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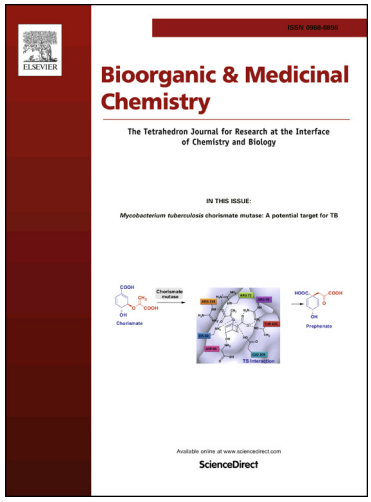

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Design, synthesis and biological evaluation of 7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one derivatives as BRD4 inhibitors

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Abstract

BRD4 is an attractive target for antitumor due to its important role in regulation of gene transcription. In this paper, we synthesized a series of 7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one derivatives as potent BRD4 inhibitors and evaluated their BRD4 inhibitory activities *in vitro* and anti-proliferation effects on tumor cells. Gratifyingly, compound **10j** exhibited robust potency of BRD4(1) and BRD4(2) inhibition with IC_{50} values of **130** and **76 nM** respectively. Docking studies were performed to explain the structure-activity relationship. Furthermore, compound **10j** potently inhibited cell proliferation in BRD4-sensitive cell lines HL-60 and MV4-11 with IC_{50} value of **0.57** and **0.18 μM** respectively. Activity on BRD4-independent K562 cell was weaker than on BRD4-sensitive lines. Overall, these results suggest that compound **10j** is a potential BRD4 inhibitor deserving further investigation for cancer treatment.

Keywords: BRD4; Bromodomain; Lysine acetylation; Antitumor

1. Introduction

BRD4 belongs to the bromodomain and extra terminal (BET) family proteins (BRD2, BRD3, BRD4, and BRDT). BRD4 has a conserved modular architecture including two N-terminal tandem bromodomain effector modules (BRD4(1) and BRD4(2)), an extra-terminal recruitment domain (ET), several conserved motifs (A, B, SEED motifs), and a C-terminal motif (CTM). Each bromodomain has a conserved architecture, comprising a four-helix bundle (helices αZ, αA, αB, and αC) linked by diverse loop regions (ZA and BC loops).[1] Lysine acetylation is a regulatory post-translational modification that, in the context of chromatin biology, is associated with open chromatin structure and transcriptional stimulation.[2] BRD4 bromodomains recognize and bind to acetylated lysine sequences found on histones or other proteins, and then regulate many downstream cellular processes such as cell cycle, growth, proliferation, and apoptosis. Upon binding to acetylated histones, BRD4 interacts with the positive transcription elongation factor complex (P-TEFb) complex to influence RNA polymerase II activity.[3] Genome-wide binding and expression profiling studies show that BRD4 controls both the expression of numerous protein-coding transcripts and of non-coding enhancer RNAs. For instance, BRD4 play a broad role in maintaining c-MYC levels,[4, 5] c-MYC is a master regulatory factor of cell proliferation, and pathologic activation of c-MYC plays a central role in cancer pathogenesis.[6] Thus, targeting BRD4 within the c-MYC transcriptional signaling network can modulate the function of c-MYC in cancer. As a result, BRD4 is becoming a novel therapeutic candidate target for antitumor drug development.[7]

Recently, a variety of small molecule compounds with potent inhibitory activity against

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BRD4 have been reported in the literatures,[8-14] such as (+)-JQ1,[15] I-BET762,[16] I-BET151,[17] OTX015,[18] and ABBV-075 (**Fig. 1**). (+)-JQ1 was the first potent BRD4 inhibitor and I-BET762 was obtained from optimization of a hit designed to identify small molecules able to enhance ApoA1 expression. I-BET762 has entered clinical trials for NUT midline carcinoma and other cancers. OTX015 has completed Phase I trials for hematologic malignancies and NUT midline carcinoma, and the results showed clinically meaningful activity at nontoxic doses.[19-21] ABBV-075 is in phase I clinical trials for the treatment of patients with solid tumors, acute myeloid leukemia or multiple myeloma, according to the strong biochemical potency for BRD4 (ki=15nM). Regardless of these BRD4 inhibitors, none has been approved. New BRD4 inhibitors with different scaffolds are still necessary to explore their therapeutic potential in different cancers. Here, 7-methylimidazo[1,5-a]pyrazin-8(7H)-one backbone was utilized to replace the heterocyclic bicyclic ring of ABBV-075 and a variety of optimal groups were employed for the design of novel BRD4 inhibitors (**Fig. 2**).[22, 23] In this paper, we report the design, synthesis and evaluation of a new series of 7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one derivatives (**10a-s**) as small molecule BRD4 inhibitors.

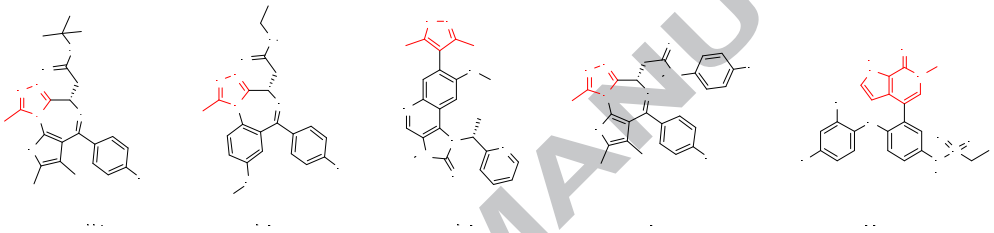


Fig. 1. Structures of known BRD4 inhibitors.

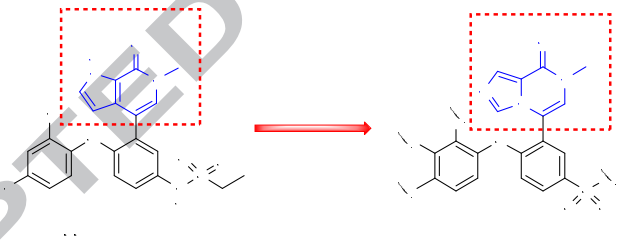


Fig. 2. Design of the novel BRD4 inhibitors.

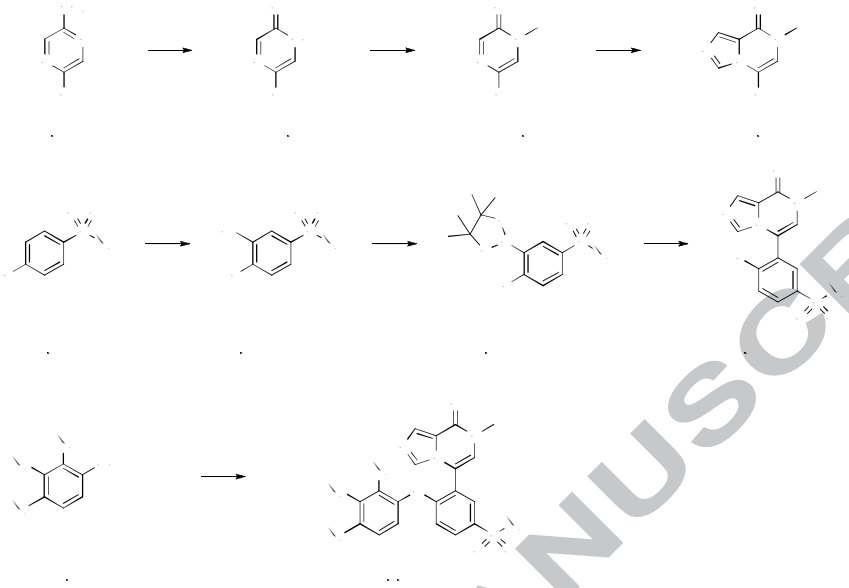
2. Results and discussion

2.1. Chemistry

Target compounds **10a-s** were synthesized as outlined in **Schemes 1-2**. All compounds were purified by flash chromatography and purity was checked by HPLC before biological evaluation (purity was > 97%). The structures were confirmed by ^{1}H NMR, ^{13}C NMR spectrum, and mass spectrometry.

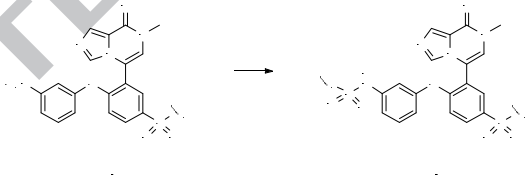
As depicted in **Scheme 1**, 5-bromopyrazin-2-amine (**1**) reacted with sodium nitrite in concentrated sulfuric acid to give 5-bromopyrazin-2(1*H*)-one (**2**). 5-Bromo-1-methylpyrazin-2(1*H*)-one (**3**) was prepared by methylation of intermediate **2** in the presence of dimethylsulfate. 5-Bromo-7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one (**4**) was obtained by cyclization of intermediate **3** with tosylmethyl isocyanide. Reaction of compounds (**5a, R1 = Me; 5b, R1 = Et**) and N-bromosuccinimide in concentrated sulfuric acid afforded intermediates (**6a, b**). The pinacol arylboronates (**7a, b**) were synthesized by coupling reaction **6a, 6b** with

dioxaborolane derivative. By Suzuki coupling reaction of intermediate **4** and **7a, 7b** intermediates, we obtained the key intermediates (**8a, b**). The target compounds (**10a-l**) were obtained by condensation of intermediates (**8a, b**) with intermediates (**9a-g**).



Scheme 1. General synthesis of **10a-l**. Reagents and conditions: (a) $NaNO_2$, conc. H_2SO_4, 0℃ to 45℃; (b) dimethylsulfate, K_2CO_3, acetonitrile, 70℃; (c) tosylmethyl isocyanide, NaH, THF, 0℃ to rt; (d) NBS, conc. H_2SO_4, 50℃; (e) 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane), Pd(dppf)$Cl_2CH_2Cl_2$, KOAc, 1,2-Dimethoxyethane, 90℃; (f) **4**, Pd(PPh_3)$_4$, K_2CO_3, acetonitrile, toluene, 80℃; (g) **8a** or **8b**, K_2CO_3, DMF, 80℃.

In addition, the target compounds (**10m-s**) were obtained by condensation of compounds (**10k, l**) with sulfonyl chlorides in the presence of pyridine as shown in Scheme 2.



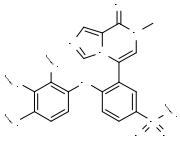
Scheme 2. General synthesis of **10m-s**. Reagents and conditions: (a) sulfonyl chloride, pyridine, CH_2Cl_2, rt.

2.2. BRD4 inhibitory activity and SAR study

BRD4 inhibitory activities of compounds (**10a-s**) were evaluated by AlphaScreen assay *in vitro*. (+)-JQ1 and ABBV-075 were used as positive control. We initially evaluated the inhibition rates of compounds against BRD4(1) at a concentration of 1 μM, the results were exhibited in **Table 1**. R_1 was replaced with methyl or ethyl (**10a** vs **10b**, **10k** vs **10l**, and **10m** vs **10n)**, the inhibition rate demonstrated that ethyl was the optimal substituent. Furthermore, compounds with high inhibition rate were selected to investigate their IC_{50} values against BRD4(1) and BRD4(2). As showed in **Table 2**, compounds **10g** and **10j** exhibited similar activity to (+)-JQ1 and ABBV-075. To understand the binding mode of this series, we performed docking experiments with Glide docking in Schrodinger. (+)-JQ1 and **10j** were docked into BRD4(1) crystal complex

(PDB id: 3P5O), and analysis of the docking conformation of (+)-JQ1 and **10j** with BRD4(1) revealed that the ligand-protein interactions were similar for these two molecules (**Fig. 3**). The key interactions of (+)-JQ1 and BRD4(1) were shown in **Fig. 3(A)**. The 3-methyl-1,2,4-triazole of (+)-JQ1 acted as a KAc mimic, with the methyl group occupying the hydrophobic pocket. One N formed a water mediated hydrogen bond to a conserved Tyr residue (TRY97), and another N accepted a hydrogen bond from the conserved Asn residue (ASN140). The key interactions of **10j** and BRD4(1) were shown in **Fig. 3(B)**. The 7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one acted as a KAc mimic, with the methyl group occupying the hydrophobic pocket. The O formed a water mediated hydrogen bond to a conserved Tyr residue (TRY97), and accepted a hydrogen bond from the conserved Asn residue (ASN140). The 4-chlorophenyl moiety of (+)-JQ1 and the phenylamino moiety of **10j** occupied a hydrophobic part of the WPF shelf. The dimethylthiophene of (+)-JQ1 and the ethylsulfonyl moiety of **10j** were directed into the ZA channel.

Table 1. Structures and BRD4(1) inhibitory activity of compounds **10a-s**.



Compound	R_1	R_2	R_3	R_4	BRD4(1) (1 μM)[a]
10a	CH_3CH_2	F	H	F	98%
10b	CH_3	F	H	F	77%
10c	CH_3CH_2	H	H	F	98%
10d	CH_3	H		H	63%
10e	CH_3	H		H	84%
10f	CH_3	H		H	67%
10g	CH_3CH_2	H		H	99%
10h	CH_3	H		H	74%
10i	CH_3	H		H	85%
10j	CH_3CH_2	H		H	100%
10k	CH_3	H	NH_2	H	78%
10l	CH_3CH_2	H	NH_2	H	89%
10m	CH_3CH_2	H		H	97%
10n	CH_3	H		H	59%
10o	CH_3CH_2	H		H	94%
10p	CH_3CH_2	H		H	92%
10q	CH_3CH_2	H		H	97%
10r	CH_3	H		H	66%
10s	CH_3	H		H	60%
(+)-JQ1[b]					100%
ABBV-075[b]					100%

[a] Inhibition rate mean values at a screening concentration of 1 μM were obtained from three independent experiments.
[b] Used as positive control.

Table 2. BRD4-BD1 and BRD4-BD2 inhibitory effects of compounds with high inhibition rate

Compound	BRD4(1) IC_{50} (μmol/L) [a]	BRD4(2) IC_{50} (μmol/L) [a]
10a	0.240±0.021	0.260±0.013
10b	0.520±0.015	0.580±0.011
10c	0.200±0.006	0.220±0.022
10e	0.380±0.011	0.180±0.021
10f	0.680±0.013	0.320±0.015
10g	0.130±0.008	0.040±0.024
10h	0.430±0.019	0.190±0.023
10i	0.350±0.015	0.180±0.019
10j	0.130±0.018	0.076±0.015
10m	0.210±0.022	0.130±0.014
10o	0.240±0.017	0.170±0.011
10p	0.390±0.009	0.280±0.007
10q	0.200±0.012	0.140±0.016
(+)-JQ1[c]	0.088±0.015	0.087±0.013
ABBV-075[c]	0.074±0.011	0.069±0.015

[a] IC_{50} values for BRD4(1) and BRD4(2) activities presented are the mean ± SD values of three independent determinations
[b] n.d. = not determined.
[c] Used as positive control.

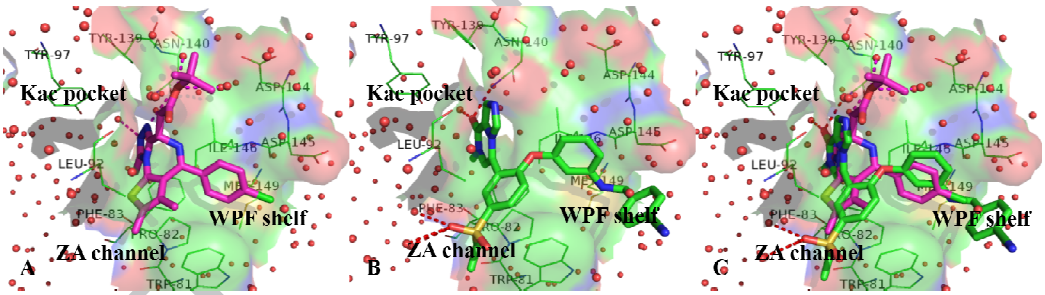


Fig. 3 (A) Docking conformation of (+)-JQ1 in BRD4(1) (PDB id: 3P5O). (B) Docking conformation of **10j** in BRD4(1) (PDB id: 3P5O). (C) Superimposition docking conformation of (+)-JQ1 (Pink) and **10j** (Green) in BRD4(1) (PDB id: 3P5O).

2.3. Anticancer evaluation against cancer cell lines *in vitro*

To investigate the effect of compound **10j** on the proliferation of leukemia cells, the reported sensitive cell lines human promyelocytic leukemia HL-60, acute myeloid leukemia MV4-11 and BRD4-independent K-562 cells were utilized to validate the cellular proliferation inhibition effects (**Table 3**).[4, 24] Most of compounds exhibited reasonable anti-proliferative activity. Especially, compound **10j** potently inhibited cell proliferation in cell line HL-60 and MV4-11 with IC_{50} value of 0.57 and 0.18 μM respectively. Activity of compound **10j** on BRD4-independent K-562 cells was weaker than on BRD4-sensitive lines. These results indicated that compound **10j** could be a promising lead compound for cancer treatment.

Table 3. Anticancer evaluation against cancer cell lines K562, HL-60 and MV4-11

Compound	K562 IC_{50} (μM)[a]	HL-60 IC_{50} (μM)[a]	MV4-11 IC_{50} (μM)[a]
10a	>10	0.62 ± 0.21	0.41± 0.11
10b	>10	2.86 ± 0.97	1.85 ± 0.98
10c	>10	0.94 ± 0.41	0.78 ± 0.31
10e	n.d[b]	n.d.	n.d.
10f	>10	1.4 ± 0.48	0.95 ± 0.32
10g	>10	2.86 ± 0.51	0.17 ± 0.05
10h	>10	1.53 ± 0.81	1.16 ± 0.74
10i	>10	6.1 ± 1.91	4.2 ± 1.22
10j	>10	0.57 ± 0.13	0.18 ± 0.07
10m	>10	3.36 ± 1.92	1.38 ± 0.94
10o	n.d.	n.d.	n.d.
10p	n.d.	n.d.	n.d.
10q	n.d.	n.d.	n.d.
(+)-JQ1[c]	9.12±0.08	0.11 ± 0.03	0.08 ± 0.02
ABBV-075[c]	>10μM	0.09 ± 0.02	0.07 ±0.03

[a] The data were expressed as the means ± SD, representing the relative levels of anti-proliferation from three independent experiments.
[b] n.d. = not determined.
[c] Used as positive control.

3. Conclusion

In summary, upon the basis of the previously reported BRD4 inhibitors, nineteen 7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one derivatives were designed and synthesized, and their BRD4-inhibitory activities and anti-proliferative activities were evaluated *in vitro*. It was found that compound **10j** exhibited robust potency of BRD4(1) and BRD4(2) inhibition with IC_{50} values of 130 and 76 nM respectively. The moderated inhibitory difference between BRD4(1) and BRD4(2) was deserving further investigation for analysis of the potential mechanisms. In addition, compound **10j** potently inhibited proliferation of leukemia cell line HL-60 and MV4-11 with IC_{50} value of 0.57 and 0.18 μM respectively. These findings demonstrated that compound **10j** could be a potent BRD4 inhibitor and worthy for further investigation.

4. Experimental section

4.1. Chemistry

All chemical reagents were commercially available and treated with standard methods before use. Solvents were dried and redistilled before use. Column chromatography (CC): silica gel 60 (200-300 mesh). Thin-layer chromatography (TLC): silica gel 60F254 plates (250 nm; Qingdao Ocean Chemical Company, Qingdao,China). m.p.: capillary tube, RY-1 capillary apparatus (Tianjin Optical Instrument Company, Tianjin, China), uncorrected. Purity of the target compounds were determined by HPLC analysis (UV detector, wavelength: 272 nm). ^{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-d6 or $CDCl_3$ unless otherwise indicated. Mass spectrometry (MS): Hewlett-Packard 1100 LC/MSD spectrometer; elemental analyses: CHNO-Rapid instrument.

4.1.1. 5-bromopyrazin-2(1*H*)-one (**2**)

To a solution of $NaNO_2$ (1.78 g, 25.86 mmol) in conc. H_2SO_4 (15 mL) was added a solution

of **1** (3.0 g, 17.24 mmol) in conc. H_2SO_4 (10 mL) dropwise at 0℃. The reaction mixture was stirred at 45℃ for 2 h. After cooling to the room temperature, the mixture was poured into ice water (200 mL) and extracted with EtOAc (300 mL). The organic layer was dried over Na_2SO_4 and concentrated to yield the title compound (1.68 g, 55.69% yield) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.24 (s, 1H), 8.10 (s, 1H), 7.92 (s, 1H).

4.1.2. 5-bromo-1 -methylpyrazin-2(1*H*)-one (**3**)

To a solution of **2** (1.51 g, 8.63 mmol) and K_2CO_3 (2.39 g, 17.26 mmol) in acetonitrile (30 mL) was added dimethylsulfate (1.63 g, 12.94 mmol). The reaction mixture was stirred at 70℃ for 4 h. After cooling to the room temperature, the mixture was filtered through a celite pad, and the filtrate was concentrated to afford the title compound (1.36 g, 83.38% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.32 (s, 1H), 3.52 (s, 3H).

4.1.3. 5-bromo-7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one (**4**)

To a solution of **3** (1.60 g, 8.47 mmol) and tosylmethyl isocyanide (1.99 g, 10.16 mmol) in THF (30 mL) was added NaH (1.02 g, 35.4 mmol) at 0℃. The reaction mixture was stirred at room temperature for 2 h. The mixture was quenched with methanol and then was concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (1.4 g, 72.52% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 3.3 Hz, 2H), 6.62 (s, 1H), 3.48 (s, 3H).

4.1.4. 2-bromo-1 -fluoro-4-(methylsulfonyl)benzene (***6a***)

To a solution of 1-fluoro-4-(methylsulfonyl)benzene **5a** (3 g, 17.22 mmol) in conc. H_2SO_4 (30 mL) was added NBS (3.07 g, 17.22 mmol). The reaction mixture was stirred at 50℃ for 4 h. After cooling to the room temperature, the mixture was poured into ice water (100 mL) and the white precipitate was filtered and washed with water. The solid was then dried under reduced pressure to give the title compound (3.67g, 84.2% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.27 (dd, J = 6.3, 2.2 Hz, 1H), 8.00 (ddd, J = 8.5, 4.5, 2.3 Hz, 1H), 7.67 (t, J = 8.7 Hz, 1H), 3.30 (s, 3H).

4.1.5. 2-bromo-4-(ethylsulfonyl)-1 -fluorobenzene (***6b***)

To a solution of 1-(ethylsulfonyl)-4-fluorobenzene **5b** (3.04 g, 16.15 mmol) in conc. H_2SO_4 (30 mL) was added NBS (2.87 g, 16.15 mmol). The reaction mixture was stirred at 50℃ for 4 h. After cooling to the room temperature, the mixture was poured into ice water (100 mL) and the white precipitate was filtered and washed with water. The solid was then dried under reduced pressure to give the title compound (3.31g, 76.72% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.22 (dd, J = 6.4, 2.2 Hz, 1H), 7.96 (ddd, J = 8.6, 4.6, 2.3 Hz, 1H), 7.68 (t, J = 8.6 Hz, 1H), 3.40 (q, J = 7.4 Hz, 2H), 1.10 (t, J = 7.4 Hz, 3H).

4.1.6. 2-(2-fluoro-5-(methylsulfonyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (***7a***)

A mixture of **6a** (3.87 g, 15.29 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (4.66 g, 18.35 mmol), Pd(dppf)$Cl_2CH_2Cl_2$ (0.34 g, 0.46 mmol) and KOAc (3 g, 30.58 mmol) in 1,2-Dimethoxyethane (50 mL) was stirred at 90℃ 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 of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and was concentrated under reduced pressure. Petroleum ether (30 mL) was added to the crude compound, the white precipitate was filtered and then dried under reduced pressure to give the title compound (3.03g, 66.02% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.19 – 8.05 (m, 2H),

7.52 – 7.39 (m, 1H), 3.23 (d, *J* = 3.8 Hz, 3H), 1.32 (s, 12H).

4.1.7. 2-(5-(ethylsulfonyl)-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**7*b***)

A mixture of **6b** (4.2 g, 15.72 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (4.79 g, 18.87 mmol), Pd(dppf)$Cl_2CH_2Cl_2$ (0.35g, 0.47 mmol) and KOAc (3.09 g, 31.45 mmol) in 1,2-Dimethoxyethane (50 mL) was stirred at 90℃ 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 of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and was concentrated under reduced pressure. Petroleum ether (30 mL) was added to the crude compound, the white precipitate was filtered and then dried under reduced pressure to give the title compound (3.84g, 77.73% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.14 – 8.02 (m, 2H), 7.48 (t, *J* = 8.7 Hz, 1H), 3.30 (q, *J* = 7.3 Hz, 2H), 1.32 (s, 12H), 1.10 (t, *J* = 7.3 Hz, 3H).

4.1.8. 5-(2-fluoro-5-(methylsulfonyl)phenyl)-7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one (**8*a***)

A mixture of **4** (1 g, 4.39 mmol), **7a** (1.58 g, 5.26 mmol), $Pd(PPh_3)_4$ (0.25g, 0.22 mmol) and K_2CO_3 (1.21 g, 8.77 mmol) in acetonitrile/toluene (30 mL, 1/5) was stirred at 80℃ under nitrogen atmosphere for 24 h. Then the mixture was diluted with CH_2Cl_2, and the insoluble material was filtered through Celite. The organic layer was concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (1.21 g, 85.88% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.24 – 8.12 (m, 2H), 8.06 (dd, *J* = 3.2, 0.6 Hz, 1H), 7.88 (d, *J* = 0.6 Hz, 1H), 7.73 (dd, *J* = 9.7, 8.7 Hz, 1H), 7.26 (s, 1H), 3.42 (s, 3H), 3.31 (s, 3H).

4.1.9. 5-(5-(ethylsulfonyl)-2-fluorophenyl)-7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one (**8*b***)

A mixture of **4** (1 g, 4.39 mmol), **7b** (1.65 g, 5.26 mmol), $Pd(PPh_3)_4$ (0.25g, 0.22 mmol) and K_2CO_3 (1.21 g, 8.77 mmol) in acetonitrile/toluene (30 mL, 1/5) was stirred at 80℃ under nitrogen atmosphere for 24 h. Then the mixture was diluted with CH_2Cl_2, and the insoluble material was filtered through celite. The organic layer was concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (1.4 g, 95.2% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.15 (d, *J* = 5.6 Hz, 2H), 8.06 (d, *J* = 2.9 Hz, 1H), 7.88 (s, 1H), 7.74 (t, *J* = 9.1 Hz, 1H), 7.26 (s, 1H), 3.46 – 3.35 (m, 5H), 1.16 (t, *J* = 7.3 Hz, 3H).

4.1.10. 5-(2-(2,4-difluorophenoxy)-5-(ethylsulfonyl)phenyl)-7-methylimidazo [1,5-a]pyrazin -8 (7*H*)-one (**10*a***)

A mixture of **8b** (0.05 g, 0.15 mmol), 2,4-difluorophenol (0.03 g, 0.22 mmol) and K_2CO_3 (0.04 g, 0.3 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.061 g, 91.85% yield) as a white solid. m.p.: 137-139℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.09 (s, 2H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.84 (s, 1H), 7.53 (d, *J* = 9.9 Hz, 2H), 7.24 (s, 2H), 7.06 (d, *J* = 8.4 Hz, 1H), 3.43 (s, 3H), 3.37 (s, 2H), 1.15 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.47, 154.54, 133.10, 132.84, 132.42, 131.93, 129.44, 124.72, 122.66, 122.47, 119.92, 114.99, 112.88, 112.57, 112.13, 106.40, 106.05, 105.73, 49.36, 34.15, 7.13; ESIMS m/z $[M + H]^+$ 446.2; Anal. calcd. For $C_{21}H_{17}F_2N_3O_4S$: C, 56.63; H, 3.85; N, 9.43. Found: C, 56.67; H, 3.82; N, 9.48.

4.1.11. 5-(2-(2,4-difluorophenoxy)-5-(methylsulfonyl)phenyl)-7-methylimidazo[1,5-a]pyrazin-8

(7*H*)-one (**10*b***)

A mixture of **8a** (0.11 g, 0.34 mmol), 2,4-difluorophenol (0.09 g, 0.68 mmol) and K_2CO_3 (0.09 g, 0.68 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.13 g, 88.02% yield) as a white solid. m.p.: > 200℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.14 (d, *J* = 2.3 Hz, 1H), 8.08 (s, 1H), 8.03 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.84 (s, 1H), 7.60 – 7.49 (m, 2H), 7.22 (d, *J* = 5.9 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 1H), 3.43 (s, 3H), 3.27 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.30, 154.46, 135.42, 132.82, 131.77, 131.08, 129.47, 124.73, 124.61, 122.67, 122.49, 119.80, 114.96, 112.89, 112.57, 112.16, 106.39, 106.10, 43.73, 34.17; ESIMS m/z $[M + H]^+$ 432.2; Anal. calcd. For $C_{20}H_{15}F_2N_3O_4S$: C, 55.68; H, 3.50; N, 9.74. Found: C, 55.73; H, 3.45; N, 9.69.

4.1.12. 5-(5-(ethylsulfonyl)-2-(4-fluorophenoxy)phenyl)-7-methylimidazo[1,5-a]pyrazin-8 (7*H*)-one (**10*c***)

A mixture of **8b** (0.13 g, 0.39 mmol), 4-fluorophenol (0.07 g, 0.58 mmol) and K_2CO_3 (0.11 g, 0.78 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.14 g, 84.49% yield) as a white solid. m.p.: > 200℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.20 (s, 1H), 8.06 (d, *J* = 2.3 Hz, 1H), 7.99 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.83 (d, *J* = 0.6 Hz, 1H), 7.30 (d, *J* = 6.5 Hz, 4H), 7.19 (s, 1H), 7.03 (d, *J* = 8.7 Hz, 1H), 3.43 (s, 3H), 3.37 (q, *J* = 7.4 Hz, 2H), 1.16 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 160.84, 154.97, 150.12, 133.67, 132.98, 132.74, 132.26, 129.88, 123.24, 123.13, 122.85, 120.86, 117.69, 117.38, 116.45, 113.02, 49.89, 34.64, 7.68; ESIMS m/z $[M + H]^+$ 428.2; Anal. calcd. For $C_{21}H_{18}FN_3O_4S$: C, 59.01; H, 4.24; N, 9.83. Found: C, 59.05; H, 4.27; N, 9.79.

4.1.13. 5-(2-(3-((4-chlorobenzyl)amino)phenoxy)-5-(methylsulfonyl)phenyl)-7-methylimidazo [1,5-a]pyrazin-8(7*H*)-one (**10*d***)

A mixture of **8a** (0.09 g, 0.28 mmol), 3-((4-chlorobenzyl)amino)phenol (0.13 g, 0.56 mmol) and K_2CO_3 (0.12 g, 0.84 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.13 g, 86.75% yield) as a white solid. m.p.: 172-174℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.04 (dd, *J* = 22.5, 5.6 Hz, 3H), 7.83 (s, 1H), 7.35 (s, 4H), 7.20 – 6.94 (m, 3H), 6.62 – 6.39 (m, 2H), 6.31 (s, 2H), 4.23 (d, *J* = 4.9 Hz, 2H), 3.41 (s, 3H), 3.25 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 160.06, 154.71, 154.45, 150.46, 138.81, 134.58, 132.94, 131.48, 131.18, 130.73, 130.42, 129.38, 129.05, 128.21, 122.65, 122.22, 120.32, 116.39, 112.67, 109.76, 107.17, 103.74, 45.52, 43.83, 34.12; ESIMS m/z $[M + H]^+$ 535.2; Anal. calcd. For $C_{27}H_{23}ClN_4O_4S$: C, 60.61; H, 4.33; N, 10.47. Found: C, 60.64; H, 4.29; N, 4.37.

4.1.14. N-(4-(((3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methylsulfonyl) phenoxy)phenyl)amino)methyl)phenyl)acetamide (**10*e***)

A mixture of **8a** (0.1 g, 0.31 mmol), N-(4-(((3-hydroxyphenyl)amino)methyl)phenyl)acetamide (0.12 g, 0.47 mmol) and K_2CO_3 (0.09 g, 0.62 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated

under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.14 g, 80.67% yield) as a white solid. m.p.: 188-190℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.89 (s, 1H), 8.07 (s, 2H), 7.99 (d, *J* = 8.9 Hz, 1H), 7.83 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.14 (s, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 6.46 (d, *J* = 8.1 Hz, 2H), 6.29 (d, *J* = 7.8 Hz, 2H), 4.16 (d, *J* = 5.5 Hz, 2H), 3.42 (s, 3H), 3.25 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 168.10, 160.09, 154.65, 154.37, 150.67, 137.97, 134.53, 134.04, 132.92, 131.45, 130.72, 130.33, 129.37, 127.57, 122.66, 122.21, 120.28, 119.02, 116.34, 112.68, 109.78, 106.95, 103.70, 45.99, 43.81, 34.11, 23.89; ESIMS m/z $[M + H]^+$ 558.3; Anal. calcd. For $C_{29}H_{27}N_5O_5S$: C, 62.46; H, 4.88; N, 12.56. Found: C, 62.40; H, 4.85; N, 12.50.

4.1.15. 5-(2-(3-((4-fluorobenzyl)amino)phenoxy)-5-(methylsulfonyl)phenyl)-7-methylimidazo [1,5-a]pyrazin-8(7*H*)-one (**10*f***)

A mixture of **8a** (0.1 g, 0.31 mmol), 3-((4-fluorobenzyl)amino)phenol (0.14 g, 0.62 mmol) and K_2CO_3 (0.13 g, 0.93 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.15 g, 96.05% yield) as a white solid. m.p.: 140-142℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.13 – 7.94 (m, 3H), 7.84 (s, 1H), 7.35 (d, *J* = 5.8 Hz, 2H), 7.19 – 6.96 (m, 5H), 6.57 – 6.39 (m, 2H), 6.31 (d, *J* = 7.7 Hz, 2H), 4.21 (s, 2H), 3.42 (s, 3H), 3.26 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 160.60, 155.20, 151.04, 136.40, 135.05, 133.45, 131.98, 131.24, 130.89, 129.88, 129.69, 129.58, 123.17, 122.72, 120.80, 116.87, 115.61, 115.34, 110.28, 107.61, 104.20, 90.08, 46.02, 44.32, 34.62; ESIMS m/z $[M + H]^+$ 519.3; Anal. calcd. For $C_{27}H_{23}FN_4O_4S$: C, 62.54; H, 4.47; N, 10.80. Found: C, 62.58; H, 4.43; N, 10.85.

4.1.16. N-(4-(((3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl) phenoxy)phenyl)amino)methyl)phenyl)acetamide (**10*g***)

A mixture of **8b** (0.11 g, 0.33 mmol), N-(4-(((3-hydroxyphenyl)amino)methyl)phenyl)acetamide (0.13 g, 0.49 mmol) and K_2CO_3 (0.09 g, 0.66 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.15 g, 80% yield) as a white solid. m.p.: 174-176℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 9.89 (s, 1H), 8.07 (s, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.94 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.82 (s, 1H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.14 (s, 1H), 7.08 (t, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 6.46 (d, *J* = 7.8 Hz, 2H), 6.30 (d, *J* = 6.8 Hz, 2H), 4.16 (d, *J* = 5.8 Hz, 2H), 3.42 (s, 3H), 3.30 (d, *J* = 7.2 Hz, 2H), 2.02 (s, 3H), 1.15 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 168.11, 160.27, 154.60, 154.31, 150.66, 137.97, 134.03, 132.94, 132.14, 131.58, 130.35, 129.35, 127.69, 127.57, 122.66, 122.20, 120.33, 119.02, 116.28, 112.66, 109.81, 106.99, 103.72, 49.46, 45.96, 34.10, 23.89, 7.16; ESIMS m/z $[M + H]^+$ 572.3; Anal. calcd. For $C_{30}H_{29}N_5O_5S$: C, 63.03; H, 5.11; N, 12.25. Found: C, 63.05; H, 5.15; N, 12.21.

4.1.17. 5-(2-(3-((3-methoxybenzyl)amino)phenoxy)-5-(methylsulfonyl)phenyl)-7-methylimidazo [1,5-a]pyrazin-8(7*H*)-one (**10*h***)

A mixture of **8a** (0.1 g, 0.31 mmol), 3-((3-methoxybenzyl)amino)phenol (0.11 g, 0.47 mmol) and K_2CO_3 (0.09 g, 0.62 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash

chromatography to give the title compound (0.13 g, 78.73% yield) as a white solid. m.p.: 122-124℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.03 (dd, *J* = 22.7, 7.5 Hz, 3H), 7.83 (s, 1H), 7.22 (t, *J* = 7.9 Hz, 1H), 7.14 – 6.95 (m, 3H), 6.88 (s, 2H), 6.78 (d, *J* = 7.1 Hz, 1H), 6.46 (d, *J* = 8.1 Hz, 2H), 6.30 (d, *J* = 8.5 Hz, 2H), 4.20 (d, *J* = 5.1 Hz, 2H), 3.71 (s, 3H), 3.41 (s, 3H), 3.25 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 160.59, 155.17, 154.79, 151.19, 141.87, 135.02, 133.42, 131.97, 131.23, 130.87, 129.87, 129.83, 123.09, 122.72, 120.79, 119.89, 116.83, 113.41, 113.05, 112.51, 110.27, 107.53, 104.19, 97.68, 55.41, 46.78, 44.32, 34.62; ESIMS m/z [M + H]$^+$ 531.3; Anal. calcd. For $C_{28}H_{26}N_4O_5S$: C, 63.38; H, 4.94; N, 10.56. Found: C, 63.35; H, 4.91; N, 10.51.

4.1.18. 4-(((3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methylsulfonyl) phenoxy)phenyl)amino)methyl)benzonitrile (**10*i***)

A mixture of **8a** (0.1 g, 0.31 mmol), 4-(((3-hydroxyphenyl)amino)methyl)benzonitrile (0.1 g, 0.47 mmol) and K_2CO_3 (0.09 g, 0.62 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.15 g, 91.71% yield) as a white solid. m.p.: 150-152℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.09 – 7.98 (m, 3H), 7.85 – 7.74 (m, 3H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.10 (dd, *J* = 16.8, 8.4 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 1H), 6.66 (s, 1H), 6.44 (d, *J* = 8.1 Hz, 1H), 6.31 (s, 2H), 4.35 (d, *J* = 5.9 Hz, 2H), 3.42 (s, 3H), 3.26 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 160.53, 155.25, 154.88, 150.80, 146.54, 135.12, 133.44, 132.73, 131.97, 131.23, 131.00, 129.88, 128.51, 123.14, 122.73, 120.83, 119.36, 116.91, 113.15, 110.22, 109.87, 107.88, 104.26, 46.33, 44.32, 34.62; ESIMS m/z [M + H]$^+$ 526.3; Anal. calcd. For $C_{28}H_{23}N_5O_4S$: C, 63.99; H, 4.41; N, 13.33. Found: C, 63.92; H, 4.45; N, 13.37.

4.1.19. 4-(((3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phenoxy)phenyl)amino)methyl)benzonitrile (**10*j***)

A mixture of **8b** (0.1 g, 0.3 mmol), 4-(((3-hydroxyphenyl)amino)methyl)benzonitrile (0.1 g, 0.45mmol) and K_2CO_3 (0.08 g, 0.59 mmol) in DMF (2 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.14 g, 87.01% yield) as a white solid. m.p.: 140-142℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 8.06 (d, *J* = 0.6 Hz, 1H), 8.02 (d, *J* = 2.3 Hz, 1H), 7.96 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.83 (d, *J* = 0.6 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.15 – 6.98 (m, 3H), 6.66 (t, *J* = 6.2 Hz, 1H), 6.44 (d, *J* = 8.0 Hz, 1H), 6.33 (d, *J* = 7.8 Hz, 2H), 4.35 (d, *J* = 6.0 Hz, 2H), 3.41 (s, 3H), 3.30 (d, *J* = 7.3 Hz, 2H), 1.15 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 160.70, 155.19, 154.75, 150.79, 146.53, 133.41, 132.72, 132.65, 132.09, 131.01, 129.86, 128.49, 128.45, 122.72, 120.83, 119.36, 116.84, 113.13, 110.18, 109.94, 109.87, 107.91, 104.27, 49.95, 46.32, 34.60, 7.68; ESIMS m/z [M + H]$^+$ 540.3; Anal. calcd. For $C_{29}H_{25}N_5O_4S$: C, 64.55; H, 4.67; N, 12.98. Found: C, 64.51; H, 4.63; N, 12.94.

4.1.20. 5-(2-(3-aminophenoxy)-5-(methylsulfonyl)phenyl)-7-methylimidazo[1,5-a]pyrazin-8(7*H*)-one (**10*k***)

A mixture of **8a** (0.3 g, 0.93 mmol), 3-aminophenol (0.2 g, 1.87 mmol) and K_2CO_3 (0.39 g, 2.8 mmol) in DMF (3 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (50 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.38 g, 99.16% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.05 (t,

J = 9.6 Hz, 3H), 7.83 (d, *J* = 2.2 Hz, 1H), 7.18 (d, *J* = 2.6 Hz, 1H), 7.05 (dd, *J* = 9.5, 7.0 Hz, 2H), 6.44 (d, *J* = 8.2 Hz, 1H), 6.28 (dd, *J* = 9.7, 6.1 Hz, 2H), 5.36 (s, 2H), 3.43 (d, *J* = 2.5 Hz, 3H), 3.26 (d, *J* = 2.4 Hz, 3H).

4.1.21. 5-(2-(3-aminophenoxy)-5-(ethylsulfonyl)phenyl)-7-methylimidazo[1,5-a]pyrazin-8(7*H*) -one (**10*l***)

A mixture of **8b** (0.4 g, 1.19 mmol), 3-aminophenol (0.2 g, 1.79 mmol) and K_2CO_3 (0.33 g, 2.39 mmol) in DMF (3 mL) was stirred at 80℃ for 12 h. Then the mixture was diluted with H_2O, extracted with EtOAc (50 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.47 g, 92.83% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.19 – 7.91 (m, 3H), 7.83 (s, 1H), 7.18 (s, 1H), 7.05 (t, *J* = 8.4 Hz, 2H), 6.44 (d, *J* = 7.9 Hz, 1H), 6.28 (d, *J* = 12.9 Hz, 2H), 5.35 (s, 2H), 3.43 (s, 3H), 3.33 – 3.23 (m, 2H), 1.15 (t, *J* = 7.3 Hz, 3H).

4.1.22. N-(3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phen oxy)phenyl)-4-methylbenzenesulfonamide (**10*m***)

To a solution of **10l** (0.1 g, 0.24 mmol) and 4-methylbenzenesulfonyl chloride (0.07 g, 0.35 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.13 g, 95.36% yield) as a white solid. m.p.: 156-158℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.40 (s, 1H), 8.01 (dd, *J* = 20.6, 8.8 Hz, 3H), 7.82 (s, 1H), 7.60 (d, *J* = 7.9 Hz, 2H), 7.40 – 7.23 (m, 3H), 7.12 (s, 1H), 6.92 (t, *J* = 8.0 Hz, 2H), 6.87 – 6.74 (m, 2H), 3.40 (s, 5H), 2.34 (s, 3H), 1.16 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.59, 154.44, 154.14, 143.51, 139.70, 136.23, 132.88, 132.33, 131.64, 131.02, 129.76, 129.41, 126.67, 122.62, 122.37, 120.81, 116.52, 115.47, 112.28, 111.24, 49.37, 34.13, 20.93, 7.18; ESIMS m/z $[M + H]^+$ 579.3; Anal. calcd. For $C_{28}H_{26}N_4O_6S_2$: C, 58.12; H, 4.53; N, 9.68. Found: C, 58.16; H, 4.49; N, 9.63.

4.1.23. 4-methyl-N-(3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methyl sulf onyl)phenoxy)phenyl)benzenesulfonamide (**10*n***)

To a solution of **10k** (0.08 g, 0.19 mmol) and 4-methylbenzenesulfonyl chloride (0.05 g, 0.28 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.1 g, 94.41% yield) as a white solid. m.p.: 182-184℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.43 (s, 1H), 8.12 (d, *J* = 2.1 Hz, 1H), 8.03 (d, *J* = 11.1 Hz, 2H), 7.83 (s, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.40 – 7.23 (m, 3H), 7.14 (s, 1H), 6.98 – 6.75 (m, 4H), 3.40 (s, 3H), 3.28 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.42, 154.42, 154.16, 143.51, 139.70, 136.16, 135.24, 132.88, 131.67, 131.02, 130.67, 129.77, 129.32, 126.67, 122.40, 120.73, 116.50, 115.28, 112.31, 111.22, 43.73, 32.53, 20.94; ESIMS m/z $[M + H]^+$ 565.2; Anal. calcd. For $C_{27}H_{24}N_4O_6S_2$: C, 57.44; H, 4.28; N, 9.92. Found: C, 57.41; H, 4.32; N, 9.87.

4.1.24. N-(3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phen oxy)phenyl)-2-fluorobenzenesulfonamide (**10*o***)

To a solution of **10l** (0.1 g, 0.24 mmol) and 2-fluorobenzenesulfonyl chloride (0.07 g, 0.35 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h.

Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.13 g, 94.71% yield) as a white solid. m.p.: 167-169℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.86 (s, 1H), 8.06 (d, *J* = 4.2 Hz, 2H), 7.98 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.86 – 7.76 (m, 2H), 7.71 (d, *J* = 6.0 Hz, 1H), 7.35 (ddd, *J* = 20.9, 17.9, 9.3 Hz, 3H), 7.14 (s, 1H), 6.98 – 6.79 (m, 4H), 3.39 (d, *J* = 9.9 Hz, 5H), 1.17 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.50, 154.40, 154.25, 139.11, 136.07, 136.07, 133.02, 132.84, 132.33, 131.66, 131.04, 130.39, 129.42, 125.03, 122.63, 122.37, 120.91, 117.48, 117.10, 116.81, 116.06, 115.47, 112.24, 110.82, 49.37, 34.11, 7.15; ESIMS m/z $[M + H]^+$ 583.2; Anal. calcd. For $C_{27}H_{23}FN_4O_6S_2$: C, 55.66; H, 3.98; N, 9.62. Found: C, 55.61; H, 3.95; N, 9.58.

4.1.25. N-(3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phen oxy)phenyl)thiophene-2-sulfonamide (**10*p***)

To a solution of **10l** (0.1 g, 0.24 mmol) and thiophene-2-sulfonyl chloride (0.06 g, 0.35 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.12 g, 89.26% yield) as a white solid. m.p.: 172-174℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.62 (s, 1H), 8.07 (d, *J* = 2.7 Hz, 2H), 8.01 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.91 (d, *J* = 5.0 Hz, 1H), 7.83 (s, 1H), 7.53 (d, *J* = 3.8 Hz, 1H), 7.33 (t, *J* = 8.1 Hz, 1H), 7.16 – 7.11 (m, 2H), 7.01 – 6.82 (m, 4H), 3.41 (s, 3H), 3.36 (d, *J* = 7.5 Hz, 2H), 1.16 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.56, 154.44, 154.25, 139.49, 139.39, 133.63, 133.01, 132.88, 132.67, 132.33, 131.69, 131.03, 129.42, 127.66, 126.02, 122.65, 122.39, 120.91, 116.93, 115.84, 112.27, 111.64, 49.38, 34.13, 7.16; ESIMS m/z $[M + H]^+$ 571.2; Anal. calcd. For $C_{25}H_{22}N_4O_6S_3$: C, 52.62; H, 3.89; N, 9.82. Found: C, 52.58; H, 3.86; N, 9.87.

4.1.26. N-(3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phen oxy)phenyl)thiophene-2-sulfonamide (**10*q***)

To a solution of **10l** (0.1 g, 0.24 mmol) and 4-methoxybenzenesulfonyl chloride (0.07 g, 0.35 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.11 g, 78.52% yield) as a white solid. m.p.: 165-167℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.36 (s, 1H), 8.07 (s, 2H), 7.98 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.84 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.15 (s, 1H), 7.06 (d, *J* = 8.9 Hz, 2H), 6.97 – 6.78 (m, 4H), 3.81 (s, 3H), 3.41 (s, 3H), 3.37 (s, 2H), 1.17 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 162.54, 159.60, 154.44, 154.14, 139.84, 132.91, 132.88, 132.33, 131.62, 130.99, 130.63, 129.42, 128.89, 122.64, 122.37, 120.83, 116.63, 116.52, 115.37, 114.44, 112.29, 111.19, 55.63, 49.40, 34.12, 7.16; ESIMS m/z $[M + H]^+$ 595.2; Anal. calcd. For $C_{28}H_{26}N_4O_7S_2$: C, 56.55; H, 4.41; N, 9.42. Found: C, 56.51; H, 4.46; N, 9.46.

4.1.27. 4-methoxy-N-(3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methyl sulfonyl)phenoxy)phenyl)benzenesulfonamide (**10*r***)

To a solution of **10k** (0.1 g, 0.24 mmol) and 4-methoxybenzenesulfonyl chloride (0.08 g, 0.37 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was

purified by flash chromatography to give the title compound (0.13 g, 91.9% yield) as a white solid. m.p.: 170-172℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.36 (s, 1H), 8.12 (d, *J* = 2.4 Hz, 1H), 8.06 (s, 1H), 8.01 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.83 (s, 1H), 7.65 (d, *J* = 8.9 Hz, 2H), 7.27 (t, *J* = 8.1 Hz, 1H), 7.14 (s, 1H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.85 (ddd, *J* = 16.1, 11.9, 5.1 Hz, 4H), 3.80 (s, 3H), 3.40 (s, 3H), 3.27 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 162.54, 159.42, 154.42, 154.16, 139.83, 135.27, 132.86, 131.68, 130.99, 130.75, 130.61, 129.45, 128.90, 122.63, 122.40, 120.75, 116.64, 116.49, 115.35, 114.44, 112.32, 111.16, 55.63, 43.74, 34.14; ESIMS m/z $[M + H]^+$ 581.2; Anal. calcd. For $C_{27}H_{24}N_4O_7S_2$: C, 55.85; H, 4.17; N, 9.65. Found: C, 55.81; H, 4.21; N, 9.61.

4.1.27. 3-methyl-N-(3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methyl sulfonyl)phenoxy)phenyl)benzenesulfonamide (**10*s***)

To a solution of **10k** (0.1 g, 0.24 mmol) and 3-methylbenzenesulfonyl chloride (0.07 g, 0.37 mmol) in CH_2Cl_2 (2 mL) was added pyridine (1 mL). The reaction mixture was stirred at rt for 2 h. Then the mixture was diluted with H_2O, extracted with EtOAc (30 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by flash chromatography to give the title compound (0.13 g, 94.5% yield) as a white solid. m.p.: 156-158℃; ^{1}H NMR (300 MHz, DMSO-d6) δ 10.48 (s, 1H), 8.13 (d, *J* = 2.3 Hz, 1H), 8.08 – 7.99 (m, 2H), 7.83 (s, 1H), 7.57 (s, 1H), 7.51 (d, *J* = 3.7 Hz, 1H), 7.44 (d, *J* = 5.4 Hz, 2H), 7.29 (t, *J* = 8.1 Hz, 1H), 7.16 (s, 1H), 6.88 (ddd, *J* = 20.8, 9.6, 5.0 Hz, 4H), 3.41 (s, 3H), 3.28 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d6) δ 159.62, 154.62, 154.16, 139.64, 139.08, 138.97, 135.25, 133.74, 132.87, 131.67, 131.56, 131.04, 130.68, 129.43, 129.17, 126.78, 123.79, 122.66, 122.40, 120.81, 116.55, 115.48, 112.31, 111.20, 43.72, 34.15, 20.76; ESIMS m/z $[M + H]^+$ 565.2; Anal. calcd. For $C_{27}H_{24}N_4O_6S_2$: C, 57.44; H, 4.28; N, 9.92. Found: C, 57.40; H, 4.25; N, 9.96.

4.2. Docking studies

All ligand molecules were drawn in ChemDraw 2014, and saved as sdf style. Then ligands were processed at a simulated pH of 7.4 ± 1.0 to generate all possible tautomers, stereoisomers, and protonation states and were finally minimized at the OPLS 2005 force field with Ligand preparation protocol of Maestro 10.2. The crystal complex (PDB id: 3P5O) was selected as the BRD4(1) docking protein. The protein was opened with Glide generation protocol of Maestro 10.2, molecules of water around the binding site were saved, and the bond orders were assigned. Hydrogen atoms were added. Then a 10-Å box centered on the geometrical center of the ligand binding site was generated for grid calculation. The docking studies were processed with the Glide docking protocol. After docking finished, only one docking conformation was saved for every compound.

4.3. Biological evaluation

4.3.1. Binding affinities of target compounds to BRD4(1) and BRD4(2) by AlphaScreen assay.

We commissioned Shanghai ChemPartner Co. to carry out the experiments. BRD4(1) (Active Motif, 44-168aa, Cat. No. 31380), BRD4(2) (Active Motif, 333-460aa, Cat. No. 31446), (+)-JQ1 (BPS, Cat. No. 27402), ABBV-075 (Lab preparation). Prepare 1x assay buffer (modified HEPES Buffer). Transfer compounds to assay plates by Echo. DMSO's final concentration is 0.1%. Prepare protein solution in 1x assay buffer (5 nM). Add peptide (H4) in 1x assay buffer to make the substrate solution. Transfer 5 μL of protein solution to assay plates or for low control transfer 5 μL of 1x assay buffer. Incubate at room temperature for 15 minutes. Add 5 μL of substrate solution

to each well to start reaction. Incubate at room temperature for 60 min. Add 15 μL acceptor and donor solution, incubate for 60 min at room temperature, subdued light. Read endpoint with EnSpire with Alpha mode. Fit the data in Excel to obtain inhibition values using equation (1). Equation (1): Inh % = (Max - Signal) / (Max - Min) × 100.Max signal was obtained from the action of Enzyme and Substrate. Min signal was obtained from the Substrate only. Fit the data in GraphPad Prism 5 statistical software to obtain 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 culture and proliferation inhibition assays.

HL-60 , MV4-11 or K562 cells were seeded in 96-well plates at a concentration of 1×10^4 cells per well. Cells were grown in 100 μL of IMDM containing 20% fetal bovine serum. After 12 h, 50 μL of which containing various concentrations of compounds (triple diluted) was added. The measurement was conducted 72 h after seeding, and 10 μL of Cell-counting kit-8(CCK-8) reagent was added to each well and incubated in 37 °C for 4 h. The spectrophotometric absorbance of each well was measured by a multi-detection microplate reader at a wavelength of 450 nm. The inhibition rate was calculated as ((A450 treated - A450 blank) / (A450 control - A450 blank)) × 100. The IC_{50} was calculated by GraphPad Prism 5 statistical software.

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