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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Development of 7-methylimidazo[1,5-a]pyrazin-8(7H)-one derivatives as a novel chemical series of BRD4 inhibitors

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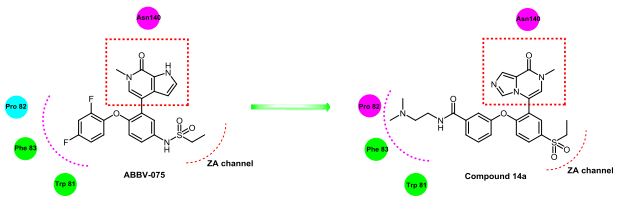
Abstract

Bromodomain protein 4 (BRD4) is a member of the bromodomain and extra-terminal domain (BET) protein family. It binds to acetylated histones that regulate gene transcription preferentially at super-enhancer regions. Now BRD4 has been involved into several types of cancers as a candidate correlated with gene transcription. In this study, we designed and synthesized 11 novel 7-methylimidazo[1,5-a]pyrazin-8(7H)-one derivatives and evaluated their BRD4 inhibitory activities. Most of these compounds exhibited moderate BRD4 inhibitory activities in vitro. It is worth noting that compound **14a** showed excellent BRD4(1) and BRD4(2) inhibitory activity with IC_{50} values of 350 and 290 nm, respectively. Meanwhile, remarkable anti-proliferative activities toward BRD4-sensitive cancer lines MV4-11 and HL-60 were also observed. In addition, molecular docking studies was utilized to elaborate the key interactions between compound **14a** and BRD4 in detail. Overall, 7-methylimidazo[1,5-a]pyrazin-8(7H)-one could be employed as a useful scaffold toward more potent BRD4 inhibitors for cancer treatments.

Graphical Abstract



Keywords Gene transcription · BRD4 · Anticancer · Molecular docking

Introduction

Histone acetylation is a significant component of epigenetic research which is very important for post-translational modification (PTM) (Zhao et al. 2010). Bromodomains (BRDs) are present in a variety of epigenetic regulatory proteins, including transcriptional activators, histone acetyltransferase (HAT), and chromatin (Filippakopoulos et al. 2012). The essence of BRDs is disclosed more and more thoroughly with the development of BRDs. Although the proteins of BRDs play a vital function in the treatment of diseases, but most thorough research at present is the BRD and extra-terminal (BET) protein. Bromodomain protein 4 (BRD4) is a member of the BET protein family (Shu and

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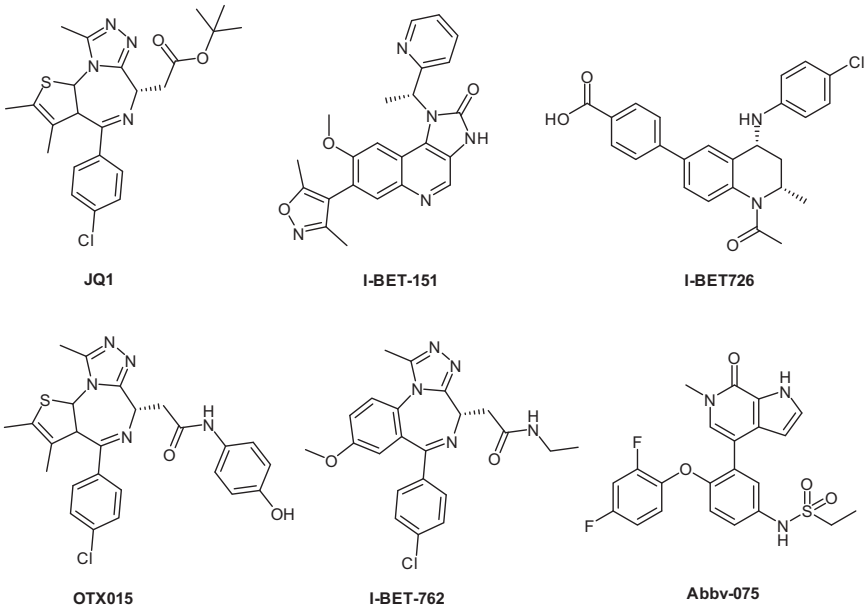


Fig. 1 Structures of unclosed BRD4 inhibitors

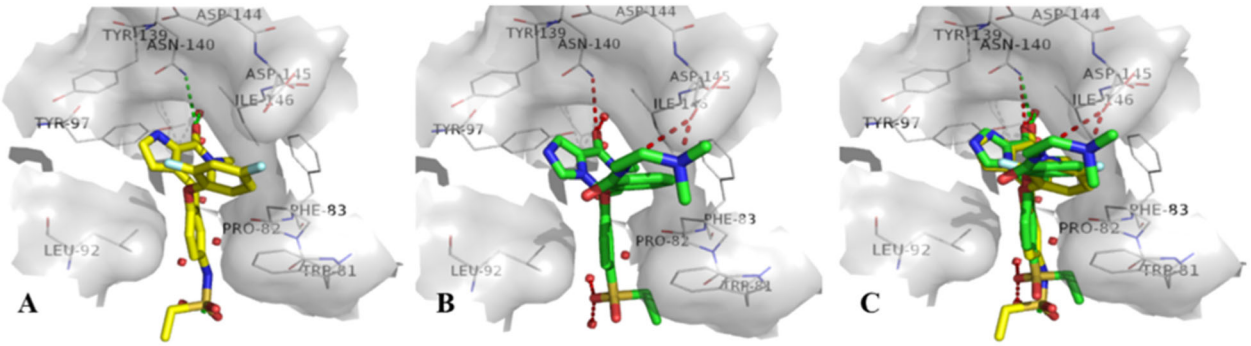
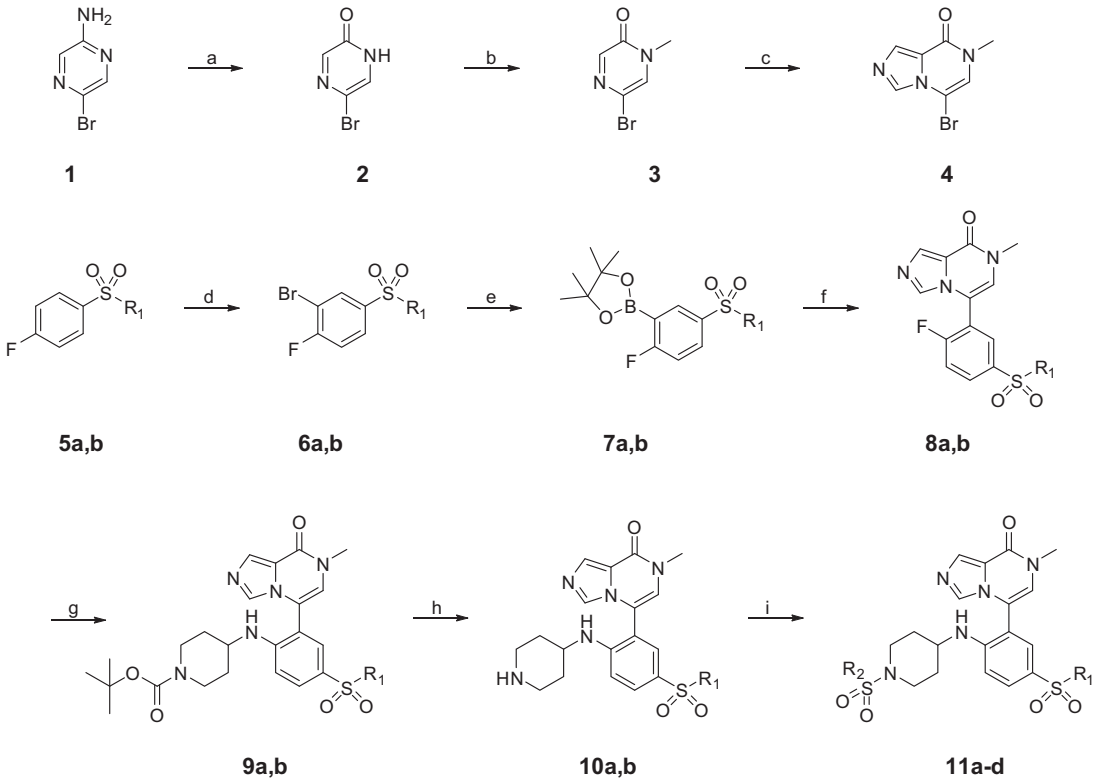


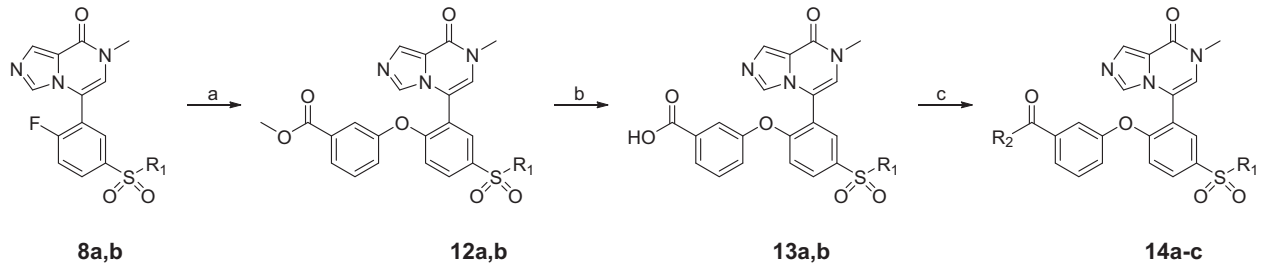
Fig. 2 **a** Docking conformation of ABBV-075 in BRD4(1) (PDB id: 3P5O). **b** Docking conformation of **14a** in BRD4(1) (PDB id: 3P5O). **c** Superimposition docking conformation of ABBV-075 (Yellow) and **14a** (Green) in BRD4(1) (PDB id: 3P5O)

Polyak 2017). The BET family of proteins plays a crucial role in regulating transcriptional programmes (Amorim et al. 2016; Lin et al. 2016). BET can recruit the positive transcription elongation factor complex (pTEFb), which is crucial for transcription elongation via their bromodomains bounding to acetylated histones (Jang et al. 2005; Mujtaba et al. 2007; Yang et al. 2005). Meanwhile, gene expression studies unclosed that BET could moderate key oncogenes important for cell cycle progression, such as MYC and E2F1 (Chapuy et al. 2013; Core et al. 2014, 2008; Dawson et al. 2011; Zhao et al. 2016). Above all, BRD4 bromodomain protein has developed to be an interesting drug target for the treatment of NUT midline carcinoma (NMC), acute myeloid leukemia (AML), multiple myeloma (MM), acute lymphoblastic leukemia (ALL), small cell lung carcinoma (SCLC), breast cancer, neuroblastoma (NB), and prostate cancer (Asangani et al. 2014; Ceribelli et al. 2014; Dawson et al. 2011; Delmore et al. 2011; Lockwood et al. 2012; Mertz et al. 2011; Zuber et al. 2011).

The main body of research in the bromodomain field has focused on the BET (bromodomain and extra terminal) family and multiple small molecule inhibitors have been disclosed, such as JQ1, I-BET 151, I-BET 726, etc. (Fig. 1). JQ1 was the first unclosed BRD4 inhibitor with IC_{50} value as tool compound for a functional role on gene transcription (Filippakopoulos et al. 2010). However, several BRD4 inhibitors have entered into clinical trials for treating different types of cancers, such as OTX015, GSK762, ABBV-075, etc. (Fig. 1) (Faivre et al. 2017; Stathis et al. 2016). ABBV-075 displayed potent antiproliferative activity in multiple models of resistance to second-generation antiandrogens (Faivre et al. 2017). However, novel chemical scaffold for BRD4 inhibitors with excellent ADMET properties will be necessary for further clinical tests. Therefore, we designed and synthesized eleven 7-methylimidazo[1,5-a]pyrazin-8(7H)-one derivatives via structural modifications of ABBV-075. Molecular docking studies were utilized to analyze the interaction with Kac pocket of



Scheme 1 General synthesis of **11a–d**. Reagents and conditions: (**a**) $NaNO_2$, conc. H_2SO_4, 0 °C to 45 °C; (**b**) dimethylsulfate, K_2CO_3, acetonitrile, 70 °C; (**c**) tosylmethyl isocyanide, NaH, THF, 0 °C to rt; (**d**) NBS, conc. H_2SO_4, 50 °C; (**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-Dimethoxye thane, 90 °C; (**f**) **4**, $Pd(PPh_3)_4$, K_2CO_3, acetonitrile, toluene, 80 °C; (**g**) 4-amino-1-Boc-piperidine, DIPEA, DMSO, 110 °C; (**h**) TFA, CH_2Cl_2, 0 °C to rt; (**i**) sulfonyl chloride, 4-methylmorpholine, DMF, rt

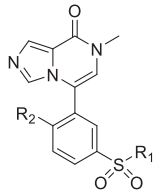


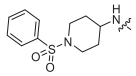
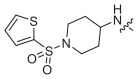
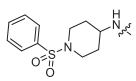
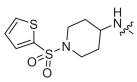
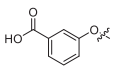
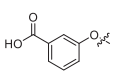
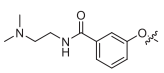
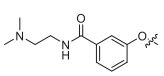
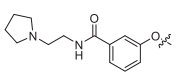
Scheme 2 General synthesis of **14a–c**. Reagents and conditions: (**a**) methyl 3-hydroxybenzoate, K_2CO_3, DMF, 85 °C; (**b**) LiOH, methanol, H_2O, rt; (**c**) amine, HBTU, DIPEA, DMF, rt

BRD4(1). Carbonyl group of 7-methylimidazo[1,5-a]pyrazin-8(7H)-one made a key hydrogen bond with Asn140 at the bottom of Kac pocket (Fig. 2). Therefore, our derivatives could be as acetylated lysine mimic occupying the pocket region.

We further assessed the inhibitory potency of our derivatives against BRD4 in vitro. Initial inhibitory activities of these compounds against BRD4(1) were estimated at a concentration of 10 μM. Most compounds exhibited moderated BRD4(1) inhibitory activities. The compounds **11d** and **14a–c** with relatively higher potency were chosen for further evaluation of inhibitory activity towards BRD4(1) and BRD4(2) and antiproliferative activities. We found that compound showed excellent BRD4(1) and BRD4(2) inhibitory activity with IC_{50} values of 350 and 290 nm, respectively. Meanwhile, compound **14a** exhibited robust antiproliferative activity in BRD4-sensitive cancer cells MV4-11 and HL-60 with IC_{50} values of 460 and 680 nm respectively. In addition, compound presented few antiproliferative activity in BRD4-independent lines K562. These findings demonstrated that compound could be a possible BRD4 inhibitor for cancer treatments.

Table 1 Structures and BRD4(1) inhibitory activity of compounds **11a-d** and **14a-c**



Compound	R_1	R_2	BRD4(1) (10μM)	BRD4(1) IC_{50} (μmol/L)	BRD4(1) IC_{50} (μmol/L)
10a	CH_3		42%	n.d[b].	n.d.
10b	CH_3CH_2		51%	n.d.	n.d.
11a	CH_3		43%	n.d.	n.d.
11b	CH_3		59%	n.d.	n.d.
11c	CH_3CH_2		67%	n.d.	n.d.
11d	CH_3CH_2		98%	0.897±0.025	0.932±0.021
13a	CH_3		67%	n.d.	n.d.
13b	CH_3CH_2		78%	n.d.	n.d.
14a	CH_3CH_2		100%	0.350±0.012	0.290±0.013
14b	CH_3		99%	0.435±0.012	0.490±0.012
14c	CH_3		100%	0.390±0.012	0.425±0.012
(+)-JQ1[c]			100%	0.103±0.022	0.098±0.013
ABBV-075[c]			100%	0.093±0.034	0.083±0.021

[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.

[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.

Results and discussion

Chemistry

The general schemes for synthesis of 7-methylimidazo[1,5-a]pyrazin-8(7H)-one derivatives **11a–d** and **14a–c** are summarized in Schemes 1 and 2. ^{1}H NMR and ^{13}C NMR spectrum, mass spectrometry were executed to verify the structures. All the derivatives were prepared by column chromatography over silica gel (200–300 mesh). The purity was further evaluated by HPLC before being tested in biological evaluation (purity was >98%).

As shown in Scheme 1, the commercial material 5-bromopyrazin-2-amine (**1**) reacted with sodium nitrite in concentrated sulfuric acid to obtain 5-bromopyrazin-2(1H)-one (**2**). Then the intermediate **2** was further converted into 5-Bromo-1-methylpyrazin-2(1H)-one (**3**) by methylation. Cyclization of intermediate **3** with tosylmethyl isocyanide prepared 5-Bromo-7-methylimi dazo [1,5-a]pyrazin-8(7H)-one (**4**). The pinacol arylboronates (**7a, b**) were obtained by bromination of compounds (**5a**, R1 = Me; **5b**, R1 = Et) in concentrated sulfuric acid, followed by coupling reaction. Then intermediate **4** reacted with intermediates (**7a, b**) by Suzuki coupling reaction to give the key intermediates (**8a, b**). The intermediates (**8a, b**) were further treated with 4-amino-1-Boc-piperidine, followed by deprotection of Boc group to get compounds (**10a, b**). In addition, compounds (**10a, b**) were condensed with different sulfonyl chlorides to prepare the target compounds (**11a–d**).

As depicted in Scheme 2, the compounds (**13a, b**) were synthesized by condensation of intermediates (**8a, b**) and methyl 3-hydroxybenzoate, followed by hydrolysis of the methyl esters. Condensation of compounds (**13a, b**) with different amines in the presence of HBTU afforded target compounds (**14a–c**).

BRD4 inhibitory activity and SAR study

AlphaScreen assay was performed to evaluate the inhibitory activities of our target compounds (**10a–b**, **11a–d**, **13a–b**, **14a–c**) against BRD4. Firstly, we assessed the inhibition rates of our targets against BRD4(1) at a concentration of 10 μM (Table 1). (+)-JQ1 and ABBV-075 were used as positive control. Most compounds exhibited moderate inhibitory activities against BRD4(1). Gratifyingly, compound **11d** and **14a–c** showed robust inhibitory potency, and their IC_{50} values against BRD4(1) and BRD4(2) were further estimated (Table 1). All compounds exhibited excellent inhibitory activities against BRD4(1) and BRD4(2), especially compound **14a** presented BRD4(1) and BRD4(2) inhibitory activity with IC_{50} values of 350 and 290 nm, respectively. These results indicated that aromatic moiety at R2 region afforded stronger potency than piperidine group. Meanwhile, the inhibition potency also demonstrated that ethyl was the optimal substituent at R2 region. To analyze the structure–activity relationship, compound **14a** was docked into BRD4(1) protein (PDB id: 3P5O) and overlapped with ABBV-075 via Glide docking protocol with root mean square deviation (RMSD) value of 0.83 in Maestro 10.2 (Fig. 2).

As shown in Fig. 2, we found that the core 7-methylimidazo[1,5-a]pyrazin-8(7H)-one could overlap with 6-methyl-1,6-dihydro-7H-pyrrolo[2,3-c]pyridin-7-one of ABBV-075, acted as a KAc mimic. The ketone group of 7-methylimidazo[1,5-a]pyrazin-8(7H)-one could interact with Asn140 by a hydrogen bond, which was vital for inhibitory potency. In addition, methyl group occupied the hydrophobic pocket adjacent to Kac region. Compound **11a** with a piperidine group at R2 position exhibited a markedly reduced inhibitory activity. Therefore, a hydrophobic group was vital for increasing the lipophilic effects with the hydrophobic WPF shelf, but the introduction of piperidine group showed unfavorable steric to decrease the binding efficiency. Based on above results, we further evaluated anti-proliferation activities of our target compounds in vitro.

In vitro anticancer evaluation against cancer cell lines

Anti-proliferation activities of compounds **11d** and **14a–c** with stronger inhibitory potency against BRD4 were further evaluated in K564, MV4-11, and HL-60 cells (Table 2). We found that all compounds showed reasonable anti-proliferation activities in BRD4-sensitive cells, MV4-11, and HL-60 cells, while these exhibited few inhibitory effects in BRD4-independent cell, K564 line. Gratifyingly, compound **14a** presented excellent anti-proliferation activities in BRD4-sensitive cells, MV4-11 and HL-60 cells with IC_{50} of 460 and 680 nm, respectively. Altogether, these results demonstrated that compound **14a** could effectively

Table 2 Anticancer evaluation against cancer cell lines K652, HL-60, and MV4-11

Compound	K562 IC_{50} (μM)[a]	HL-60 IC_{50} (μM)[a]	MV4-11 IC_{50} (μM)[a]
11d	>10	1.864 ± 0.152	1.243 ± 0.121
14a	>10	0.680 ± 0.143	0.460 ± 0.114
14b	>10	2.862 ± 0.173	1.854 ± 0.187
14c	>10	1.944 ± 0.415	0.785 ± 0.013
(+)-JQ1[b]	9.12 ± 0.08	0.158 ± 0.037	0.096 ± 0.021
ABBV-075[b]	>10 μM	0.142 ± 0.022	0.088 ± 0.034

[a]The data were expressed as the means ± SD, representing the relative levels of anti-proliferation from three independent experiments.

[b]Used as positive control.

inhibit BRD4 and induce apoptosis in BRD4-sensitive cells, MV4-11 and HL-60 cells.

Conclusion

In this study, we report our structural modification chemical synthesis and evaluation inhibitory potency of BRD4 in vitro. Eleven novel 7-methylimidazo[1,5-a]pyrazin-8 (7H)-one derivatives were synthesized. Meanwhile, the inhibitory potency of BRD4 and anti-proliferation activity were assessed in protein and cell level, respectively. Gratifyingly, compound **14a** exhibited robust BRD4(1) and BRD4(2) inhibitory effect with IC_{50} of 350 and 290 nm, respectively. In addition, compound **14a** has remarkable anti-proliferative activities with IC_{50} of 460 and 680 nm in BRD4-sensitive cells, MV4-11 and HL-60 cells, respectively. Thus, further research aiming at the potential mechanisms behind the inhibitory of bromodomian is requisite when it comes to developing novel BRD4 inhibitors for cancer treatment.

Experimental section

Chemistry

All chemical reagents were commercially available and used without further purification.

^{1}H spectra (DMSO-d_6) were measured on Bruker AV-300 spectrometer at 25 °C and referenced to TMS. Purity of the target compounds were confirmed 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 stated. High-resolution mass spectra were recorded using an Agilent QTOF 6520 (Beijing, China).

5-bromopyrazin-2(1H)-one (2)

Yellow solid (to a solution of $NaNO_2$ (1.78 g, 25.86 mmol) in conc., then H_2SO_4 (15 mL) was added a solution of **1** (3.0 g, 17.24 mmol) in conc. The reaction mixture was stirred at 45 °C for 2 h. After the reaction completed, the mixture was diluted with water (200 mL) and extracted with EtOAc (300 mL). The organic layer was dried over Na_2SO_4 and concentrated to give Yellow solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 12.24$ (s, 1H, –NH–C=O), 8.10 (s, 1H, Ar–H), 7.92 (s, 1H, Ar–H).

5-bromo-1-methylpyrazin-2(1H)-one (3)

Yellow solid (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), then stirred at 70 °C for 6 h. After the reaction completed, the mixture was filtered by a celite pad, and the filtrate was obtained.); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.97$ (s, 1H, Ar–H), 7.32 (s, 1H, Ar-H), 3.52 (s, 3H, –CH_3).

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

Yellow solid (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 °C. After the reaction completed, the mixture was concentrated under reduced pressure to get yellow solid without further purification.); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.08$ (d, $J =$ 3.3 Hz, 2H, Ar–H), 6.62 (s, 1H, Ar–H), 3.48 (s, 3H, –CH_3).

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

White solid (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 °C for 4 h. After the reaction completed, the mixture was diluted with ice water (100 mL) and the white precipitate was filtered and washed with water. Then dried the solid was under reduced pressure to get a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.27$ (dd, $J = 6.3$, 2.2 Hz, 1H, Ar–H), 8.00 (ddd, $J = 8.5$, 4.5, 2.3 Hz, 1H, Ar–H), 7.67 (t, $J = 8.7$ Hz, 1H, Ar–H), 3.30 (s, 3H, –CH_3).

2-bromo-4-(ethylsulfonyl)-1-fluorobenzene (6b)

White solid (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 °C for 4 h. After the reaction completed, the mixture was diluted with ice water (100 mL) and the white precipitate was filtered and washed with water. Then the solid was dried under reduced pressure to get a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.22$ (dd, $J =$ 6.4, 2.2 Hz, 1H, Ar–H), 7.96 (ddd, $J = 8.6$, 4.6, 2.3 Hz, 1H, Ar–H), 7.68 (t, $J = 8.6$ Hz, 1H, Ar–H), 3.40 (q, $J = 7.4$ Hz, 2H, –CH_2–), 1.10 (t, $J = 7.4$ Hz, 3H, –CH_3).

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

White solid (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 °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 of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and was concentrated under reduced pressure to get a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.19–8.05$ (m, 2H, Ar–H), 7.52–7.39 (m, 1H, Ar–H), 3.23 (d, $J = 3.8$ Hz, 3H, –CH_3), 1.32 (s, 12H, –$C(CH_3)_2$–$C(CH_3)_2$–).

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

White solid (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)$Cl_2CH_2Cl_2$ (0.35 g, 0.47 mmol) and KOAc (3.09 g, 31.45 mmol) in 1,2-dimethoxyethane (50 mL) was stirred at 90 °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 of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and was concentrated under reduced pressure to get a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.14 – 8.02$ (m, 2H, Ar–H), 7.48 (t, $J = 8.7$ Hz, 1H, Ar–H), 3.30 (d, $J =$ 7.3 Hz, 2H, –CH_2–), 1.32 (s, 12H, –$C(CH_3)_2$–$C(CH_3)_2$–), 1.10 (t, $J = 7.3$ Hz, 3H, –CH_3).

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

White solid (A mixture of **4** (1 g, 4.39 mmol), **7a** (1.58 g, 5.26 mmol), $Pd(PPh_3)_4$ (0.25 g, 0.22 mmol), and K_2CO_3 (1.21 g, 8.77 mmol) in acetonitrile/toluene (30 mL, 1/5) was stirred at 80 °C under nitrogen atmosphere for 24 h. Then the mixture was diluted with CH_2Cl_2 and then was concentrated under reduced pressure to get a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.24–8.12$ (m, 2H, Ar–H), 8.06 (dd, $J = 3.2$, 0.6 Hz, 1H, Ar–H), 7.88 (d, $J =$ 0.6 Hz, 1H, Ar–H), 7.73 (dd, $J = 9.7$, 8.7 Hz, 1H, Ar–H), 7.26 (s, 1H, Ar–H), 3.42 (s, 3H, –CH_3), 3.31 (s, 3H, –CH_3).

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

White solid (This compound was prepared by **7b**.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.15$ (d, $J = 5.6$ Hz, 2H, Ar–H), 8.06 (d, $J = 2.9$ Hz, 1H, Ar–H), 7.88 (s, 1H, Ar–H), 7.74 (t, $J = 9.1$ Hz, 1H, Ar–H), 7.26 (s, 1H, Ar–H), 3.46–3.35 (m, 5H, –CH_2–, –CH_3), 1.16 (t, $J = 7.3$ Hz, 3H, –CH_3).

Tert-butyl 4-((2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methylsulfony l)phenyl)amino)piperidine-1-carboxylate (9a)

White solid (A mixture of **8a** (0.5 g, 1.56 mmol.), 4-amino-1-Boc-piperidine (0.78 g, 3.89 mmol) and DIPEA (0.6 g, 4.67 mmol) in DMSO (5 mL) was stirred at 110 °C for 24 h. Then the mixture was poured into water (50 mL) and the white precipitate was filtered and washed with water. The solid was then dried under reduced pressure to get a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.83–7.73$ (m, 2H, Ar–H), 7.68 (d, $J = 2.4$ Hz, 1H, Ar–H), 7.54 (s, 1H, Ar–H), 7.08 – 6.97 (m, 2H, Ar–H), 5.88 (d, $J = 7.9$ Hz, 1H, –NH–), 3.87 (s, 2H, –CH_2–), 3.68 (s, 1H, –CH–), 3.40 (d, J = 2.4 Hz, 3H, –CH_3), 3.12 (d, $J = 2.4$ Hz, 3H, –CH_3), 2.81 (s, 2H, –CH_2–), 1.75 (s, 2H, –CH_2–), 1.36 (d, $J = 2.4$ Hz, 9H, –$C(CH_3)_3$), 1.25–1.13 (m, 2H, –CH_2–).

Tert-butyl tert-butyl 4-((4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihy droimi dazo[1,5-a]py razin-5-yl)phenyl)amino) piperidine-1 -carboxylate (9b)

White solid (A mixture of **8b** (0.5 g, 1.49 mmol), 4-amino-1-Boc-piperidine (0.75 g, 3.73 mmol) and DIPEA (0.58 g, 4.47 mmol) in DMSO (5 mL) was stirred at 110 °C for 24 h. Then the mixture was poured into water (50 mL) and the white precipitate was filtered and washed with water. The solid was then dried under reduced pressure to get a white solid.)

7-methyl-5-(5-(methylsulfonyl)-2-(piperidin-4-ylamino) phenyl)imidazo [1,5-a]pyrazin-8(7H)-one (10a)

White solid (This compound was prepared by **9a**); m.p. > 200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.84–7.70$ (m, 2H, Ar–H), 7.66 (d, $J = 2.4$ Hz, 1H, Ar–H), 7.52 (s, 1H, Ar–H), 7.00 (dd, $J = 15.8$, 5.9 Hz, 2H, Ar–H), 5.90 (d, $J =$ 7.6 Hz, 1H,–NH–), 3.40 (d, $J = 2.9$ Hz, 6H, –CH_3, –CH_3), 3.11 (d, $J = 2.9$ Hz, 3H, –CH_2–, –NH–), 2.88 (d, $J =$ 11.3 Hz, 2H, –CH_2–), 1.71 (s, 2H, –CH_2–), 1.21 (d, $J =$ 12.6 Hz, 2H, –CH_2–). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$ 155.1 (CH, C-6′), 149.7 (C=O, C-6), 135.3 (CH, C-2), 133.1 (CH,C-4′), 131.5 (CH, C-4), 128.6 (C, C-5′), 128.3 (CH, C-8), 127.3 (C, C-5), 126.5 (CH, C-6′), 123.7 (C, C-1′), 115.7 (C, C-9), 110.3 (CH, C-3′), 62.4 (CH_2, Ar-$\underline{C}H_2$-N), 50.1 (CH_2, –N–CH_2–$\underline{C}H_2$–), 45.1(CH_2, –N–$\underline{C}H_2$–CH_2–), 44.2 (CH_3, –S(O_2)–$\underline{C}H_3$), 36.0 (CH_3, N-CH_3). MS (ESI, *m/z*): 402.3 $[M+H]^+$. Anal. calcd. for $C_{25}H_{27}N_5O_5S_2$: C, 56.84; H, 5.77; N, 17.44. Found: C, 56.81; H, 5.74; N, 17.40.

5-(5-(ethylsulfonyl)-2-(piperidin-4-ylamino)phenyl)-7-methylimidazo[1,5-a]pyrazin-8(7H)-one (10b)

White solid (This compound was prepared by **9b**); m.p. > 200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.84–7.70$ (m, 2H, Ar–H), 7.54 (d, $J = 2.5$ Hz, 1H, Ar–H), 7.48 (s, 1H, Ar–H), 6.88 (dd, $J = 13.9$, 5.2 Hz, 2H, Ar–H), 5.78 (d, $J = 7.1$ Hz, 1H, –NH–), 3.25 (d, $J = 1.9$ Hz, 6H, $–CH_3$, $–CH_3$), 3.28 (q, $J = 6.2$ Hz, 2H, $–CH_2–$), 2.54 (d, $J = 9.2$ Hz, 2H, $–CH_2–$), 1.65 (s, 2H, $–CH_2–$), 1.19 (d, $J = 11.3$ Hz, 2H, $–CH_2–$), 1.13 (t, $J = 5.3$ Hz, 3H, $–CH_2–$). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 154.9$ (CH, C-6′), 149.8 (C=O, C-6), 135.3 (CH, C-2), 133.3 (CH,C-4′), 131.4 (CH, C-4), 128.7 (C, C-5′), 128.5 (CH, C-8), 127.7 (C, C-5), 126.6 (CH, C-6′), 123.7 (C, C-1′), 115.8 (C, C-9), 110.3 (CH, C-3′), 62.4 (CH_2, Ar–$\underline{C}H_2$–N), 48.3 (CH_2, –$\underline{C}H_2$–CH_3), 45.1 (CH_2, –N–CH_2–$\underline{C}H_2$–), 40.5(CH_2, –N–$\underline{C}H_2$–CH_2–), 36.7 (CH_3, N–CH_3), 10.4 (CH_3, $–CH_2–\underline{C}H_3$). MS (ESI, *m/z*): 416.3 $[M+H]^+$. Anal. calcd. for $C_{25}H_{27}N_5O_5S_2$: C, 57.81; H, 6.06; N, 16.86. Found: C, 57.82; H, 5.98; N, 16.80.

7-methyl-5-(5-(methylsulfonyl)-2-((1-(phenylsulfonyl) piperidin-4yl)amino) phenyl)imida zo[1,5-a]pyrazin-8(7H)-one (11a)

White solid (This compound was prepared by **10a**); m.p. > 200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.79–7.63$ (m, 8H, Ar–H), 7.51 (s, 1H, Ar–H), 7.03–6.95 (m, 2H, Ar–H), 5.91 (d, $J = 8.5$ Hz, 1H, –NH–), 3.58 (s, 2H, $–CH_2–$), 3.55–3.45 (m, 1H, –CH–), 3.39 (s, 3H, $–CH_3$), 3.10 (s, 3H, $–CH_3$), 2.34 (d, $J = 6.4$ Hz, 2H, $–CH_2–$), 1.81 (d, $J = 12.2$ Hz, 2H, $–CH_2–$), 1.39 (s, 2H, $–CH_2–$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 155.0$ (C=O, C-6), 149.7 (CH, C-2′), 135.3 (C, –N–SO_2–C), 133.1 (CH, C-2), 131.5 (CH, Ar–C), 130.2 (CH, C-4′), 129.3 (CH, C-4), 128.9 (CH, Ar–C), 127.3 (CH, Ar–C), 126.2 (C, C-5), 123.3 (CH, C-8), 122.1 (CH, C-6′), 120.4 (C, C-5′), 117.2, (C, C-1′), 113.6 (C, C-9), 110.6 (CH, C-3′), 48.1 (CH, –NH–$\underline{C}$H–), 45.1 (CH_3, $–SO_2–CH_3$), 44.2 (CH_2, –NH–$\underline{C}H_2$– CH_2–), 34.0 (CH_3, N-CH_3), 30.5(CH_2, –NH–CH_2– $\underline{C}H_2$–). MS (ESI, *m/z*): 542.3 $[M+H]^+$. Anal. calcd. for $C_{25}H_{27}N_5O_5S_2$: C, 55.44; H, 5.02; N, 12.93. Found: C, 55.41; H, 5.07; N, 12.90.

7-methyl-5-(5-(methylsulfonyl)-2-((1-(thiophen-2-ylsulfonyl) piperidin-4-yl)amino)phenyl) imidazo[1,5-a]pyrazin-8(7H)-one (11b)

White solid (This compound was prepared by **10a**); m.p. > 200 °C; ^{1}H NMR (300 MHz, DMSO-d6): $\delta = 8.09–8.03$ (m, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 7.71 (dd, $J = 8.9$, 2.3 Hz, 1H, Ar–H), 7.66 (d, $J = 2.2$ Hz, 1H, Ar–H), 7.63–7.59 (m, 1H, Ar–H), 7.52 (s, 1H, Ar–H), 7.33 – 7.26 (m, 1H, Ar–H), 7.00 (d, $J = 7.0$ Hz, 2H, Ar–H), 5.91 (d, $J = 7.9$ Hz, 1H, –NH–), 3.57 (s, 3H, $–CH_3$), 3.39 (s, 3H, $–CH_3$), 3.10 (s, 3H, $–CH_2–$, –CH–), 2.42 (d, $J = 9.4$ Hz, 2H, $–CH_2–$), 1.87 (t, $J = 12.7$ Hz, 2H, $–CH_2–$), 1.42 (d, $J = 11.4$ Hz, 2H, $–CH_2–$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 155.0$ (–C=O, C-6), 149.7 (CH, C-2′), 135.3 (C, –C–SO_2–N–), 133.6 (CH, C-2), 132.8 (CH, C-4′), 131.5 (CH, –$\underline{C}$H = C–S–), 130.2 (CH, C-4), 129.0 (CH, –C=), 129.0 (C, =C–S–), 128.2 (C, C-5), 126.3 (CH, C-8), 123.4 (CH, C-6′), 122.1 (C, C-5′), 114.0 (C, C-1′), 113.2 (C, C-9), 110.6 (CH, C-3′), 48.0 (CH, –NH–CH–), 45.3 (CH_3, $–SO_2–CH_3$), 45.2 (CH_2, $–SO_2–N–\underline{C}H_2–$), 34.0 ($CH_3$, $–N–CH_3$), 30.4 (CH_2, $–N–CH_2–\underline{C}H_2–$). MS (ESI, *m/z*): 548.2 $[M+H]^+$. Anal. calcd. for $C_{23}H_{25}N_5O_5S_3$: C, 50.44; H, 4.60; N, 12.79. Found: C, 50.41; H, 4.63; N, 12.76.

5-(5-(ethylsulfonyl)-2-((1-(phenylsulfonyl)piperidin-4-yl) amino)phenyl)-7-methylimidazo [1,5-a]pyrazin-8(7H)-one (11c)

White solid (This compound was prepared by **10b**); m.p. > 200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.82–7.57$ (m, 8H, Ar-H), 7.50 (s, 1H, Ar–H), 6.99 (d, $J = 11.7$ Hz, 2H, Ar–H), 5.92 (d, $J = 7.8$ Hz, 1H, –NH–), 3.59 (s, 3H, $–CH_3$), 3.39 (s, 3H, $–CH_2–$, –CH–), 3.21–3.10 (m, 2H, $–CH_2–$), 2.34 (s, 2H, $–CH_2–$), 1.84 (s, 2H, $–CH_2–$), 1.39 (s, 2H, $–CH_2–$), 1.08 (t, $J = 7.3$ Hz, 3H, $–CH_3$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 155.1$ (–C=O, C-6), 149.8 (C, C-2′), 135.5 (C, –N–SO_2–C), 133.0 (CH, C-2), 132.2 (CH, Ar–C), 131.5 (CH, C-4′), 131.0 (CH, C-4), 129.3 (CH, Ar–C), 128.9 (C, Ar–C), 127.3 (C, C-5), 123.6 (CH, C-8), 123.4 (CH, C-6′), 122.1 (C, C-5′), 114.1 (C, C-1′), 113.2 (C, C-9), 110.6 (CH, C-3′), 49.7 (CH, –NH–$\underline{C}$H–), 48.1 (CH_3, $–SO_2–CH_3$), 45.2 (CH_2, –NH–$\underline{C}H_2–CH_2–$), 34.0 (CH_3, $–N–CH_3$), 30.4 (CH_2, $–N–CH_2–\underline{C}H_2–$), 7.3 ($CH_3$, $-CH_2–CH_3$). MS (ESI, *m/z*): 556.3 $[M+H]^+$. Anal. calcd. for $C_{26}H_{29}N_5O_5S_2$: C, 56.20; H, 5.26; N, 12.60. Found: C, 56.17; H, 5.23; N, 12.64.

5-(5-(ethylsulfonyl)-2-((1-(thiophen-2-ylsulfonyl)piperidin-4-yl)amino)phenyl)-7-methyl imidazo[1,5-a]pyrazin-8(7H)-one (11d)

White solid (This compound was prepared by **10b**); m.p. > 200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.06$ (dd, $J = 5.0$, 1.3 Hz, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 7.66 (dd, $J = 8.8$, 2.2 Hz, 1H, Ar–H), 7.63–7.58 (m, 2H, Ar–H), 7.50 (s, 1H, Ar–H), 7.29 (dd, $J = 5.0$, 3.8 Hz, 1H, Ar–H), 7.01 (t, $J = 4.5$ Hz, 2H, Ar–H), 5.92 (d, $J = 8.3$ Hz, 1H, –NH–), 3.57 (s, 3H, $–CH_3$), 3.39 (s, 3H, $–CH_2–$, –CH–), 3.16 (q, $J = 7.2$ Hz, 2H, $–CH_2–$), 2.42 (d, $J = 9.9$ Hz, 2H, $–CH_2–$), 1.87 (t, $J = 12.8$ Hz, 2H, $–CH_2–$), 1.42 (s, 2H, $–CH_2–$), 1.09 (t, $J = 7.3$ Hz, 3H, $–CH_3$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$

154.9(–C=O, C-6), 149.8 (C, C-2′), 135.3 (C, –C–SO_2–N–), 133.6 (CH, C-2), 132.8 (CH, C-4′), 132.2 (CH, –CH=C–S–), 131.5 (CH, C-4), 129.0 (CH, –C=), 131.0 (C, =C–S–), 129.2 (C, C-5), 128.2 (CH, C-8), 123.7 (C, C-6′), 122.1 (C, C-5′), 114.1 (C, C-1′), 113.2 (C, C-9), 110.6 (CH, C-3′), 49.7 (CH, –NH–CH–), 48.0 (CH_3, –SO_2–CH_3), 45.3 (CH_2, –SO_2–N–CH_2–), 34.0 (CH_3, N-CH_3), 30.4 (CH_2, –N–CH_2–CH_2–), 7.3 (CH_3, –CH_2–CH_3). MS (ESI, *m/z*): 562.3 $[M+H]^+$; Anal. calcd. for $C_{24}H_{27}N_5O_5S_3$: C, 51.32; H, 4.85; N, 12.47. Found: C, 51.37; H, 4.81; N, 12.41.

Methyl 3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a] pyrazin-5-yl)-4-(methylsulfonyl) phenoxy)benzoate (12a)

White solid (A mixture of **8a** (0.4 g, 1.24 mmol), methyl 3-hydroxyben zoate (0.28 g, 1.87 mmol) and K_2CO_3 (0.34 g, 2.49 mmol) in DMF (3 mL) was stirred at 85 °C for 6 h. Then the mixture was diluted with H_2O, extracted with EtOAc (60 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 a white solid.); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.22 (d, J = 2.9 Hz, 1H, Ar–H), 8.15 (d, J = 2.4 Hz, 1H, Ar–H), 8.06 (dd, J = 8.7, 2.5 Hz, 1H, Ar–H), 7.83 (d, J = 7.7 Hz, 2H, Ar–H), 7.69 (s, 1H, Ar–H), 7.59 (t, J = 7.9 Hz, 1H, Ar–H), 7.46 (d, J = 7.6 Hz, 1H, Ar–H), 7.19 (d, J = 2.9 Hz, 1H, Ar–H), 7.11 (dd, J = 8.7, 2.8 Hz, 1H, Ar–H), 3.85 (d, J = 2.9 Hz, 3H, –CH_3), 3.41 (d, J = 2.9 Hz, 3H, –CH_3), 3.28 (d, J = 2.9 Hz, 3H, –CH_3).

Methyl 3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)phe noxy)benzoate(12b)

White solid (This compound was prepared by **8b**); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.23 (s, 1H, Ar-H), 8.08 (d, J = 2.3 Hz, 1H, Ar–H), 8.00 (dd, J = 8.7, 2.4 Hz, 1H, Ar–H), 7.86–7.78 (m, 2H, Ar–H), 7.71 (d, J = 2.1 Hz, 1H, Ar–H), 7.58 (t, J = 7.9 Hz, 1H, Ar–H), 7.48 (dd, J = 7.8, 1.9 Hz, 1H, Ar–H), 7.19 (s, 1H, Ar–H), 7.09 (d, J = 8.7 Hz, 1H, Ar–H), 3.84 (s, 3H, –CH_3), 3.40 (s, 3H, –CH_3), 3.36 (s, 2H, –CH_2–), 1.18–1.12 (m, 3H, –CH_3).

3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methylsulfonyl)phenox y)benzoic acid (13a)

White solid (This compound was prepared by **12a**); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.25 (s, 1H, –COOH), 8.20 (s, 1H, Ar–H), 8.14 (s, 1H, Ar–H), 8.05 (d, J = 8.8 Hz, 1H, Ar–H), 7.81 (s, 2H, Ar–H), 7.66 (s, 1H, Ar–H), 7.55 (t, J = 7.8 Hz, 1H, Ar–H), 7.42 (d, J = 8.2 Hz, 1H, Ar–H), 7.19 (s, 1H, Ar–H), 7.12 (d, J = 8.6 Hz, 1H, Ar–H), 3.41 (s, 3H, –CH_3), 3.28 (s, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.1 (C=O, COOH), 155.0 (C=O, C-6), 153.1 (CH, –O–CH=), 149.9 (CH, C-2′), 136.1 (CH, C-4′), 133.6 (CH, C-2), 132.7 (C, C-5′), 130.6 (CH, C-4), 129.4 (CH, =CH-COOH), 128.7 (CH, Ar–C), 127.6 (C, C-5), 126.6 (CH, C-6′), 123.4 (C, C-9), 122.5 (CH, C-8), 120.6 (C, C-1′), 120.6 (CH, Ar–C), 118.7 (CH, Ar–C), 118.3 (CH, C-3′), 116.9 (CH, Ar–C), 45.3 (CH_3, –SO_2–CH_3), 34.2 (CH_3, N-CH_3). MS (ESI, *m/z*): 440.3 $[M+H]^+$. Anal. calcd. for $C_{25}H_{27}N_5O_5S_2$: C, 57.40; H, 3.90; N, 9.56. Found: C, 57.32; H, 3.84; N, 9.50.

3-(4-(Ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo [1,5-a]pyrazin-5-yl)phenoxy) benzoic acid (13b)

White solid (This compound was prepared by **13b**); m.p. 162–164 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.27 (s, 1H, –COOH), 8.23 (s, 1H, Ar–H), 8.08 (d, J = 2.4 Hz, 1H, Ar–H), 8.00 (dd, J = 8.7, 2.4 Hz, 1H, Ar–H), 7.82 (d, J = 6.0 Hz, 2H, Ar–H), 7.69 (s, 1H, Ar–H), 7.56 (t, J = 7.9 Hz, 1H, Ar–H), 7.45 (dd, J = 8.2, 1.5 Hz, 1H, Ar–H), 7.20 (s, 1H, Ar–H), 7.10 (d, J = 8.7 Hz, 1H, Ar–H), 3.41 (s, 3H, –CH_3), 3.36 (q, J = 7.3 Hz, 2H, –CH_2–), 1.16 (t, J = 7.3 Hz, 3H, –CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.0 (C=O, COOH), 154.9 (C=O, C-6), 153.1 (CH, –O–CH=), 149.9 (CH, C-2′), 136.2 (CH, C-4′), 133.6 (CH, C-2), 132.6 (C, C-5′), 130.6 (CH, C-4), 129.4 (CH, =CH-COOH), 128.8 (CH, Ar–C), 127.7 (C, C-5), 126.6 (CH, C-6′), 123.3 (C, C-9), 122.5 (CH, C-8), 120.7 (C, C-1′), 120.6 (CH, Ar–C), 118.4 (CH, Ar–C), 118.2 (CH, C-3′), 116.9 (CH, Ar–C), 45.3 (CH_3, –SO_2–CH_3), 34.2 (CH_3, N-CH_3), 7.4 (CH_3, –CH_2–CH_3). MS (ESI, *m/z*): 454.3 $[M+H]^+$. Anal. calcd. for $C_{25}H_{27}N_5O_5S_2$: C, 58.27; H, 4.22; N, 9.27. Found: C, 58.25; H, 4.20; N, 9.31.

N-(2-(dimethylamino)ethyl)-3-(4-(ethylsulfonyl)-2-(7-methyl-8-oxo-7,8-dihydroimidazo [1,5-a]pyrazin-5-yl) phenoxy)benzamide (14a)

Yellow solid (This compound was prepared by **13b**); m.p. 164–166 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.57 (s, 1H, –NH–C=O), 8.18 (s, 1H, Ar–H), 8.09 (d, J = 2.4 Hz, 1H, Ar–H), 8.02 (dd, J = 8.7, 2.4 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 7.74 (d, J = 7.8 Hz, 1H, Ar–H), 7.63 (s, 1H, Ar–H), 7.56 (t, J = 7.9 Hz, 1H, Ar–H), 7.43 (d, J = 9.7 Hz, 1H, Ar–H), 7.21 (s, 1H, Ar–H), 7.07 (d, J = 8.7 Hz, 1H, Ar–H), 3.49–3.34 (m, 9H, –CH_3, –CH_2–, –CH_2–, –CH_2–), 2.45 (s, 6H, –N(CH_3)$_2$), 1.16 (t, J = 7.3 Hz, 3H, –CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.7 (C, –NH–C=O), 155.2 (C=O, C-6), 154.8 (CH, –O–CH=), 154.1 (C, C-2′), 136.9 (CH, –CO–CH=), 133.5 (CH, C-4′), 133.3 (CH, C-9), 132.8 (CH, C-4), 132.2 (C, C-5′), 130.9 (CH, C-6′), 129.8 (CH, Ar–C), 124.8 (C, C-1), 123.7 (C, C-9), 123.1 (CH, C-8), 122.9 (CH, Ar–C), 121.1 (C, C-1′), 119.6 (CH,

Ar–C), 117.1 (CH, C-3′), 112.8 (CH, Ar–C), 57.5 (CH_2, –CH_2–NH–), 49.9 (CH_2, –SO_2–CH_2–), 44.4 (CH_2, –CH_2–NH–), 36.6 (CH_3, –NH– (CH_3)$_2$), 34.6 (CH_3, N-CH_3), 7.6 (CH_3, –CH_2–CH_3); MS (ESI, *m/z*): 524.3 $[M+H]^+$; Anal. calcd. for $C_{26}H_{29}N_5O_5S$: C, 59.64; H, 5.58; N, 13.38. Found: C, 59.61; H, 5.55; N, 13.41.

N-(2-(dimethylamino)ethyl)-3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methylsulfonyl) phenoxy)benzamide (14b)

Yellow solid (This compound was prepared by **13a**); m.p. 194-196 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.09$ (d, $J =$ 2.3 Hz, 1H, –NH–C=O), 8.03–7.97 (m, 2H, Ar–H), 7.75 (s, 1H, Ar–H), 7.59 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.52 (s, 1H, Ar-H), 7.42 (t, $J = 7.9$ Hz, 1H, Ar–H), 7.09 (dd, $J = 8.1$, 3.4 Hz, 2H, Ar–H), 7.01 (s, 1H, Ar–H), 6.55 (s, 1H, Ar–H), 3.54–3.47 (m, 5H, –CH_3, –CH_2–), 3.13 (s, 3H, –CH_3), 2.52 (t, $J = 5.8$ Hz, 2H, –CH_2–), 2.26 (s, 6H, –N(CH_3)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 165.6$ (C, –NH–C=O), 155.1 (C=O, C-6), 154.9 (CH, –O–CH=), 154.3 (C, C-2′), 136.6 (CH, –CO–CH=), 133.4 (CH, C-4′), 133.3 (CH, C-9), 132.8 (CH, C-4), 132.3 (C, C-5′), 130.9 (CH, C-6′), 129.9 (CH, Ar-C), 124.9 (C, C-1), 123.5 (C, C-9), 123.2 (CH, C-8), 122.7 (CH, Ar–C), 121.5 (C, C-1′), 119.6 (CH, Ar–C), 117.5 (CH, C-3′), 112.8 (CH, Ar–C), 57.6 (CH_2, –CH_2–NH–), 44.5 (CH_2, –CH_2–NH–), 44.2 (CH_3, –S(O_2)–CH_3), 36.7 (CH_3, –NH– (CH_3)$_2$), 34.6 (CH_3, N-CH_3); MS (ESI, *m/z*): 510.3 $[M+H]^+$; Anal. calcd. for $C_{25}H_{27}N_5O_5S$: C, 58.93; H, 5.34; N, 13.74. Found: C, 58.97; H, 5.31; N, 13.69.

3-(2-(7-methyl-8-oxo-7,8-dihydroimidazo[1,5-a]pyrazin-5-yl)-4-(methylsulfonyl)phenox y)-N-(2-(pyrrolidin-1 -yl)ethyl) benzamide (14c)

Yellow solid (This compound was prepared by **13a**); m.p. 187–189 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.70$ (s, 1H, –NH–C=O), 8.16 (s, 2H, Ar–H), 8.11–8.03 (m, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 7.74 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.63–7.52 (m, 2H, Ar–H), 7.45 (d, $J = 8.7$ Hz, 1H, Ar–H), 7.20 (s, 1H, Ar–H), 7.10 (d, $J = 8.8$ Hz, 1H, Ar–H), 3.56 (d, $J = 5.3$ Hz, 4H, –CH_2–, –CH_2–), 3.42 (s, 3H, –CH_3), 3.32–3.29 (m, 2H, –CH_2–), 3.28 (s, 3H, –CH_3), 3.05 (s, 2H, –CH_2–), 2.01 (s, 2H, –CH_2–), 1.85 (s, 2H, –CH_2–); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 165.9$ (C, –NH–C=O), 155.2 (C=O, C-6), 154.7 (CH, –O–CH=), 154.4 (C, C-2′), 136.9 (CH, –CO–CH=), 133.5 (CH, C-4′), 133.3 (CH, C-9), 132.8 (CH, C-4), 132.1 (C, C-5′), 130.9 (CH, C-6′), 129.7 (CH, Ar–C), 124.8 (C, C-1), 123.7 (C, C-9), 123.1 (CH, C-8), 122.9 (CH, Ar–C), 121.5 (C, C-1′), 119.6 (CH, Ar–C), 117.1 (CH, C-3′), 112.8 (CH, Ar–C), 54.0 (CH_2, –CH_2–NH–), 53.7 (CH_2, –CH_2-NH–), 44.2 (CH_3, –S(O_2)–CH_3), 36.3 (CH_2, pyrrolidine–N–CH_2–), 34.6 (CH_3, N-CH_3), 22.9 (CH_2, pyrrolidine–CH_2–CH_2–); MS (ESI, *m/z*): 536.3 $[M+H]^+$; Anal. calcd. for $C_{27}H_{29}N_5O_5S$: C, 60.55; H, 5.46; N, 13.08. Found: C, 60.51; H, 5.41; N, 13.11.

Docking studies

All compounds were drawn in ChemDraw, and exported as sdf style. Ligand preparation protocol of Maestro 10.2 was utilized to generate all possible tautomers, stereoisomers, and protonation states of molecules at a simulated pH of 7.4 ± 1.0, then minimization was proceeded at the OPLS force field (Yang et al. 2016a, b). In our study, BRD4(1) docking protein was from the crystal complex (PDB id: 3P5O). The Glide protein was created with Glide generation protocol of Maestro 10.2 and the bond orders were assigned. Seven molecules of water around the binding site were saved (i.d.: HOH18, HOH27, HOH33, HOH256, HOH267, HOH268, HOH358). Hydrogen atoms were added. Finally, a 10 Å box centered on the geometrical center of the ligand binding site was created for grid docking. The docking research was executed with the Glide docking protocol. After Glide docking completed, only one conformation was saved for each molecular ligand (Yang et al. 2016a, b).

Biological evaluation

Binding effects of target compounds to BRD4(1) and BRD4 (2)

We commissioned Shanghai ChemPartner Co. to proceed 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 1× assay buffer (modified HEPES buffer). Transfer compounds to assay plates by Echo. DMSO's final concentration is 0.1%. Prepare protein solution in 1× assay buffer (5 nM). Add peptide (H4) in 1× assay buffer to make the substrate solution. Transfer 5 µL of protein solution to assay plates or for low control transfer 5 µL of 1× assay buffer, followed with incubation at room temperature for 15 min. 5 µL of substrate solution was added into each well, followed with incubation at room temperature for 60 min. Lastly, 15 µL acceptor and donor solution was added, followed with incubation for 60 min at room temperature, subdued light (Yang et al. 2016a, b). The IC_{50} values were calculated from the endpoints of EnSpire with Alpha mode.

Cell proliferation assay

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

cultured in 100 μL of IMDM containing 20% fetal bovine serum. After 12 h, 50 μL of various concentrations of compounds (triple diluted) were added. The measurement was executed 72 h after seeding, and 10 μL of cell-counting kit-8 (CCK-8) reagent was added to each well, followed by incubation at 37 °C for 4 h. A multi-detection microplate was utilized to assess the spectrophotometric absorbance of each well at a wavelength of 450 nm (Yang et al. 2016a, b). The cell proliferation assay was executed to give the IC_{50} for the compounds **14a–c** and reference compounds reported in Table 2. GraphPad Prism 5 was used to calculate the IC_{50} values of target compounds (Tagde et al. 2016).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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