhibited moderate improvement. The biochemical activities of compound **36** and **37** with mono bromo substituent were also limited. These results clearly indicated the importance of the moderate groups located at WPF shelf for the affinity. In addition, ethyl sulfonamide was preferred over methyl sulfonamide based upon its modestly improved potency. With these encouraging results in hand, the anti-proliferation activities were further assessed in several tumor cell lines (**Table 2**).

Table 2. Biochemical and cellular activities of compound **25-37**

ID	R_1	R_2	BRD4/BD1(IC_{50}nM)[a]	BRD4/ BD2 (IC_{50}nM)	HL60[b] IC_{50}(μM)	Raji Inh%(2μM)	BxPC3 Inh% (2μM)
25	2,4- di-F-phenyl	Me	49	76	0.25	40.8	20.3
26	3-pyridyl	Et	61	73	7.7	42.1	2.2
27	m-F-phenyl	Et	33	27	0.2	49.3	46.1
28	2,4-di-F-phenyl	Et	33	25	0.11	46.23	43.2
29	3-pyridyl	Me	120	280	6.1	31.4	9.4
30	3,4,5-tri-F-phenyl	Me	90	520	1.5	45.8	7.9
31	o-F- phenyl	Me	46	NT	0.2	42.9	27.5
32	m-F-phenyl	Me	52	NT	0.64	45.2	27.8
33	2-Me-5-F- phenyl	Me	37	110	0.38	49.9	27.9

34	2-F-5-Me- phenyl	Me	74	NT	1.00	49.7	22.4
35	p-isopropylphenyl	Me	100	84	0.39	27.1	30.4
36	p-Br-phenyl	Et	72	NT	0.27	41.3	25.8
37	m-Br-phenyl	Et	52	NT	0.55	38.7	24.3
JQ1			87	79	0.87	47.9	31.1
ABBV-075			39	47	0.12	51.2	42.7

[a] BRD4 AlphaScreen IC_{50} values are reported as the mean ± SD value of three independent determinations.

[b] IC_{50} values presented are the mean derived from three measurements.

[c] The percent of residue via liver microsomes and the mean derived from three measurements.

2.2 Anti-proliferation in cancer cell lines

Furthermore, we assessed the effects of compounds on cancer cell proliferation in the lymphoblast-like cell line (Raji), promyelocytic cell line (HL-60) and human pancreatic cancer cell line (BxPC-3). As our previous reports, BET inhibitors show more robust anti-proliferation activities in hematologic malignancies than solid tumor[25]. In this work, all compounds exhibited preferred effects in HL-60 cell lines. It is intriguing that compounds **27** and **28** exhibited satisfying anti-proliferation in solid cancer cell, especially in human pancreatic cancer cell line (BxPC-3). Among them, compound **28** showed anti-proliferation Inh% of 43.2 in BxPC-3 cell line with 2μM (**Table 2**). In addition, cell cycle analysis was performed to characterize the molecular mechanisms underlying the cytotoxic effect of compound **28** in BxPC-3 cell. BxPC-3 cell was cultured with DMSO control or varied concentration of compound **28** (2, 4, 8 μM) respectively. As shown in **Figure 4A**, compound **28** treatment resulted in a remarkable increase in G1 cells at 48 h, which was consistent with previous report[20]. These results indicated that compounds **27** and **28** were demonstrated promising effects in the therapy of pancreatic cancer.

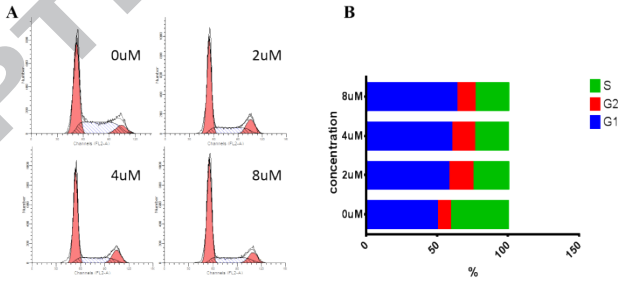


Figure 4. Cellular effects of compound **28** in BxPC-3 cell lines. (A) Cell cycle analysis was performed on BxPC-3 cell cultured with DMSO or varied concentration of compound **28** (2, 4, 8 μM) for 48 h. (B) Data was demonstrated by graph.

2.3 Induce apoptosis via the mitochondrial pathways in pancreatic cancer cells

To further clarify the mechanism of compound **28** triggering apoptosis in pancreatic cancer cells, we examined the expression pattern of BRD4 and several key proteins in the intrinsic apoptotic pathway. The results showed that both compound **28** and **JQ1** upregulated BRD4 protein, perhaps related to enrichment occurred in signaling regulation. Compound **28** downregulated c-Myc at 24 hours, consistent with **JQ1**. Meanwhile, compound **28** downregulated BcL-xL in BxPc3 pancreatic cancer cell lines to trigger apoptosis by modulating the intrinsic apoptotic pathway. Early apoptotic events such as caspase activation (caspase 3 and caspase 9) and PARP cleavage were already apparent at the low concentration of compound **28** (**Figure 5**). In addition, the result exhibited that the hexamethylene bisacetamide

inducible protein 1 (HEXIM1), well-characterized PD markers reported by Lin et.al, was significantly up-regulated [26]. Taken together, these results indicated that compound **28** can effectively trigger apoptosis in BxPc3 cells by modulating the intrinsic apoptotic pathway.

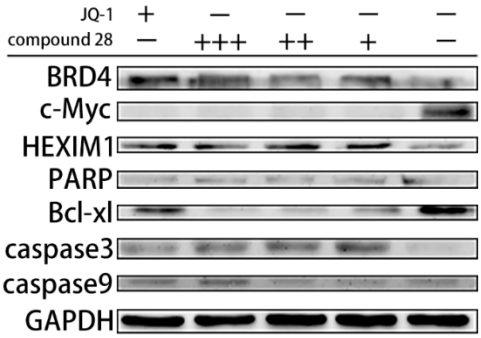


Figure 5. Induce apoptosis via the mitochondrial pathways in pancreatic cancer cells. BxPc3 cells were treated with compound **28** for 24 h, and then the expression of BRD4, c-Myc, PARP, Bcl-xl, caspase 9, caspase 3 and HEXIM1 were examined by western blot. "+" means 5 μM, "++"means 10 μM, "+++" means 15 μM.

Conclusion

We have designed and synthesized a series of BET inhibitors with 7-methylimidazo[1,5-a]pyrazin-8(7H)-one scaffold. Optimization of bidentate contact afforded compounds with high BRD4 potency and excellent physicochemical properties. Compound **28** exhibited excellent BRD4-inhibitory activity with IC_{50} value of **33 nM** and anti-proliferation potency with IC_{50} value of **110 nM** in HL-60 cell lines. Western Blot (WB) indicated that compound **28** can effectively trigger apoptosis in BxPc3 cells by modulating the intrinsic apoptotic pathway. All of these results demonstrated that compound **28** exhibited excellent preclinical development properties and was progressed into further investigation.

4. Experimental section

4.1 Chemistry. General Methods.

All chemical reagents were commercially purchased and used without further purification. Column chromatography was performed on silica gel 60 (200-300 mesh). Thin-layer chromatography (TLC): silica gel 60F254 plates (250 nm; Qingdao Ocean Chemical Company, Qingdao, China) were used to monitor the reactions. Capillary tubes were used for the measurement of melting points. HPLC analysis (UV detector, wavelength: 272 nm) was employed to evaluate purity of the target compounds and all final compounds were purified to ≥95% purity. ^{1}H and ^{13}C NMR spectra: Bruker ACF-300Q apparatus (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR), in DMSO-d_6 or $CDCl_3$ unless otherwise indicated. The data of the chemical shifts were showed in δ values (ppm) and the coupling constants (*J*) in Hz. Mass spectrometry (MS): Hewlett-Packard 1100 LC/MSD spectrometer; elemental analyses: CHNO-Rapid instrument.

*4-Amino-5-bromopyrimidin-2(1H)-one (**9**).* To a solution of 4-aminopyrimidin-2(1H)-one (2g, 18mmol) in acetic acid (10 mL) was added bromine (3.6 g, 22.5mmol). The reaction mixture was stirred at 70 °C for 8 h. The reaction mixture was diluted with H_2O (30 mL) and the light yellow precipitate was filtered and washed with water. The solid was then dried under reduced pressure to give the title compound (2.91g, 84.8% yield). ^{1}H NMR (300 MHz, DMSO-d6) δ 10.86 (s, 1H), 7.75 (s, 1H), 6.85 (s, 1H).

*8-Bromoimidazo[1,2-c]pyrimidin-5(6H)-one (**10**).* To a solution of **9** (1 g, 5.26 mmol) in DMF (10

mL) was added 40% chloroacetaldehyde (1.24 g, 6.32 mmol) at rt. The reaction mixture was stirred at 100 °C for 10 h. The reaction mixture was diluted with H_2O (20 mL) and the white precipitate was filtered and washed with water. The solid was then dried under reduced pressure to give the title compound (0.73g, 64.8% yield). ^{1}H NMR (300 MHz, DMSO-d6) δ 11.92 (s, 1H), 7.89 (d, *J* = 1.0 Hz, 1H), 7.65 (s, 1H), 7.42 (d, *J* = 1.0 Hz, 1H).

*8-Bromo-6-methylimidazo[1,2-c]pyrimidin-5(6H)-one (**11**).* A solution of **10** (0.25 g, 1.17 mmol) and K_2CO_3 (0.32 g, 2.34 mmol) in acetonitrile (20 mL) was stirred at ambient temperature. To this solution was added dimethylsulfate (0.17 g, 0.14 mmol) and the reaction mixture was stirred at 70 °C for 4 h. After cooling to the room temperature, the mixture was filtered through a celite pad, and the filtrate was concentrated under reduced pressure. The crude compound was purified by flash chromatography (silica gel, PE/EA = 10:1~5:1) to afford the title compound (0.13 g, 48.8% yield). ^{1}H NMR (300 MHz, DMSO-d6) δ 7.92 (d, *J* = 12.7 Hz, 2H), 7.42 (s, 1H), 3.50 (s, 3H).

*8-(2-Fluoro-5-nitrophenyl)-6-methylimidazo[1,2-c]pyrimidin-5(6H)-one (**12**).* A mixture of compound **11** (0.3 g, 1.32 mmol), (2-fluoro-5-nitrophenyl)boronic acid (0.495 g, 1.97 mmol), $Pd(PPh_3)_4$ (0.152 g, 0.133mmol), and Na_2CO_3 (0.418 g, 3.95 mmol) in toluol (3 mL), water (3 mL) and EtOH (1 mL) and methanol (1.5 mL) was stirred at 110 °C under nitrogen atmosphere for 24 h Then the mixture was diluted with H_2O and EtOAc, and the insoluble material was filtered through Celite. The organic layer was separated and purified by flash chromatography (silica gel, PE/EA = 5:1~2:1) to afford the title compound (0.26 g, 68.57% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.85 (d, *J* = 2.9 Hz, 1H), 8.38 (d, *J* = 3.1 Hz, 1H), 7.96 (s, 2H), 7.69 (d, *J* = 9.3 Hz, 1H), 7.47 (d, *J* = 1.4 Hz, 1H), 3.61 (s, 3H).

*8-(2-(2,6-Dimethylphenoxy)-5-nitrophenyl)-6-methylimidazo[1,2-c]pyrimidin-5(6H)-one (**13a**).* A mixture of **12** (0.14 g, 0.49 mmol), 2,6-dimethylphenol (0.12 g, 0.97 mmol) and K_2CO_3 (0.13 g, 0.97 mmol) in DMF (4 mL) was stirred at 80 °C for 12 h. Then the mixture was diluted with H_2O and the white precipitate was filtered and washed with water. The solid was then dried under reduced pressure to give the title compound (0.16 g, 84.4% yield). ^{1}H NMR (300 MHz, DMSO-d6) δ 8.63 (d, *J* = 2.8 Hz, 1H), 8.21 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.96 – 7.84 (m, 2H), 7.45 (d, *J* = 1.5 Hz, 1H), 7.25 – 7.10 (m, 3H), 6.55 (d, *J* = 9.1 Hz, 1H), 3.62 (s, 3H), 2.07 (s, 6H).

*8-(5-Amino-2-(2,6-dimethylphenoxy)phenyl)-6-methylimidazo[1,2-c]pyrimidin-5(6H)-one (**14a**).* To a solution of **13a** (0.1 g, 0.26 mmol) and NH_4Cl (0.054 g, 1.02 mmol) in 50% EtOH was added Iron powder (0.057 g, 1.02 mmol) at ambient temperature. Then the mixture was stirred at 100 °C for 2 h. Then the mixture was diluted with H_2O and EtOAc, and the insoluble material was filtered through Celite. The organic layer was separated and purified by flash chromatography (silica gel, PE/EA = 8:1~2:1) to afford the title compound (0.077 g, 83.41%). The crude product was further reacted directly for next step without further purification.

*N-(4-(2,6-Dimethylphenoxy)-3-(6-methyl-5-oxo-5,6-dihydroimidazo[1,2-c]pyrimidin-8-yl) pheny l)methanesulfonamide (**15**).* To a solution of compound **14a** (0.12 g, 0.332 mmol) and triethylamine (0.13 g, 1.33 mmol) in dichloromethane (6.0 mL) was added dropwise methanesulfonyl chloride (0.13 g, 0.99 mmol) at ice bath. The reaction mixture was stirred for 5 h at ambient temperature. Then the solution was concentrated under reduced pressure. The residue was diluted with a mixture of 1,4-dioxane (6 mL) and 2 M aqueous sodium hydroxide (3 mL) and heated for 30min at 70 °C. Then the mixture was diluted with H_2O and EtOAc, brought to pH 7 with 1 M HCl. The organic layer was separated and purified by

flash chromatography (silica gel, PE/EA = 3:1~1:1) to afford the title compound (0.12 g, 81%) as a white solid. m.p.: 126-128 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.53 (s, 1H), 7.86 (s, 1H), 7.71 (s, 1H), 7.55 (s, 1H), 7.42 (s, 1H), 7.09 (d, *J* = 8.4 Hz, 4H), 6.33 (d, *J* = 8.8 Hz, 1H), 3.61 (s, 3H), 3.00 (s, 3H), 2.06 (s, 6H).^{13}C NMR (75 MHz, DMSO) δ 152.43, 150.83, 146.38, 145.12, 133.68, 132.89, 131.85, 131.19, 129.48, 125.99, 125.71, 123.25, 122.27, 113.13, 107.93, 60.21, 40.86, 40.59, 40.31, 40.03, 39.75, 39.47, 39.20, 36.97, 21.20, 16.41, 14.53. MS (ESI, m/z): 439.4[M + H]$^+$; Anal. calcd. for $C_{22}H_{22}N_4O_4S$: C, 60.26; H, 5.06; N, 12.78. Found: C, 60.40; H, 5.11; N, 12.58.

*N-(4-(2,6-Dimethylphenoxy)-3-(6-methyl-5-oxo-5,6-dihydroimidazo[1,2-c]pyrimidin-8-yl)ph eny l)ethanesulfonamide (**16**)*. Compound **16** was prepared in a similar procedure of compound **15**. A white solid was provided. Yield: 84.9%. m.p.: 130-132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.82 (dd, *J* = 12.3, 2.1 Hz, 2H), 7.56 – 7.43 (m, 2H), 7.26 (s, 1H), 7.08 (s, 3H), 6.82 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.22 (d, *J* = 8.8 Hz, 1H), 3.67 (s, 3H), 3.15 (q, *J* = 7.4 Hz, 2H), 2.03 (s, 6H), 1.41 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 185.60, 177.60, 152.24, 150.86, 146.38, 145.12, 133.67, 132.89, 131.84, 131.18, 129.49, 125.66, 122.88, 122.27, 113.15, 113.09, 107.91, 105.33, 90.13, 54.72, 45.47, 40.87, 40.59, 40.32, 40.04, 39.76, 39.48, 39.21, 36.98, 16.42, 8.49. MS (ESI, m/z): 453.3[M + H]$^+$; Anal. calcd. for $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38. Found: C, 61.18; H, 5.41; N, 12.63.

*N-(4-(2,4-Difluorophenoxy)-3-(6-methyl-5-oxo-5,6-dihydroimidazo[1,2-c]pyrimidin-8-yl)ph eny l)ethanesulfonamide (**17**)*. Compound **17** was prepared in a similar procedure of compound **15**.. A white solid was provided. Yield: 83.2%; m.p.: 129-131 °C;^{1}H NMR (300 MHz, DMSO-d6) δ 9.85 (s, 1H), 7.83 (s, 1H), 7.69 (s, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 0.9 Hz, 2H), 7.20 (dd, *J* = 15.3, 8.8 Hz, 2H), 7.04 (d, *J* = 7.9 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 3.55 (s, 3H), 3.12 (dd, *J* = 14.4, 7.1 Hz, 2H), 1.23 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (75 MHz, DMSO-d6) δ 199.63, 188.26, 147.35, 145.11, 144.54, 134.25, 133.97, 133.14, 127.32, 124.55, 123.21, 122.76, 122.64, 120.04, 117.18, 112.94, 112.27, 109.68, 106.38, 105.97, 96.93, 78.57, 56.16, 45.61, 44.89, 40.80, 40.60, 40.49, 40.33, 40.22, 40.05, 39.93, 39.77, 39.59, 39.49, 39.34, 37.43, 36.84, 31.43, 19.45, 11.77, 8.45. MS (ESI, m/z): 461.3[M + H]$^+$; Anal. calcd. for $C_{21}H_{18}F_2N_4O_4S$: C, 54.78; H, 3.94; N, 12.17. Found: C, 54.18; H, 4.11; N, 12.23.

The synthesis methods of **19-21** would not be shown here because their preparation has been presented in detail in our previous study [25].

*5-Bromopyrazin-2(1H)-one (**19**)*. Yellow solid; Yield: 55.7%; ^{1}H NMR (300 MHz, DMSO-d6) δ 12.24 (s, 1H), 8.10 (s, 1H), 7.92 (s, 1H).

*5-Bromo-1-methylpyrazin-2(1H)-one (**20**)*. Yellow solid; Yield: 83.4%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.32 (s, 1H), 3.52 (s, 3H).

*5-Bromo-7-methylimidazo[1,5-a]pyrazin-8(7H)-one (**21**)*. Yellow solid; Yield: 72.5%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, *J* = 3.3 Hz, 2H), 6.62 (s, 1H), 3.48 (s, 3H).

*5-(2-Fluoro-5-nitrophenyl)-7-methylimidazo[1,5-a]pyrazin-8(7H)-one (**22**)*. The synthesis method of **22** was similar to the preparation of **12**. White solid; Yield: 68.3%; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.57 (d, *J* = 5.7 Hz, 1H), 8.51 (dd, *J* = 8.6, 3.9 Hz, 1H), 8.15 (s, 1H), 7.91 (s, 1H), 7.75 (t, *J* = 9.1 Hz, 1H), 7.30 (s, 1H), 3.42 (s, 3H).

*5-(2-(2,4-Difluorophenoxy)-5-nitrophenyl)-7-methylimidazo[1,5-a]pyrazin-8(7H)-one (**23a**)*. The synthesis method of **23a** was similar to the preparation of **13a**. White solid; Yield: 95.2%; The crude product was further reacted directly for next step without further purification.

*5-(5-Amino-2-(2,4-difluorophenoxy)phenyl)-7-methylimidazo[1,5-a]pyrazin-8(7H)-one (**24a**)*. The synthesis method of **24a** was similar to the preparation of **14a**. White solid; Yield: 83.4%;^{1}H NMR (300 MHz, DMSO-d6) δ 7.83 (s, 1H), 7.76 (s, 1H), 7.28 – 7.21 (m, 1H), 7.04 – 6.98 (m, 1H), 6.94 – 6.89 (m,

2H), 6.81 (d, *J* = 9.5 Hz, 1H), 6.76 – 6.71 (m, 2H), 5.21 (s, 2H), 3.35 (s, 3H).

The synthesis method of **25-37** was similar to the preparation of **15-17**.

*N-(4-(2,4-Difluorophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)methanesulfonamide (**25**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.85 (s, 1H), 7.98 (s, 1H), 7.82 (s, 1H), 7.47 – 7.27 (m, 4H), 7.10 (s, 2H), 6.94 (d, *J* = 8.7 Hz, 1H), 3.40 (s, 3H), 3.05 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.89, 152.40, 139.16, 138.96, 134.57, 133.04, 129.89, 124.66, 124.49, 123.64, 123.50, 123.20, 122.09, 120.77, 117.56, 113.28, 112.72, 112.42, 106.09, 40.85, 40.57, 40.29, 40.02, 39.74, 39.46, 39.19, 34.61. MS (ESI, m/z): 447.0[M+H]$^+$; Anal. calcd. for $C_{20}H_{16}F_2N_4O_4S$: C, 53.81; H, 3.61; N, 12.55. Found: C, 53.97; H, 3.51; N, 12.42.

*N-(3-(7-Methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(pyridin-3-yloxy)phenyl) ethanesulfonamide (**26**)*. m.p.: 128-130 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.98 (s, 1H), 8.29 (dd, *J* = 4.6, 1.2 Hz, 1H), 8.24 (d, *J* = 2.7 Hz, 1H), 8.03 (s, 1H), 7.78 (s, 1H), 7.42 – 7.29 (m, 4H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.04 (s, 1H), 3.35 (s, 3H), 3.18 (q, *J* = 7.3 Hz, 2H), 1.24 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.81, 153.11, 151.10, 145.23, 140.95, 135.40, 133.13, 129.91, 125.94, 124.94, 123.78, 123.16, 122.39, 122.15, 120.56, 113.17, 45.75, 40.86, 40.58, 40.31, 40.03, 39.75, 39.47, 39.19, 34.58, 8.53. MS (ESI, m/z):426.1[M+H]$^+$; Anal. calcd. for $C_{20}H_{19}N_5O_4S$: C, 56.46; H, 4.50; N, 16.46. Found: C, 56.58; H, 4.91; N, 16.53.

*N-(4-(3-Fluorophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)ethanesulfonamide (**27**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.95 (s, 1H), 7.99 (s, 1H), 7.77 (s, 1H), 7.42 – 7.37 (m, 2H), 7.29 (dd, *J* = 15.2, 8.2 Hz, 1H), 7.17 – 7.11 (m, 1H), 7.00 (s, 1H), 6.93 – 6.85 (m, 1H), 6.84 – 6.71 (m, 2H), 3.34 (s, 3H), 3.18 (q, *J* = 7.3 Hz, 2H), 1.24 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.81, 150.96, 135.40, 133.12, 131.67, 131.54, 129.87, 123.72, 123.17, 122.55, 122.11, 120.98, 114.58, 113.20, 110.97, 110.69, 106.34, 106.01, 45.76, 40.86, 40.58, 40.30, 40.02, 39.74, 39.47, 39.19, 34.56, 8.52. MS (ESI, m/z):443.0[M+H]$^+$; Anal. calcd. for $C_{21}H_{19}FN_4O_4S$: C, 57.01; H, 4.33; N, 12.66. Found: C, 56.84; H, 4.25; N, 12.89.

*N-(4-(2,4-Difluorophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)ethanesulfonamide (**28**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.89 (s, 1H), 7.94 (s, 1H), 7.80 (s, 1H), 7.45 – 7.24 (m, 4H), 7.08 (s, 2H), 6.93 (d, *J* = 8.8 Hz, 1H), 3.40 (s, 3H), 3.15 (q, *J* = 7.2 Hz, 2H), 1.23 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 154.88, 152.18, 134.61, 132.95, 129.89, 124.38, 124.17, 123.99, 123.63, 123.48, 123.21, 122.06, 120.78, 117.61, 113.26, 112.33, 106.45, 106.09, 105.79, 45.73, 40.87, 40.59, 40.31, 40.03, 39.75, 39.48, 39.20, 34.62, 8.51.MS (ESI, m/z): 461.0[M+H]$^+$; Anal. calcd. for $C_{21}H_{18}F_2N_4O_4S$: C, 54.78; H, 3.94; N, 12.17. Found: C, 53.58; H, 3.91; N, 12.13.

*N-(3-(7-Methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(pyridin-3-yloxy)phenyl)methanesulfonamide (**29**)*. m.p.: 115-117 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.91 (s, 1H), 8.29 (dd, *J* = 4.4 Hz, 1.2 Hz,1H), 8.25 (d, *J* = 2.6 Hz, 1H), 8.04 (s, 1H), 7.78 (s, 1H), 7.44 – 7.29 (m, 4H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.05 (s, 1H), 3.35 (s, 3H), 3.07 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.80, 153.17, 151.29, 145.18, 140.89, 135.45, 133.08, 129.92, 125.84, 124.87, 124.34, 124.30, 123.21, 122.47, 122.18, 120.66, 113.15, 40.96, 40.68, 40.41, 40.13, 39.92, 39.85, 39.57, 39.29, 34.54. MS (ESI, m/z): 412.0 [M+H]$^+$; Anal. calcd. for $C_{19}H_{17}N_5O_4S$: C, 55.47; H, 4.16; N, 17.02. Found: C, 55.52; H, 4.21; N, 17.38.

*N-(3-(7-Methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(3,4,5-trifluorophenoxy)phenyl) methanesulfonamide (**30**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.93 (s, 1H), 8.06 (s, 1H), 7.80 (s, 1H), 7.42 – 7.36 (m, 2H), 7.18 (d, *J* = 9.2 Hz, 1H), 7.10 (dd, *J* = 9.0, 6.1 Hz, 2H), 7.04 (s, 1H), 3.37 (s, 3H), 3.07 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.84, 150.99, 135.62, 133.19, 129.94, 124.13, 123.18, 122.35, 122.19, 120.59, 112.92, 104.67, 104.36, 40.81, 40.53, 40.25, 39.97, 39.81, 39.70,

39.42, 39.14, 34.61. MS (ESI, m/z):487.0[M+Na]$^+$; Anal. calcd. for $C_{20}H_{15}F_3N_4O_4S$: C, 51.72; H, 3.26; N, 12.06. Found: C, 52.02; H, 3.18; N, 12.21.

*N-(4-(2-Fluorophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)methanesulfonamide (**31**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.93 (s, 1H), 8.06 (s, 1H), 7.80 (s, 1H), 7.42 – 7.36 (m, 2H), 7.18 (d, *J* = 9.2 Hz, 1H), 7.10 (dd, *J* = 9.0, 6.1 Hz, 2H), 7.04 (s, 1H), 3.37 (s, 3H), 3.07 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.87, 152.16, 142.68, 142.52, 134.61, 133.01, 129.88, 125.90, 124.56, 124.46, 123.17, 122.27, 122.08, 121.02, 118.08, 117.67, 117.43, 113.31, 40.81, 40.53, 40.26, 39.98, 39.78, 39.70, 39.42, 39.14, 34.62. MS (ESI, m/z):487.0[M+Na]$^+$; Anal. calcd. for $C_{20}H_{17}F_1N_4O_4S$: C, 56.07; H, 4.00; N, 13.08. Found: C, 56.05; H, 4.11; N, 13.12.

*N-(4-(3-Fluorophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)methanesulfonamide (**32**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.88 (s, 1H), 8.00 (s, 1H), 7.78 (s, 1H), 7.46 – 7.36 (m, 2H), 7.30 (dd, J = 15.4, 8.0 Hz, 1H), 7.16 (d, 1H), 7.01 (s, 1H), 6.95 – 6.85 (m, 1H), 6.85 – 6.72 (m, 2H), 3.35 (s, 3H), 3.07 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.81, 151.14, 135.45, 133.08, 131.63, 131.50, 129.89, 124.25, 123.22, 122.64, 122.13, 121.10, 114.54, 113.18, 110.91, 110.63, 106.26, 105.93, 40.96, 40.69, 40.41, 40.13, 39.93, 39.85, 39.57, 39.29, 34.52. MS (ESI, m/z):429.0[M+H]$^+$; Anal. calcd. for $C_{20}H_{17}F_1N_4O_4S$: C, 56.07; H, 4.00; N, 13.08. Found: C, 55.98; H, 4.13; N, 12.95.

*N-(4-(5-Fluoro-2-methylphenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl) phenyl)methanesulfonamide (**33**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.87 (s, 1H), 8.01 (s, 1H), 7.79 (s, 1H), 7.44 – 7.36 (m, 2H), 7.18 (t, J = 7.6 Hz, 1H), 7.11 – 7.03 (m, 2H), 6.86 – 6.76 (m, 1H), 6.73 – 6.66 (m, 1H), 3.35 (s, 3H), 3.07 (s, 3H), 1.91 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.84, 151.18, 135.13, 133.25, 132.56, 132.43, 129.88, 124.37, 124.30, 123.14, 122.23, 121.91, 120.34, 113.25, 110.70, 110.42, 105.41, 105.08, 40.86, 40.58, 40.30, 40.03, 39.83, 39.75, 39.47, 39.19, 34.57, 15.21. MS (ESI, m/z):457.1[M+H]$^+$; Anal. calcd. for $C_{21}H_{19}F_1N_4O_4S$: C, 51.07; H, 4.33; N, 12.66. Found: C, 51.08; H, 4.23; N, 12.34.

*N-(4-(2-Fluoro-5-methylphenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl) phenyl)methanesulfonamide (**34**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.84 (s, 1H), 7.95 (s, 1H), 7.79 (s, 1H), 7.42 – 7.33 (m, 2H), 7.20 – 7.10 (m, 1H), 7.07 (s, 1H), 7.02 – 6.90 (m, 3H), 3.38 (s, 3H), 3.06 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.85, 152.27, 142.12, 135.38, 134.59, 132.87, 129.85, 126.38, 126.29, 124.69, 124.53, 123.23, 122.30, 122.03, 120.98, 118.31, 117.06, 116.83, 113.23, 40.97, 40.68, 40.41, 40.13, 39.85, 39.57, 39.29, 34.53, 20.57. MS (ESI, m/z): 443.0 [M+H]$^+$; Anal. calcd. for $C_{21}H_{19}F_1N_4O_4S$: C, 51.07; H, 4.33; N, 12.66. Found: C, 51.12; H, 4.31; N, 12.54.

*N-(4-(4-Isopropylphenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl) methanesulfonamide (**35**)*. m.p.: 108-110 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.83 (s, 1H), 7.99 (s, 1H), 7.78 (s, 1H), 7.36 (d, *J* = 9.4 Hz, 2H), 7.15 (d, *J* = 7.7 Hz, 2H), 7.01 (d, *J* = 5.5 Hz, 2H), 6.87 (d, *J* = 7.7 Hz, 2H), 3.36 (s, 3H), 3.05 (s, 3H), 2.88 – 2.76 (m, 1H), 1.14 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d6) δ 154.83, 154.16, 152.56, 144.43, 134.45, 133.16, 129.76, 128.03, 124.60, 123.12, 121.99, 121.70, 119.63, 119.00, 113.74, 40.76, 40.48, 40.20, 39.93, 39.65, 39.37, 39.09, 34.57, 33.17, 24.39. MS (ESI, m/z):453.1[M+H]$^+$; Anal. calcd. for $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38. Found: C, 61.07; H, 5.41; N, 12.61.

*N-(4-(4-Bromophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)ethanesulfonamide (**36**)*. m.p. > 220-222 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.95 (s, 1H), 7.98 (s, 1H), 7.79 (s, 1H), 7.42 (dd, *J* = 20.5, 8.1 Hz, 4H), 7.11 (d, *J* = 9.2 Hz, 1H), 7.00 (s, 1H), 6.89 (d, *J* = 8.9 Hz, 2H), 3.36 (s, 3H), 3.17 (d, *J* = 7.3 Hz, 2H), 1.24 (t, *J* = 7.3 Hz, 3H); MS (ESI, m/z): 500.90 [M-H]$^-$. Anal.

calcd. for $C_{21}H_{19}B_rN_4O_4S$: C, 50.11; H, 3.80; N, 11.13. Found: C, 50.05; H, 3.41; N, 11.34.

*N-(4-(3-Bromophenoxy)-3-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenyl)ethan esulfonamide (**37**).* m.p.: 108-110 °C; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.95 (s, 1H), 8.00 (s, 1H), 7.77 (s, 1H), 7.39 (s, 2H), 7.20 (d, *J* = 29.3 Hz, 4H), 6.97 (d, *J* = 23.3 Hz, 2H), 3.35 (s, 3H), 3.18 (s, 2H), 1.23 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 156.77, 154.30, 150.51, 134.77, 132.67, 131.53, 129.36, 126.56, 123.19, 122.62, 122.03, 121.81, 121.66, 121.08, 120.19, 117.32, 112.72, 45.23, 34.09, 8.03; MS (ESI, m/z): 500.90 $[M-H]^-$. Anal. calcd. for $C_{21}H_{19}B_rN_4O_4S$: C, 50.11; H, 3.80; N, 11.13. Found: C, 50.15; H, 3.79; N, 11.22.

4.2 Docking studies

These docking small molecules were processed with Ligand preparation protocol in Maestro 10.2. Then all ligands were minimized with OPLS 2005 force field [27]. Glide generation protocol of Maestro 10.2 was performed to process the BRD4 (BD1) protein (PDB id: 3P5O), which was downloaded from PDB (http://www.rcsb.org/pdb). Seven molecules of water around the Kac-pocket were reserved (id: HOH18, HOH27, HOH33, HOH256, HOH267, HOH268, HOH358), and the bond orders were assigned [25]. After that, a glide box using the centroid of the bound ligand and a maximum size of 12 Å was generated following hydrogen atoms modified. The optimal docking conformations were saved.

4.3. Biological evaluation

4.3.1. AlphaScreen bromodomain binding assay.

The AlphaScreen bromodomain binding assay was performed by Shanghai ChemPartner Co. Firstly 1x assay buffer (modified HEPES Buffer) was prepared for this experiment. Small molecules were transferred to assay plates with Echo. DMSO's final concentration is 0.1% following protein solution prepared in 1x assay buffer (5 nM). Then, to generate the substrate solution, peptide (H4) was added in 1x assay buffer. 5 μL of protein solution or 5 μL of 1x assay buffer was transferred to assay plates. After that, these assay plates were incubated at room temperature for 15 minutes. To each well 5 μL of substrate solution was added for reaction. Next, these assay plates were incubated at room temperature for one hour. In subdued light environment, 15 μL acceptor and donor solution was added and incubated for another one hour at room temperature. The Alpha mode of EnSpire was performed to show the endpoint. Finally, the data was imported into Excel to obtain inhibition values using equation (1). Equation (1): Inh % = (Max - Signal) / (Max - Min) × 100. Meanwhile, max signal was provided from the action of Enzyme and Substrate and min signal was acquired from the Substrate. GraphPad Prism 5 was used to get IC_{50} values using equation (2). Equation (2): Y = Bottom + (Top - Bottom) / (1 + 10^ (($LogIC_{50}$ - X) × Hill Slope)). Y is %inhibition and X is compound concentration.

4.3.2. Cell proliferation assay

The anti-proliferation activities of these compounds in cancer cell proliferation lines, lymphoblast-like cell line(Raji), promyelocytic cell line (HL-60) and human pancreatic cancer cell line(BxPC-3) were evaluated using MTT colorimetric assay and the procedure and conditions were described in our previous work[25, 27]. Raji and HL-60 cell line from Key laboratory of SATCM for Empirical Formulae Evaluation and Achievements Transformation were grown in RPMI 1640 supplemented with 16% fetal bovine serum (FBS). BxPC-3 cell line from Nanjing Tech University were grown in Dulbecco's Modifie d Eagle Medium (DMEM) supplemented with10% FBS. HL-60 DNA content analysis was performed by flow cytometry (BD, LSR Fortessa TM, San Diego, CA, USA). The percentage of cellsin different cell cycle phases was determined with modfit LT 2.0 software (BD Biosciences, Pharmingen, San Diego, CA, USA). Cell cycle analysis was performed following standard protocols.

4.3.3. Western Blot Analysis

Cell lysates were prepared in cell lysis buffer (RIPA Lysis Buffer，Beyotime，P0013B) with protease inhibitor cocktail (Roche,5892970001). 30μg of total protein was resolved on a 12% SDS polyacrylamide gel and probed with anti-cMyc (Abcam Ab32072), anti-BRD4 (Cell Signaling #13440), anti-HEXIM1(Abcam,Ab-25388), anti-PARP (Cell Signaling #9542), anti-Bcl-xl (Abcam Ab-32370), anti-caspase3 (Cell Signaling # 9665), anti-caspase9 (Cell Signaling # 9508), GAPDH loading control Cell Signaling#5174. Western blotting was performed following standard protocols.

Acknowledgments

This study was supported by the Natural Science Foundation of Jiangsu Province (No. BK 20141349) and the China National Key HiTech Innovation Project for the R&D of Novel Drugs (No.2013ZX09301303-002).

AUTHOR INFORMATION

*E-mail: cl.xu81@gmail.com (C. Xu).

*E-mail: zhanghb80@163.com (H. Zhang).

*E-mail: jpzhou668@163.com (J. Zhou).

ABBREVIATIONS

BET, Bromodomain and Extraterminal Domain; DMPK, drug metabolism and pharmacokinetics; P-TEFb, positive transcription elongation factor; CDK9, cyclin dependent kinase; PDA, Pancreatic ductal adenocarcinoma; AlphaScreen, Amplified Luminescent Proximity Homogeneous; HEXIM1, hexamethylene bisacetamide inducible protein 1; IHC, immunohistochemical; WB, Western Blot

References

[1] T. Kouzarides. Cell 128(2007) 693-705.

[2] S.R. Haynes, Dollard, C., Winston, F., Beck, S., Trowsdale, J., and Dawid, I.B. Nucleic Acids Res 20(1992) 2603.

[3] C. Dhalluin, Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.M. Nature 399(1999) 491-6.

[4] G.V. Denis. Front Biosci 6(2001) D1065-8.

[5] P. Filippakopoulos, Picaud, S., Mangos, M., Keates, T., Lambert, J.P., Barsyte-Lovejoy, D., Felletar, I., Volkmer, R., Muller, S., Pawson, T., Gingras, A.C., Arrowsmith, C.H., and Knapp, S. Cell 149(2012) 214-31.

[6] Z. Sun, Zhang, H., Chen, Z., Xie, Y., Jiang, H., Chen, L., Ding, H., Zhang, Y., Jiang, H., Zheng, M., and Luo, C. Bioorg Med Chem Lett 27(2017) 2003-2009.

[7] S. Rahman, Sowa, M.E., Ottinger, M., Smith, J.A., Shi, Y., Harper, J.W., and Howley, P.M. Mol Cell Biol 31(2011) 2641-52.

[8] G. LeRoy, Rickards, B., and Flint, S.J. Mol Cell 30(2008) 51-60.

[9] Z. Yang, Yik, J.H., Chen, R., He, N., Jang, M.K., Ozato, K., and Zhou, Q. Mol Cell 19(2005) 535-45.

[10] S. Picaud, Da Costa, D., Thanasopoulou, A., Filippakopoulos, P., Fish, P.V., Philpott, M., Fedorov, O., Brennan, P., Bunnage, M.E., Owen, D.R., Bradner, J.E., Taniere, P., O'Sullivan, B., Muller, S., Schwaller, J., Stankovic, T., and Knapp, S. Cancer Res 73(2013) 3336-46.

[11] R. Gosmini, Nguyen, V.L., Toum, J., Simon, C., Brusq, J.M., Krysa, G., Mirguet, O., Riou-Eymard, A.M., Boursier, E.V., Trottet, L., Bamborough, P., Clark, H., Chung, C.W., Cutler, L., Demont, E.H., Kaur, R., Lewis, A.J., Schilling, M.B., Soden, P.E., Taylor, S., Walker, A.L., Walker, M.D., Prinjha, R.K., and Nicodeme, E. J Med Chem 57(2014) 8111-31.

[12] I.A. Asangani, Dommeti, V.L., Wang, X., Malik, R., Cieslik, M., Yang, R., Escara-Wilke, J.,

Wilder-Romans, K., Dhanireddy, S., Engelke, C., Iyer, M.K., Jing, X., Wu, Y.M., Cao, X., Qin, Z.S., Wang, S., Feng, F.Y., and Chinnaiyan, A.M. Nature 510(2014) 278-82.

[13] P. Filippakopoulos, Qi, J., Picaud, S., Shen, Y., Smith, W.B., Fedorov, O., Morse, E.M., Keates, T., Hickman, T.T., Felletar, I., Philpott, M., Munro, S., McKeown, M.R., Wang, Y., Christie, A.L., West, N., Cameron, M.J., Schwartz, B., Heightman, T.D., La Thangue, N., French, C.A., Wiest, O., Kung, A.L., Knapp, S., and Bradner, J.E. Nature 468(2010) 1067-73.

[14] S. Amorim, Stathis, A., Gleeson, M., Iyengar, S., Magarotto, V., Leleu, X., Morschhauser, F., Karlin, L., Broussais, F., Rezai, K., Herait, P., Kahatt, C., Lokiec, F., Salles, G., Facon, T., Palumbo, A., Cunningham, D., Zucca, E., and Thieblemont, C. Lancet Haematol 3(2016) e196-204.

[15] O. Mirguet, Gosmini, R., Toum, J., Clement, C.A., Barnathan, M., Brusq, J.M., Mordaunt, J.E., Grimes, R.M., Crowe, M., Pineau, O., Ajakane, M., Daugan, A., Jeffrey, P., Cutler, L., Haynes, A.C., Smithers, N.N., Chung, C.W., Bamborough, P., Uings, I.J., Lewis, A., Witherington, J., Parr, N., Prinjha, R.K., and Nicodeme, E. J Med Chem 56(2013) 7501-15.

[16] L. Chen, Yap, J.L., Yoshioka, M., Lanning, M.E., Fountain, R.N., Raje, M., Scheenstra, J.A., Strovel, J.W., and Fletcher, S. ACS Med Chem Lett 6(2015) 764-9.

[17] K.F. McDaniel, Wang, L., Soltwedel, T., Fidanze, S.D., Hasvold, L.A., Liu, D., Mantei, R.A., and Pratt, J.K. 60(2017) 8369-8384.

[18] M.H. Bui, Lin, X., Albert, D.H., Li, L., Lam, L.T., Faivre, E.J., Warder, S.E., Huang, X., Wilcox, D., Donawho, C.K., Sheppard, G.S., Wang, L., Fidanze, S., Pratt, J.K., Liu, D., Hasvold, L., Uziel, T., Lu, X., Kohlhapp, F., Fang, G., Elmore, S.W., Rosenberg, S.H., McDaniel, K.F., Kati, W.M., and Shen, Y. Cancer Res 77(2017) 2976-2989.

[19] E.J. Faivre, Wilcox, D., Lin, X., Hessler, P., Torrent, M., He, W., Uziel, T., Albert, D.H., McDaniel, K., Kati, W., and Shen, Y. Mol Cancer Res 15(2017) 35-44.

[20] K.T. Siu, Ramachandran, J., Yee, A.J., Eda, H., Santo, L., Panaroni, C., Mertz, J.A., Sims Iii, R.J., Cooper, M.R., and Raje, N. Leukemia 31(2017) 1760-1769.

[21] P.L. Garcia, Miller, A.L., Kreitzburg, K.M., Council, L.N., Gamblin, T.L., Christein, J.D., Heslin, M.J., Arnoletti, J.P., Richardson, J.H., Chen, D., Hanna, C.A., Cramer, S.L., Yang, E.S., Qi, J., Bradner, J.E., and Yoon, K.J. Oncogene 35(2016) 833-45.

[22] R.L. Siegel, Miller, K.D., and Jemal, A. CA Cancer J Clin 66(2016) 7-30.

[23] L. Rahib, Smith, B.D., Aizenberg, R., Rosenzweig, A.B., Fleshman, J.M., and Matrisian, L.M. Cancer Res 74(2014) 2913-21.

[24] X. Zhang, Lee, H.C., Shirazi, F., Baladandayuthapani, V., Lin, H., Kuiatse, I., Wang, H., Jones, R.J., Berkova, Z., Singh, R.K., Lu, J., Qian, Y., Raina, K., Coleman, K.G., and Crews, C.M. (2018).

[25] L. Zhao, Yang, Y., Guo, Y., Yang, L., Zhang, J., Zhou, J., and Zhang, H. Bioorg Med Chem 25(2017) 2482-2490.

[26] X. Lin, Huang, X., Uziel, T., Hessler, P., Albert, D.H., Roberts-Rapp, L.A., McDaniel, K.F., Kati, W.M., and Shen, Y. Mol Cancer Ther 16(2017) 388-396.

[27] Y. Yang, Zhao, L., Xu, B., Yang, L., Zhang, J., Zhang, H., and Zhou, J. Bioorg Chem 68(2016) 236-44.

Novel 7-methylimidazo[1,5-a]pyrazin-8(7H)-one BRD4 inhibitors were designed and synthesized.

·Molecular docking was performed.

·Cell proliferation inhibition was evaluated in the Raji, HL-60 and BxPC-3 cell lines.

·Induce apoptosis via the mitochondrial pathways in BxPC-3 cells.