Journal of
Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • Publication Date (Web): 30 Nov 2019

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of Benzo[*cd*]indol-2(1*H*)-ones and Pyrrolo[4,3,2-*de*]quinolin-2(1*H*)-ones as Bromodomain and Extra-Terminal Domain (BET) Inhibitors with Selectivity for the First Bromodomain with Potential High Efficiency Against Acute Gouty Arthritis

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ABSTRACT

The bromodomain and extra-terminal domain (BET) family of proteins are readers which specifically recognize histone acetylated lysine residues. Each BET bromodomain protein contains two highly homologous domains: the first bromodomain (BD1) and the second bromodomain (BD2), respectively. Pan-BET bromodomain inhibition is a potential therapy for various cancers and immune-inflammatory diseases, but only few reported inhibitors show selectivity within the BET family. Herein, we identified a series of benzo[*cd*]indol-2(1*H*)-ones and pyrrolo[4,3,2-*de*]quinolin-2(1*H*)-ones with good selectivity for BET BD1. Through structure-based optimization, highly active and selective compounds are ultimately obtained. The representative compounds are the first reported inhibitors with selectivity more than 100-fold for BRD4(1) over BRD4(2). Among them, we further show that **68** (LT052) mediates BRD4/NF-κB/NLRP3 signaling inflammatory pathways with comparable protein expression and significantly improves symptoms of gout arthritis in a rat model. Therefore, selective pharmacological modulation of individual bromodomains could represent a strategy for the treatment of acute gouty arthritis.

INTRODUCTION

Gout is the most common type inflammatory arthritis with increasing prevalence in recent years, which is characterized by the precipitation of monosodium urate (MSU) crystals in the joint[1]. Pro-inflammatory cytokine IL-1β is a critical manifestation in response to MSU crystal attack. IL-1β secretion is dependent on a thoroughly studied inflammasome, nucleotide-binding oligomerization domain-like receptor, pyrin domain containing 3 (NLRP3)[2-5]. The activation of NLRP3 involves a two-step process. The first signal triggers NF-κB-dependent pro-IL1β and NLRP3 expression[6]. The second signal triggers the formation of inflammasome multimers by microbial molecules or dangerous signals (Such as MSU) of different structures. NLRP3 induces self-cleavage and activation of caspase-1. Activated caspase-1 promotes the hydrolysis and maturation of IL-1β and IL-18, and the formation of pores on the cell membrane and triggers proinflammatory cell death, pyroptosis[7, 8].

In recent years, Bromodomain-containing proteins (BRDs) have been found to play an important role in the development and progression of inflammation and cancer as epigenetic targets. Bromodomain-containing proteins (BRDs) are epigenetic reader proteins of histone acetylation involved in chromatin remodeling and transcriptional regulation, which recruit transcriptional regulator complexes to chromatin and binding to acetylated lysine (KAc) residues on the *N*-terminal tails of histones[9-11]. So far, 61 bromodomains have been identified in 46 human proteins, which are classified into eight families[12]. The second family of bromodomain and extraterminal (BET) includes BRD2, BRD3, BRD4, and BRDT, each of which contains two bromodomains (BD1 and BD2). The BD2 domains in these four BET proteins display a high degree

of structural similarity, as do the four BD1 domains.

BET family members recruit different partners. BRD4 recruits positive transcriptional elongation factor complex (P-TEFb), which plays an essential role in the regulation of transcription by RNA polymerase II (RNA Pol II) in eukaryotes[13]. BET proteins facilitate the transcription of tumor-growth-promoting and antiapoptotic genes, including c-Myc, and their dysfunction plays critical roles in a variety of human diseases[14-16]. Small-molecule BET inhibitors have shown efficacy against a range of cancers, and several inhibitors have entered clinical trials in oncology[17]. BET inhibition has been shown to potently modulate inflammatory autoimmune gene expression[18]. BET bromodomains control the differentiation of $CD4^+$ T cells to inflammatory Th17 cells through genomic localization and their effects on cytokine regulation[19]. Jiang Yafei et al[20] reported that BRD4 ligands have dual effects on the HMGB1 and NF-κB signalling pathways and BRD4 is a potential therapeutic target for osteoarthritis. Meanwhile, BRD4 proteins regulate gene transcription during lineage-specific differentiation of naïve $CD4^+$ T cells to produce mature T-helper cells. Pan-BET bromodomain (BRD) inhibitors have distinct immune functions, thus limiting their therapeutic potential[19]. In human monocytes, BET inhibition suppressed the transcriptional response of HMGB1 and NF-κB pathway to cytokine stimulation, and downregulates pro-inflammatory cytokine production in macrophages[21], dendritic cells[22], and rheumatoid arthritis synovial fibroblasts[23]. In vivo, BET inhibitors exhibit therapeutic effects in a variety of anti-inflammatory disease models, such as colitis[19], arthritis[24], psoriasis[25], and pathologic bone loss[26].

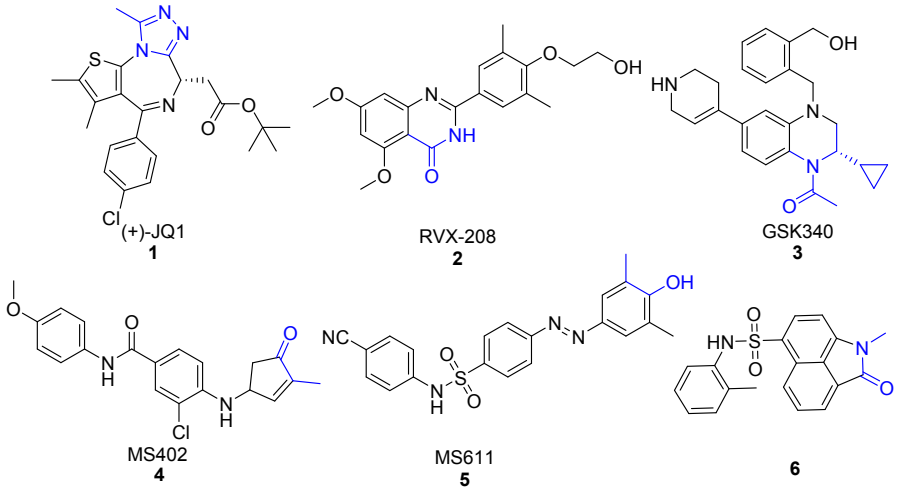


Figure 1. Representative BET bromodomain family selective inhibitors

In the past decade, multiple structural cores that mimic acetylated lysine (KAc) binding have been discovered. Among BET inhibitors, the triazolodiazepine chemotype has been extensively explored. With (+)-JQ1 (**1**)[27] as a representative widely used in biological mechanism research, its derivatives have been advanced into clinical trials, including i-BET762[28], OTX-015[29], TEN-10[30] and CPI-0610[31]. Except RVX-208 (**2**)[32], these clinical-stage molecules exhibit little or no selectivity between the eight bromodomains of the BET family. Given the fact that eight bromodomains in BET family are highly homologous, it is still a challenge to obtain highly selective inhibitors. It's still not clear if side effects and toxicity were related to their selectivity in biological function[33]. Therefore, the development of highly selective inhibitors is urgently needed.

Currently, only a few selective BET inhibitors were reported. Among them, RVX-208 (**2**) is a compound with high degree of selectivity for BET BD2 and good response in the treatment of metabolic diseases in clinical trials[34]. Based on AlphaScreen data, RVX-208 (2) has about 170-fold selectivity towards BD2 over BD2 (IC_{50} values of 87±10 μM and 0.510±0.041 μM for BD1 of BRD3 and BD2 of BRD3, respectively)[32]. The sequence alignment (Figure 2A-C) shows that the BC

loop-specific DDI of the first Bromodomain of the four BET proteins is different from the BC loop HDV or HEV sequence of the second Bromodomain. RVX-208 (**2**) selectively binds to the second Bromodomain by utilizing the fine structural differences of the BC loop-specific histidine in the two Bromodomains[35]. Compound **3** selectively binds to the second bromodomain, and His433, which is unique to BRD4 BD2, is utilized to form hydrogen bond interactions to increase selectivity[36]. Weak (~10-fold) BRD4 BD1 bias is also observed for the cyclopent-2-en-1-one inhibitor MS402 (**4**), which blocked Th17 maturation from mouse naive $CD4^+$ T cells, with limited or no effects on Th1, Th2, or Treg cells[37]. Experimental models of mouse colitis induced by T cell transfer have been observed to potentially treat inflammatory bowel diseases. The diazobenzene MS611 (**5**) was reported to be moderately active for BRD4 BD1 with K_d = 0.41 μM and 100-fold selectivity over BRD4 BD2, where the phenol acts as KAc mimetic[38].

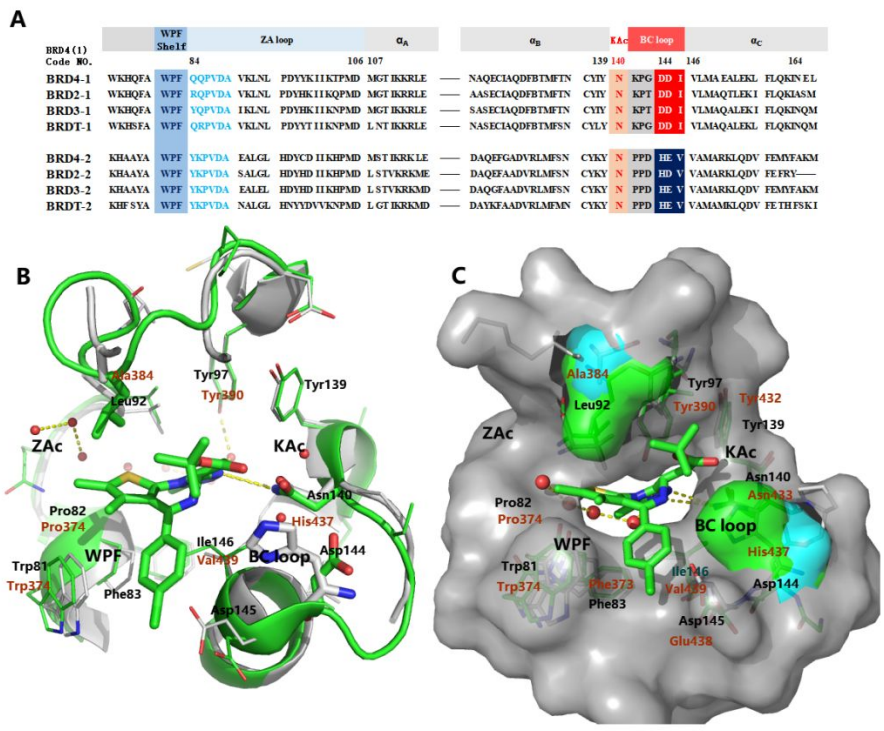


Figure 2. (**A**) Sequence alignment of BET bromodomains with the residues highlighted. (**B**)

Comparison of BRD4 BD1 (3MXF, green) and BD2 (5uf0, gray) with (+)-JQ1 (**1**). The cartoons and lines show the binding pockets of BD1 and BD2, and the sticks are shown to highlight key differential residues. (**C**) Comparison of BRD4 BD1 (3MXF, green) and BD2 (5uf0, gray) with (+)-JQ1 (**1**). The surfaces and lines show the binding pockets of BD1 and BD2, and the color surface is shown to highlight the volume of the space differential residues.

There are currently very few small molecule inhibitors that selectively bind to a single bromodomain of the BET family proteins. It remains a challenge to identify highly potent and selective-bromodomain small-molecule BET inhibitors. Importantly, Ke Ding et al[39] reported a novel chemical core of 1-ethylbenzo[*cd*]indol-2(1*H*)-one based on virtual screening which showed promising BRD4 binding activity. Subsequently, Hua Chen et al[40] also reported that this core has excellent BRD4 activity. Although derivatives of this core only displayed moderate anti-cancer cell proliferation activity in vitro compared to triazole derivatives (e.g., JQ1), they show great potential for the development of anti-cancer therapeutics. Further investigation on benzo[*cd*]indol-2(1*H*)-ones may stimulate discovery of selective-bromodomain BET inhibitors .

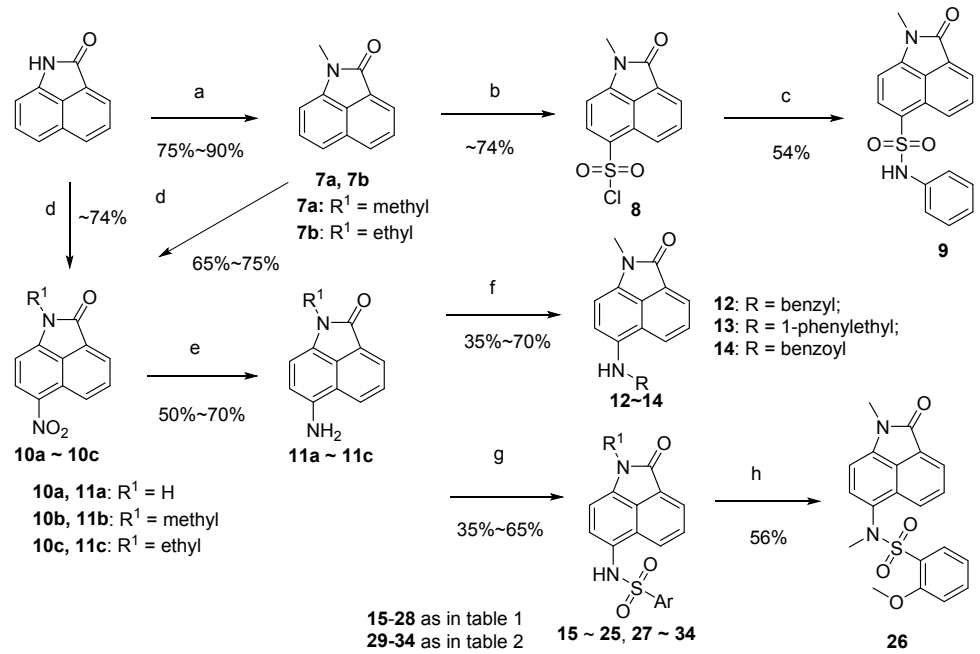
A previous fragment-based screen carried out in our laboratories identified benzo[*cd*]indol-2(1*H*)-one **6** (Figure 1), which bound weakly but with unique structural features to BRD4 BD1 (IC_{50} = 15.53 μM and ΔTm = 2.23 ℃). Through a series of structural modifications, we obtained a class of inhibitors that specifically bind to BRD4 BD1. Among them, we identified a lead compound with nanomolar potency towards BD1, and more importantly, it displayed more than 100-fold selectivity towards BD1 over BD2. The lead compound has a potential in the treatment of BRD4/NF-κB/NLRP3-mediated gout arthritis, providing a very useful tool for the

disease phenotype of subsequent selective-bromodomain BET inhibitors.

CHEMISTRY

The synthesis of compound **9** and **12**-**34** was shown in Scheme 1. Benzo[*cd*]indol-2(1*H*)-one was used as the starting material and compound **9** was prepared through methylation, electrophilic aromatic substitution with chlorosulfonic acid and nucleophilic addition with aniline. Compounds **11a**-**11c** are synthesized by alkylation, nitration reaction followed by hydrogenation. With starting from the intermediate **11a**, the title compounds **12**-**14** were prepared through the nucleophilic aromatic substitution with appropriate halides or benzoyl chlorides. Further, the title compounds **15**-**34** were prepared by appropriate benzenesulfonyl chlorides with **11a**-**11b** in the presence of a small amount of pyridine.

Scheme 1. Synthetic routes of compounds **9** and **12**-**14**

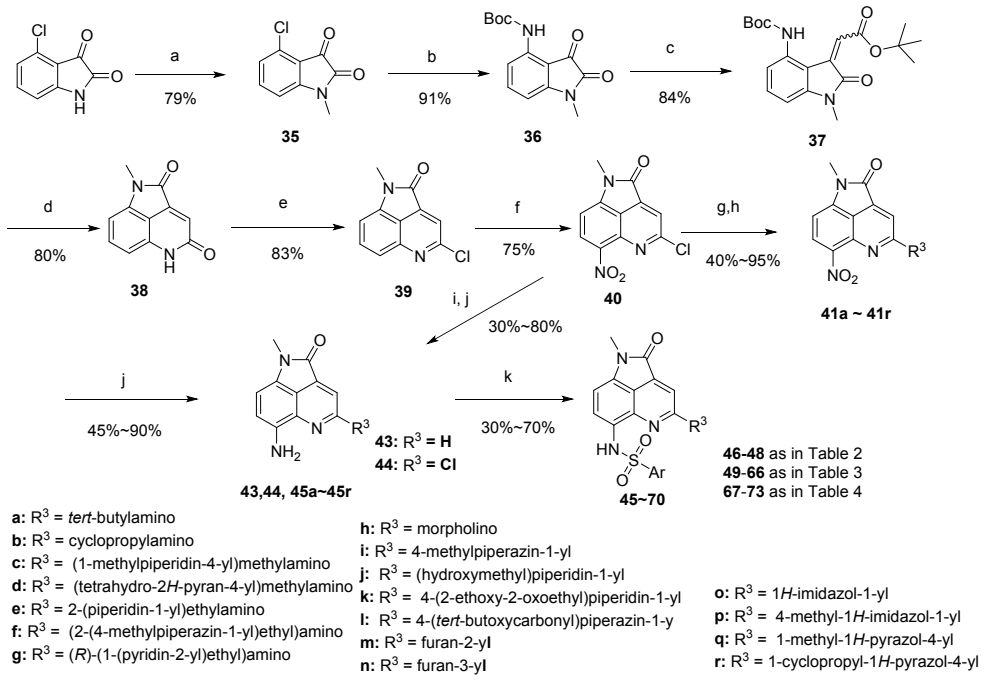


Reagents and conditions: (a) appropriate Iodide, 60% NaH, DMF; (b) $ClSO_3H$, 0 °C; (c) aniline,

pyridine, DCM, r.t; (d) 69% HNO_3, AcOH, r.t; (e) H_2, 10% Pd/C, MeOH, THF, r.t; (f) appropriate bromide or benzoyl chloride, 60% NaH, DMF, r.t; (g) appropriate sulfonyl chlorides, pyridine, DCM, r.t; (h) CH_3I, 60% NaH, DMF.

In scheme **2**, benzo[*cd*]indol-2(1*H*)-one derivatives were prepared (Table 3 and Table 4). The synthesis of intermediate **39** is no longer described in the same way as reported by Sheng-Wei Yang et al[41] Starting from inexpensively available 4-chloroindoline-2,3-dione intermediate was nitrated with nitric acid sulfuric acid system from -25 °C to -10 °C to afford compound **40**. Intermediates **43** and **44** were synthesized from **39** by hydrogenation and dechlorination. Meanwhile, **43a**-**43n** was prepared from **40** with different amines by nucleophilic reaction followed by hydrogenation of nitro group and **43o**-**43v** was prepared from **40** upon a Suzuki–Miyaura cross coupling reaction with different boronic acids/esters, followed by hydrogenation of nitro group. Finally, the title compounds **46**-**70** were prepared with different benzene sulfonyl chloride in the presence of a small amount of pyridine.

Scheme 2. Synthetic routes of compounds **46**-**70**

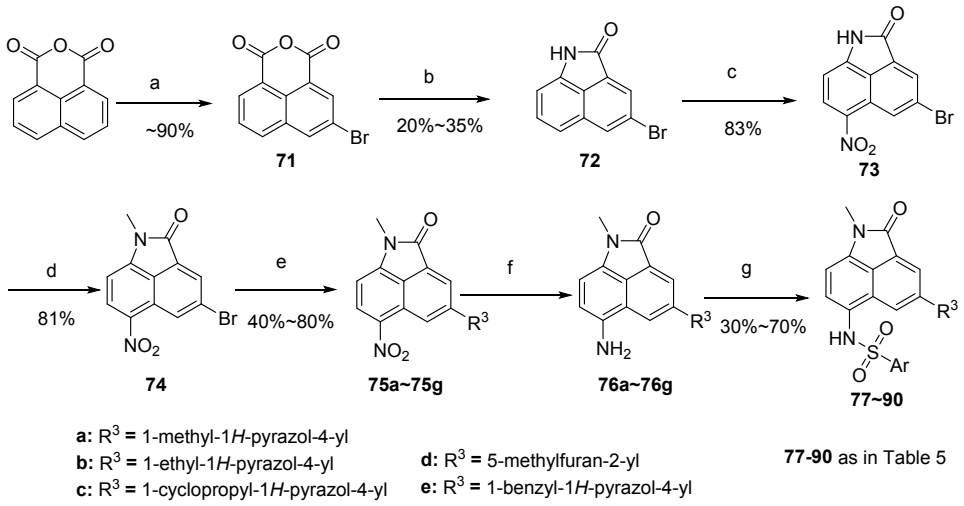


Reagents and conditions: (a) CH_3I, 60% NaH, DMF; (b) $BocNH_2$, $Pd(AcO)_2$, x-PHOS, K_2CO_3, anhydrous 1,4-dioxane, 80 °C, 10 h; (c) *tert*-butyl 2-(triphenyl-λ^5-phosphanylidene)acetate, DMF, r.t; (d) TFA, 100 °C; (e) $POCl_3$, 100 °C ;(f) H_2SO_4, HNO_3, -25 - -10 °C; (g) appropriate boronic ester, $Pd(PPh_3)_4$, 1,4-dioxane, N_2, 80 °C; (h) appropriate amine derivatives, anhydrous 1,4-dioxane, 100 °C; (i) K_2CO_3, 10% P/C, H_2, THF; (j) 10% P/C, H_2, THF, MeOH; (K) appropriate sulfonyl chlorides, pyridine, DCM, r.t.

In scheme 3, benzo[*cd*]indol-2(1*H*)-one derivatives were prepared (Table 4). Starting from inexpensively available 1,8-Naphthalic anhydride, intermediate **71** was synthesized through halogenation reaction with bromine and silver sulfate in concentrated sulfuric acid. Two key steps, nucleophilic substitution and strong base decarboxylation, were carried out in one pot in acceptable yields to obtain **72**. Subsequently, **75a**-**75g** were synthesized through a three-step reaction including methylation with methyl iodide, nitrification under acetic acid-nitric acid conditions and Suzuki coupling reaction with different boric acid or borate esters. The

intermediates were subjected to hydrogenation and were used directly for a next step without further purification. Finally, the title compounds **77**-**90** were prepared with different benzene sulfonyl chlorides and **76a**-**76g** in the presence of a small amount of pyridine.

Scheme 3. Synthetic routes of compounds **77**-**90**



Reagents and conditions: (a) Br_2 , H_2SO_4, Ag_2SO_4, 60 °C; (b) i: NH_2OH HCl, TsCl, pyridine, 90 °C; ii:NaOH, HCl, EtOH, H_2O; (c) HNO3, AcOH, 60 °C; (d) CH_3I, 60% NaH, DMF; (e) appropriate boronic este, $Pd(PPh_3)_4$, 1,4-dioxane, N_2, 80 °C; (f) Fe, NH_4Cl, EtOH, 80 °C; (g) appropriate sulfonyl chlorides, pyridine, DCM, r.t.

RESULTS AND DISCUSSION

Optimization of the Linker to the WPF Shelf

To improve the potency of benzo[*cd*]indol-2(1*H*)-one-based BET bromodomain inhibitors such as **6**, we explored interactions between the inhibitor core and the conserved Asn140 residue and the WPF shelf of the BET protein. Compounds were evaluated using differential scanning fluorimetry (DSF) and amplified luminscent proximity homogeneous assay

(AlphaScreen). The DSF binding assay and AlphaScreen assay were used to determine the thermal stability (ΔTm) and the affinities (IC_{50}) of compounds for a construct containing the two bromodomains of BRD4. All derivatives were tested for their inhibitory potency toward recombinant human BRD4 BD1 His and BRD4 BD2 His.

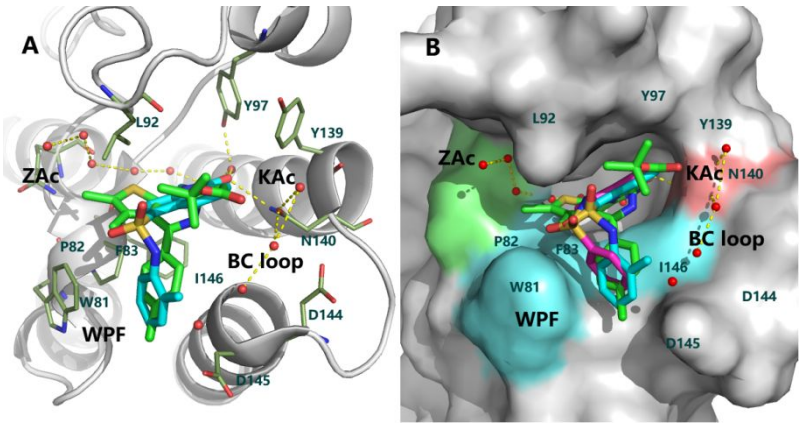
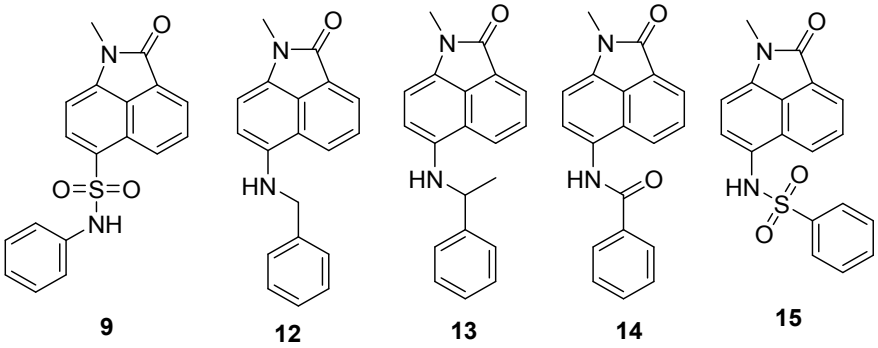


Figure 3. Binding mode analysis of compound **6** and **15** to BRD4 BD1 protein (PDB code 3MXF). (A) Compound **6** (shown as blue) docked into BRD4 BD1 overlaid **JQ1** (shown as green) with BRD4(1) protein. (B) Compound **6** (shown as blue) and **15** (shown as purple) docked into BRD4 BD1 overlaid **JQ1** (shown as green) with BRD4(1) protein (displayed as gray surface).

From the binding mode of **6** with BRD4 BD1 (Figure 3A), we can see that the carbon-based oxygen of the benzo[*cd*]indol-2(1*H*)-one forms a molecular key hydrogen bond with the amino group of Asn140 and indirectly forms another molecular key hydrogen bond with the water molecule and Tyr97. The phenylmethyl group of compound **6** extended to the WPF shelf via a sulfonamide linker, not in the WPF hydrophobic center region. To better fit the WPF shelf, we examined a series of linkers in compounds **9**, **12**-**15**, including methylene, ethylene, amide, and back-linked sulfonamides. As shown in Table 1, methylene, ethylene, and amide did not achieve desired effect and instead had a large increase in the non-BET family. It is very exciting that the

value of ΔTm of **15** was elevated by 3.53 ℃ over the forward sulfonyl linker in compound **9**, still remaining selective for the BET family. The binding mode (Figure 3B) showed that the benzene ring of **15** has a high degree of fit with the space of the benzene ring of JQ1 (**1**) at the center of the WPF shelf.

Table 1. Structures and biological evaluation of target compounds



9 **12** **13** **14** **15**

Compounds	△Tm (℃)[a]			
	BRD4(1)	**BRD9(1)**	**CECR2(1)**	**CREBBP(1)**
9	2.19	0.47	-0.03	0.53
12	2.70	-0.90	1.92	1.60
13	3.15	4.33	0.13	0.55
14	2.17	4.10	2.17	0.93
15	5.72	1.93	-0.31	1.51

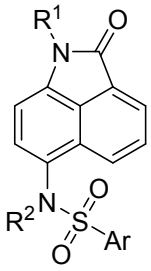
[a] using the differential scanning fluorimetry (DSF) at 10 μM concentration

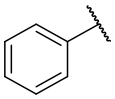
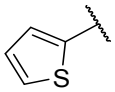
Optimization of the WPF Shelf and the KAc pocket

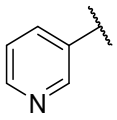
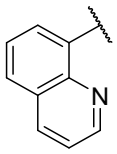
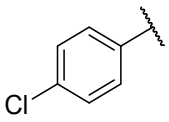
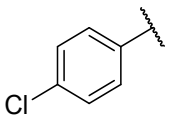
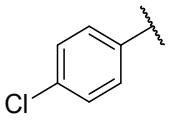
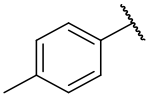
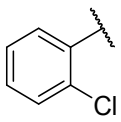
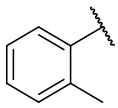
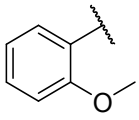
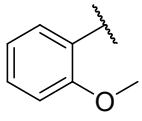
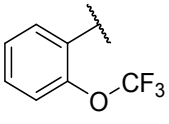
With these encouraging results, the benzene ring moiety of **15** was investigated with a suitable aromatic ring and a substituted ortho or para-monosubstituted benzene ring (Table 2) while maintaining the sulfonamide linker of **15**. From the comparison of BRD4 BD1 protein and

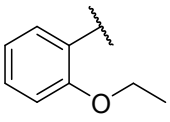
BRD4 BD2 sequence (Figure 1A), it was found that the amino acids (Tyr, Pro and Phe) constituting the WPF region were significantly different in terms of space volume, electrical property and flexibility. First, the spatial volume and electrical properties of the WPF shelf were explored using five-membered thiophene in **16**, electron-deficient pyridine in **17** and bulkier quinoline ring in **18**. Among them, the IC_{50} values of **15** are 709.1±4.5 nM and 2621±10 nM for BD1 and BD2, respectively, suggesting about 3.7-fold selectivity (Table 2). The selectivity was reduced by the increase in the size of R^1 and the activity of the methyl group (**19**) appears to be the best. Changes in methylation of **22**, selectivity and binding were not significant compared to **15**.

Table 2. Structures and biological evaluation of target compound **15**-**28.**



Cpd.	R^1	R^2	Ar	BRD4(1) ΔTm (℃)[a]	BRD4 IC_{50} (% protein activity at 2 μM)[b]		Selectivity
					BD1 (nM)[d]	BD2 (nM)	BD2/BD1
15	Me	H		5.72	709.1±4.5	2621±10	3.7
16	Me	H		4.02	3724±46	nd.[c]	---

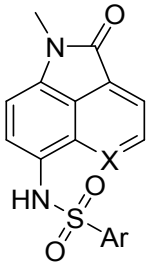
17	Me	H		2.3	2436±290	13675±1029	5.6
18	Me	H		4.13	328.6±21.3	260.3±15.9	0.8
19	Me	H		6.5	167.1±14.3	2717±401	16
20	H	H		3.73	769.2±114.3	15502±125	20.1
21	Et	H		6.36	237.4±9.3	2717±401	11.4
22	Me	H		7.36	545.2±23.4	2283±132	4.1
23	Me	H		5.37	1529±41	6048±67	3.9
24	Me	H		5.1	708.7±9.1	nd.	---
25	Me	H		8.72	49.5±10.3	2479±170	50.5
26	Me	Me		1.06	(90.39±1.3%)	nd.	---
27	Me	H		4.73	3888±130	nd.	---

28	Me	H		5.51	172.1±12.8	1640±68	9.5

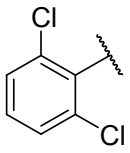
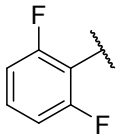
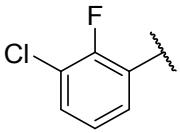
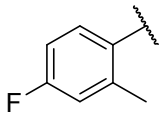
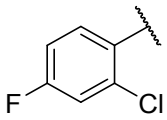
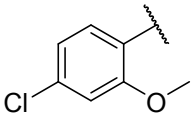
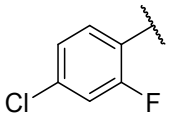
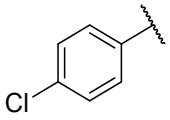
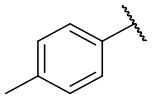
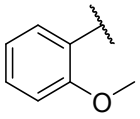
[a] using the differential scanning fluorimetry (DSF) at 10 μM concentration; [b]Using AlphaScreen assay, the values are the mean ± SD from two independent experiments. [c] nd: not determined. [d] n = 2.

To investigate the effect of ortho substituents on the potency and selectivity, we designed chloro, methyl, ethoxy trifluoromethoxy and ethoxy substituents. The increase in the potency is very limited with the 2-position benzene ring replaced with electron-withdrawing chlorine (**23**), trifluoromethoxy (**27**), and electron-donating methyl (**24**) and ethoxy groups (**28**). Surprisingly, a breakthrough level in the selectivity and the affinity was found in compound **25** (IC_{50}, 49.5 ± 10.3 nM and 2479 ± 170 nM for BD1 and BD2, respectively thus about 50-fold selectivity) with a methoxy group at the ortho-phenyl.

Table 3. Structures and biological evaluation of target compound **29**-**28.**



Cpd.	**X**	**Ar**	**BRD4(1) ΔTm (℃)**	**BRD4 IC_{50} (% protein activity at 2 μM)**		**Selectivity**
				BD1 (nM) [d]	**BD2 (nM)**	**BD2/BD1**

29	C		2.75	(91.12±3.3%)	nd.	---
30	C		3.73	9065±14	nd.	---
31	C		4.83	1696±33	nd.	---
32	C		4.32	810.4±17.4	nd.	---
33	C		6.23	91.3±3.3	3828±48	42
34	C		7.1	299.3±23.6	3468±37	11.5
45	N		2.31	(87.36±0.45%)	nd.	---
46	N		6.53	3466±89	20540±258	5.9
47	N		5.12	1569±55	6623±76	4.2
48	N		7.64	205.2±22.4	4599±53	22.4

[a] using the differential scanning fluorimetry (DSF) at 10 μM concentration; [b]Using AlphaScreen assay, the values are the mean ± SD from two independent experiments. [c] nd: not determined. [d] n = 2.

We further designed a series of derivatives with multiple substitutions on the phenyl ring and examined their effects on activity and selectivity of BD1 and BD2 as shown in Table 3. The introduction of 2,6-dichloro (**29**) and 2,6-difluoro (**30**) substitutions in WPF shelf has a greater decreasing effect on the activity , while the activity of the 2,3-substituents (**31**) on the same side of the benzene ring is restored. In addition, it was found that 2,4-substituent groups (**32**-**34**) are more selective to BRD4 BD1. Among them, the IC_{50} values of **33** were 91.3±3.3 nM and 3828±48 nM for BD1 and BD2, respectively, thus about 42-fold selectivity (Table 3). Taking into account the physical and chemical properties, we have moderately decreased the activity and selectivity of the 5-position C of the benzofluorene ring with N in compound **48**, and its physical and chemical properties (**48** predicted tPSA: 88 and CLogP: 1.29; **25** predicted tPSA: 75 and CLogP: 2.03) are improved.

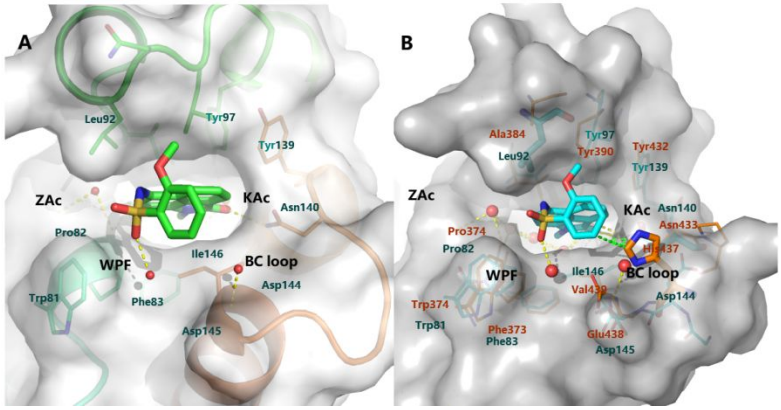


Figure 4. The conformation of compound **25** in the BRD4 BD1 and BRD4 BD2 proteins. (A) X-ray crystal structure of compound **25** bound to BRD4 BD1 (PDB ID: 6JJB, Authors will release the atomic coordinates and experimental data upon article publication). The ligands are shown as sticks models, and the protein is shown as surface and a cartoon with line model. (B) X-ray crystal structure of **25** (Surface and line, PDB ID: 6JJB) bound to BRD4 BD1, overlaid with BRD4 BD2 (stick and line, PDB ID: 5uf0).

To clarify the outstanding activity and selectivity of compound **25**, we determined crystal structure of compound **25** with BRD4 BD1 as shown in Figure 4A. The phenyl group of compound **25** differs from the conformation of the phenyl group of JQ1 (**1**) or other above derivatives (**6** and **15**, the binding mode in Figure 3) and does not completely occupy the WPF shelf, but is more adjacent to the region between Asp144 and Lue92. As shown in Figure 4B, steric hindrance may exist between the 2-methoxyphenyl of **25** and the imidazole of BD2-specific His437 (with a distance of around 3.2 Å from **25**), which suggests a very unfavorable conformation binding to BRD4 BD2. The 2-position methoxy group may play a crucial role in the selective mechanism, and this hypothesis was verified by the selection and activity in Table 2. The 2-methoxyphenyl module was moved forward in further optimization and new modules were added to further improve selectivity and activity.

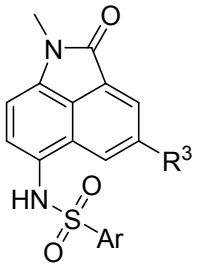
Optimization of the BC loop solvent region

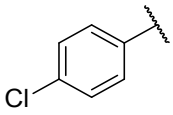
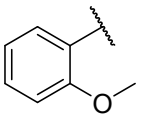
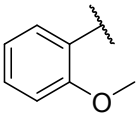
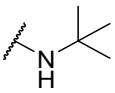
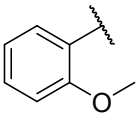
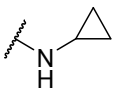
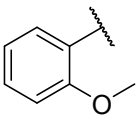
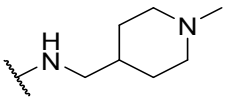
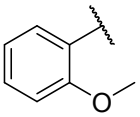
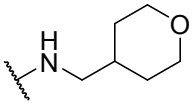
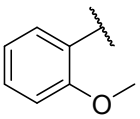
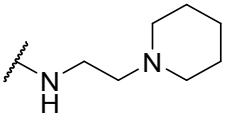
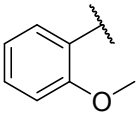
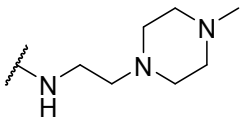
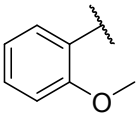
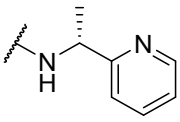
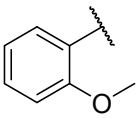
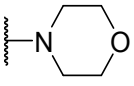
The BC loop solvent region formed by B loop and C loop was found to have a large difference in residues between the two domains BD1 and BD2, although the two domains are highly homologous in the BRD4 protein. The greatest differences lie in the BC loop, with Asp144 in BD1 changed to His437 in BD2 and an aspartic to glutamic acid switch of the adjacent residue. It was suspected that the introduction of appropriate hydrophilic groups in this region may be beneficial for activity and selectivity. Therefore, a series of derivatives in Table 3 were synthesized and evaluated for the binding potency of BRD4 BD1 and BRD4 BD2. First, **49** and **50** moderately decreased BRD4 BD1 efficacy compared to 5-position chlorine substitutions in **46** and **48**. Cyclic amine had good potency in different volumes of amino groups including

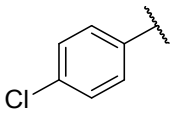
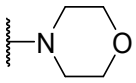
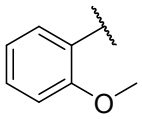
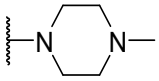
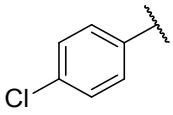
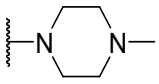
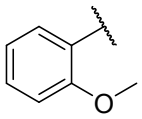
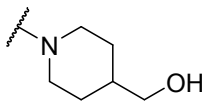
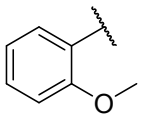
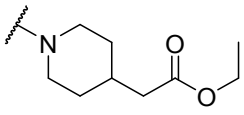
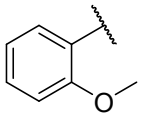
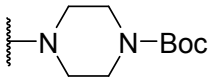
tert-butylamine, and cyclopropylamine.

Inspired by this structure-activity relationship, the difference in binding potency tolerance between BD1 and BD2 was examined using groups (different chain lengths and hydrophilic contributions at the 5-position R^3). The IC_{50} values from the AlphaScreen data showed that these derivatives had approximately a 5-fold change in potency (except **57**) for BRD4 BD1, but their binding potency to BRD4 BD2 was greatly affected. The methylpiperidine of **53** was more suitable for the solvent region of BRD4 BD1 than for the tetrahydro-2*H*-pyran of **54**. Interestingly, subtle changes of nitrogen atoms in **55** – **57** could obtain unexpected results. Among them, the IC_{50} values of **55** are 328 ±7.8 nM and 7068±69 nM for BD1 and BD2, respectively, thus about 21-fold selectivity. In contrast, the introduction of moderately hydrophobic pyridine **57** was nearly 20-fold lower for BD1 on potency. In order to further explore the characteristics of BD1 and BD2 in the solvent accessible region of BC, we designed a larger volume of six-membered cyclic hydrophilic fragments directly linked to the pyridine 2-position. As shown in the AlphaScreen assay of Table 4, the morpholine ring and the nitrogen methylpiperazine ring exhibited good potency against BD1 over BD2. However, the *N*-methyl group of *N*-methylpiperazine (**60** - **61**) didn't show improvement when it was replaced by other groups.

Table 3. Optimization of the hydrophilic group in the solvent accessible region



Cpd.	Ar	R^3	BRD4 IC_{50} (% protein activity at 2 μM)[a]		Selectivity
			BD1 (nM)[c]	BD2 (nM)	BD2/BD1
49		Cl	2598±49	nd.[b]	--
50		Cl	655.80±84.6	nd.	---
51			573.1±35.8	2085±47	3.6
52			271.2±22.8	660.2±22.6	2.4
53			113.0±1.8	1284±62	11.4
54			503.2±15.1	nd.	---
55			328 ±7.8	7068±69	21.5
56			223±15.3	1520±61	6.8
57			2226±46	nd.	---
58			95.2±12.3	808.4±26.9	8.5

59			217.1±32.5	nd.	---
60			100.0 ±6.8	2117±42	21.1
61			391.3±7.9	nd.	---
62			169.1±27.8	29638.1	17.5
63			709.4±73.2	nd.	---
64			334.2±7.8	2167±45	6.5

[a] Using AlphaScreen assay, the values are the mean ± SD from two independent experiments.

[b] nd: not determined. [c] n = 2.

To better understand the mechanism of selective binding of compound **60** to BRD4 BD1 over BRD4 BD1, we determined a co-crystal structure of compound **60** with BRD4 BD1. In Figure 5A, the 2-methoxyphenyl group of **60** differs from the selective dominant conformation of **25**, with deflection fully entering the WPF shelf. Therefore, the selectivity decrease of **60** is due to the fact that R^3 *N*-methylpiperazinyl changes the original conformation of 2-oxyphenyl group, resulting in reduced steric hindrance to imidazole H437 in BD2 (Figure 5B) without affecting the binding of BD1. Although the selective contribution of 2-methoxyphenyl was weakened in **60**, a new steric hindrance effect was obtained between *N*-methylpiperazinyl and imidazole H437 in

BD2 (the closest distance of 1.5 Å, Figure 5B). As shown in Table 3, the selectivity of **60** was 21-fold lower than **25** for BD1 over BD2.

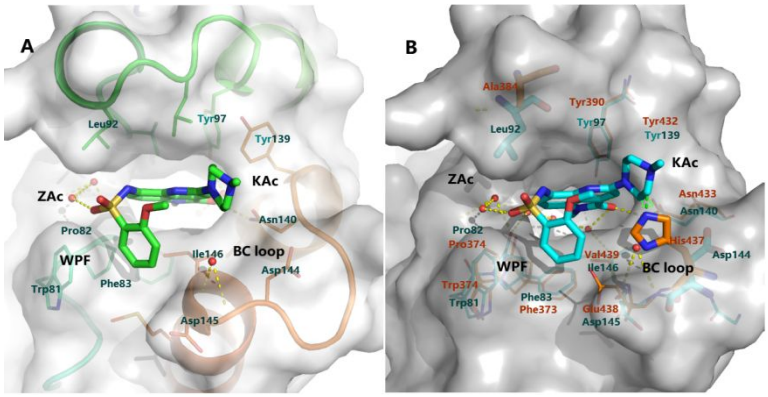
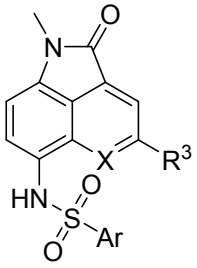


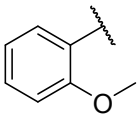
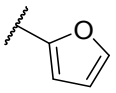
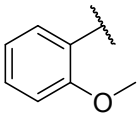
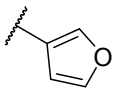
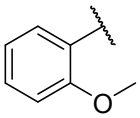
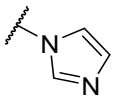
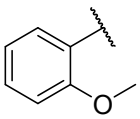
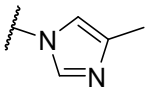
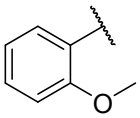
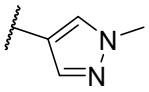
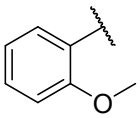
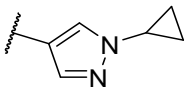
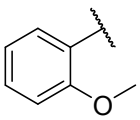
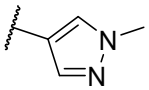
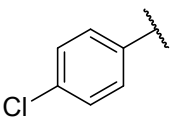
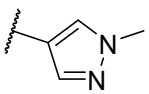
Figure 5. The conformation of compound **60** in the BRD4 BD1 and BRD4 BD2 proteins. (A) X-ray crystal structure of compound **60** bound to BRD4 BD1 (PDB ID: 6JJ3, Authors will release the atomic coordinates and experimental data upon article publication). The ligands are shown as sticks models, and the protein is shown as surface and a cartoon with line model. (B) X-ray crystal structure of **60** (stick) bound to BRD4 BD1 (line and cartoon, PDB ID: 6JJ3), overlaid with BRD4 BD2 (line and cartoon, PDB ID: 5uf0).

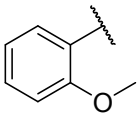
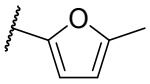
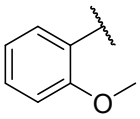
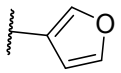
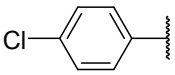
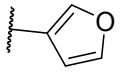
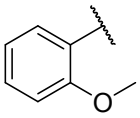
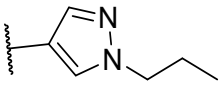
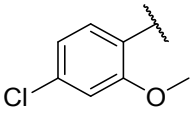
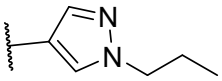
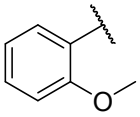
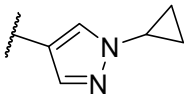
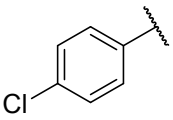
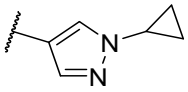
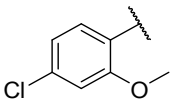
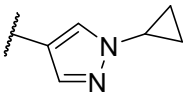
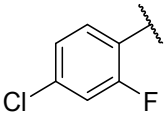
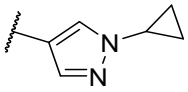
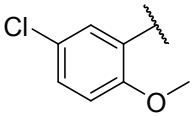
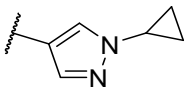
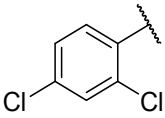
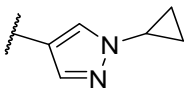
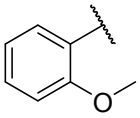
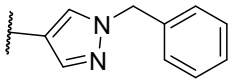
Based on the structure-activity relationship, we made an assumption that an appropriate rigid group substitution in the solvent region of R^3 may render a ligand with a high selectivity for BRD4 BD1 over BRD4 BD2. The rigid ring is more likely to have a repulsive effect with the imidazole ring of the His437 residue in BD2 and reduce the affinity for BD2. We designed multiple rigid five-membered heterocycles to validate this hypothesis. The activity evaluation results showed excellent protein binding ability and selectivity (Table 3). When X is a quinoline core and R^3 is unsubstituted 2-furan (**65**) and 3-furan (**66**), the IC_{50} values for BD1 are 35.1±2.2 nM and 37.0±1.3 nM, respectively. Next, we introduced an imidazole in **67** and a

methylimidazole ring in **68** with IC_{50} values of 889.0±32.9 nM and 87.7±4.9 nM for BD1, respectively. Excitingly, the IC_{50} values of Compound **68** are 87.7±4.9 nM and 12130±308 nM for BD1 and BD2, respectively, thus about 138-fold selectivity. The methyl group of **68** resulted in a significant steric hindrance effect between the methylimidazole (**68**) and the H437 residue of BD2, which in turn increased selectivity without affecting the binding of BD1. Compound **69** merely changes the position of the nitrogen atom compared to compound **68** resulting in a loss of selectivity of more than 100-fold. However, when 5-methylfuran-2-yl was introduced in **79**, the IC_{50} values suggested 857-fold selectivity (IC_{50} 54.8±6.7 nM and 47000±542 nM for BD1 and BD2). The hetero five-membered ring and a methyl group at R^3 in (**68**, **69**, **77** and **79**) showed good selectivity and activity. This also shows that the volume is favorable for the steric hindrance effect of the His437 of BRD4(2). In addition, due to the imidazole electron-deficient ring of His437, the hetero atom position of R^3 has a great contribution to the selectivity (**68** and **79**). The affinity of **80** for BD1 was reduced by more than 3-fold by the introduction of 3-furyl. When Ar is an 2-methoxyphenyl group in the WPF shelf and R^3 is a methyl (**69** and **77**), propyl (**82**), cyclopropyl (**70** and **84**) and benzyl (**90**) substituted pyrazole, its IC_{50} value for BD1 was between 14.4±5.7 nM and 205.2±16.2 nM, its selectivity being within 25-fold. Furthermore, when Ar in the WPF shelf is substituted by other substituted phenyl in **85-89** (except 2-methoxy-4-chlorophenyl), the activity is drastically reduced or even lost.

Table 3. Optimization of the hydrophilic group in the solvent accessible area



Cpd.	X	Ar	R^3	BRD4 IC_{50} (% protein activity at 2 μM)		Selectivity
				BD1 (nM)[c]	BD2 (nM)	BD2/BD1
65	N			35.1±2.2	981.7±42.7	27.3
66	N			37.0±1.3	458.7±52.5	12.4
67	N			889.0±32.9	4110±56	4.6
68	N			87.7±4.9	12130±308	138.3
69	N			12.6±2.7	289.3±53.6	22.9
70	N			16.6±9.3	107.1±8.5	6.5
77	C			17.4±5.6	434.9±7.3	25.0
78	C			348.2±1.1	nd.	---

79	C			54.8±6.7	47000±542	857.2
80	C			163.7±19.3	nd.	---
81	C			(56.59±1.31 %)	nd.	---
82	C			43.7±12.6	265.6±7.9	6.2
83	C			106.7±8.5	320.2±46.7	3.0
84	C			14.4±5.7	237.6±7.4	16.9
85	C			665.7±25.7	nd.	---
86	C			37.5±6.8	434.9±72.6	11.6
87	C			(65.17±3.27%)	nd.	---
88	C			(64.88± 0.54%)	nd.	---
89	C			(61.84±0.23 %)	nd.	---
90	C			205.2±16.2	nd.	---

[a] Using AlphaScreen assay, the values are the mean ± SD from two independent experiments.

[b] nd: not determined. [c] n = 2.

In vitro evaluation of selected compounds on anti-inflammatory activity

To investigate the role of single Bromodomains in the development of inflammatory diseases, we evaluated the selective-bromodomain BET inhibitors and pan-BET inhibitors to inhibit NF-κB activity in HUVECs cells and inhibit nitric oxide (NO) production in RAW264.7 cells. Detection of NO in macrophages is widely used in the screening of anti-inflammatory drugs[42, 43]. Ana S. Leal etl etc.[44] reported that pan-BRD4 inhibitors play an important role in the tumor microenvironment. These drugs suppressed the production of NO in RAW264.7 cells. Therefore, we selected 17 compounds at 1 μM to evaluate NO inhibition rate treated with LPS 1 μg/mL in macrophages. From the results shown (Table 4), most of the compounds showed significant activity to reduce the concentration of NO production. The inhibition rate of 7 compounds was greater than 90% and the selectivity of compounds **33** and **68** was more than 40-fold. Remarkably, the NO inhibition rate of RVX-208 with selective binding to BD2 was significantly lower than the NO inhibition rate of inhibitors with high activity for BD1 (JQ1 and other compounds). In addition, the highly selective compounds **25** and **79** failed to decrease in NO production which may be due to poor permeability (**79** with low permeability 0.060 nM/s and **68** with moderate permeability 1.172 nM/s in Table S2).

Table 4. In RAW264.7 cells the downregulate NO production activities of selected compounds at 1 μM treated with LPS 1 μg/mL

Cpd.	BRD4 IC_{50} (nM)	Sel.	At 1 μM

	BD1	BD1	BD2/BD1	NO Inhibition rate (%)[a]
19	167±14	2717±401	16.2	15.09±8.64%
25	49.5±10.3	2479±170	50.1	51.37±4.13%
28	172.1±12.8	1640±68	9.5	101.89±0.05%
33	91.3±3.3	3828±48	41.9	98.11±3.27%
48	205.2±22.4	4599±53	22.4	94.34±3.26%
53	113.0±1.8	1284±62	11.4	47.17±18.20%
55	328 ±7.8	7068±69	21.5	43.40±8.65%
56	223±15.3	1520±61	6.8	39.62±1.05%
58	95.2±12.3	808.4±26.9	8.5	100.00±3.28%
60	100.0 ±6.8	2117±42	21.1	92.45±3.22%
65	35.1±2.2	981.7±42.7	27.3	64.86±8.14%
66	37.0±1.3	458.7±52.5	12.37	66.28±2.98%
68	87.7±4.9	12130±308	138.3	101.89±1.01%
77	17.4±5.6	434.9±7.3	24.99	101.89±5.66%
79	54.8±6.7	47000±542	857.2	58.49±17.29%
82	43.7±12.6	265.6±7.9	6.2	105.66±3.27%
84	14.4±5.7	237.6±7.4	16.9	83.73±1.10%
JQ1	42.24±5.08	24.71±1.01	0.5	47.56±0.55%
RVX-208 [b]	1800	40	0.02	9.17±0.41%

[a] Using Griess reagents method, the values are the mean ± SDs from three independent experiments, n = 3. NO inhibition rate % = (model group NO concentration – Compound group NO concentration) / (model group NO concentration– control group NO concentration) ×

100%. [b] The data was assayed by TR-FRET[45].

The dual luciferase reporter gene assay data (Figure 7 and Table S3) showed that the selective BD1 inhibitors and pan-BET inhibitor (JQ1) were significantly more potent in down-regulating the NF-κB activity than the selective BD2 inhibitor (RVX-208). Compound **68** of NF-κB transfection activity was superior to compounds **65** and **79**, comparable to JQ1 inhibitory activity. Obviously, these results suggest that inhibitors selectively binding to BD1 have a better anti-inflammatory activity than inhibitors selectively binding to BD2 in vitro.

In the evaluation of in vitro inflammatory activity, the selective BET BD1 inhibitor **68** (inhibition rate: 101.89±1.01%) was significantly superior to the pan-BET inhibitor JQ1 (inhibition rate: 47.56±0.55%) in inhibiting the NO production of macrophage RAW264.7. The NO production is regulated by multiple inflammatory signaling pathways, and NF-κB signaling pathway is one of the important pathways regulating NO production. Selective BET BD1 inhibitor **68** of NF-κB transfection activity was comparable to pan-BET inhibitor JQ1 inhibitory activity. It is demonstrated that the selective BET BD1 inhibitor (**68** BRD4(1): 87.7±4.9 nM; BRD4(2): 12130±308 nM) still maintains comparable or better anti-inflammatory activity than the pan-BET inhibitor (JQ1 BRD4(1): 42.24±5.08 nM; BRD4(2): 24.71±1.01 nM) compared to the protein weak activity. Based on our analysis, compound **68** was considered for further studies in biological mechanisms and diseases.

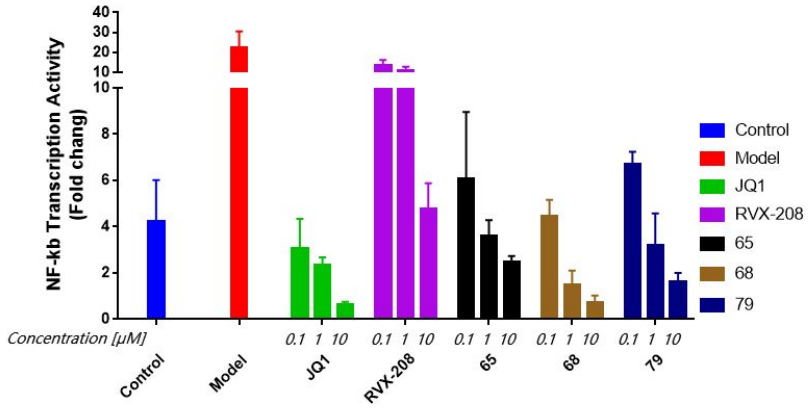


Figure 7. Examination for the NF-κB transcriptional activity in HUVECs undergoing three concentration degrees (0.1μM, 1 μM and 10 μM) treated JQ1, RVX-208 and compounds for 24 h, respectively. The models were established by TNF-A 50 ng/mL. The values of fold changes are the mean ± SDs from three independent experiments, n = 3.

Bromodomains activity profile of compound 68

The lead compound **68** displayed highest inhibitory activity against BRD4(1) (IC_{50}: 87.7±4.9 nM), BRD3(1) (IC_{50}: 246.3±20.2 nM) and BRDT(1) (IC_{50}: 357.1±8.3 nM). It should be noted that **68** also has inhibitory activities against BRPF1b (IC_{50}: 567.5±16.9 nM). In addition, compound **68** showed 102-fold selectivity for BRD4(1) over BRD2(1). The Kd values from the BROMOscan™ Kd data (Figure S2) suggests 238-fold selectivity towards BD1 over BD2 (Kd 105±5 nM and > 25 μM for BD1 and BD2, respectively). Therefore, compound **68** was identified as a highly selective BET BD1 inhibitor and the inhibition of single bromodomain by compound **68** may be beneficial to its anti-inflammatory efficiency and reduce potential toxicity.

Table 5. Inhibitory activities of compound **68**, toward BRD2(1), BRD2(2), BRD3(1), BRD3(2), BRD4(1), BRD4(2), BRDT(1) and BRPF1b.

BRDs	IC_{50} (nM) [a]	Selectivity BRDs/BRD4(1)	BRDs	IC_{50} (nM) [a]	Selectivity BRDs /BRD4(1)	Selectivity BD2/BD1
BRD2(1)	8948±457	**102.3**	**BRD2(2)**	21450±127	244.5	2.4
BRD3(1)	246.3±20.2	**2.8**	**BRD3(2)**	49770±258	567.5	202.1
BRD4(1)	87.7±4.9	**1.0**	**BRD4(2)**	12130±308	138.3	138.3
BRDT(1)	357.1±8.3	**4.1**	**BRPF1b**	567.5±16.9	6.5	----

[a] Using AlphaScreen assay, the values are the mean ± SD from two independent experiments, n = 2.

Pharmacokinetics of 68 in Liver Microsomes of Multiple Species

The preliminary metabolic stability study of compound **68** were performed by using human liver microsomes (HML), rat liver microsomes (RLM), mouse liver microsomes (MLM), dog liver microsomes (DLM) and monkey liver microsomes (CLM) (Table 6 and Table S1). The terminal $t_{1/2}$ was moderate in human liver microsomes, rat liver microsomes, mouse liver microsomes and monkey liver microsomes (ranged between 9.4 min and 14.8 min). Compound **68** has a high clearance rate in the range of 93.517 μL/min/mg proteins to 146.685 μL/min/mg proteins for different species. Overall, compound **68** exhibited moderately stable levels of in vitro liver microsomal metabolism. Therefore, intra-articular injections for the treatment of acute gout arthritis were superior to oral and intravenous injections.

Table 6. Pharmacokinetics of compound **68** in liver microsomes of multiple species

Compounds	Species	R^2 [a]	$T_{1/2}$ (min)[b]	$CL_{int, microsome}$ ($\mu L \cdot min^{-1} \cdot mg^{-1}$ proteins)[c]	$CL_{int, in\ vivo}$ (mL/min/kg)[d]

68	Human	0.9840	14.821	93.517	88.373
	Monkey	0.9918	10.263	135.043	194.461
	Dog	0.9105	10.409	133.159	191.748
	Rat	0.9635	9.449	146.685	264.034

[a] R^2 is the correlation coefficient of the linear regression for the determination of kinetic constant.[b] $T_{1/2}$ is the half life, [c] C_{Lint} is the intrinsic clearance, [d] $C_{Lint,\ in\ vivo}$ is the intrinsic hepatic clearance;

Compound 68 inhibited MSU-induced pyroptosis of THP-1 cells through BRD4/NF-κB/NLRP3 signaling pathways

To investigate the anti-inflammatory mechanisms, we explored the relationship between compound **68** with high selectivity for BET BD1 in inflammation-related pyroptosis and signaling pathways in vitro. Pyroptosis was defined to activate in the presence of propidium iodide (PI) and active Caspase-1 double positivity by flow cytometry. MSU exposure in the model group exhibited a significant increment in the amount of PI and Caspase-1 double positive cells in comparison with normal group, which could be reversed by pretreatment of compound **68** at 1 μM (Figure 8A). More importantly, as shown in Figure 8B, MSU stimulation (500 μg/mL) contributed to the up-regulation of BRD4, phosphorylation of NF-κB p65 and activation of NLRP3 inflammasome compared with normal group. These increased levels were notably attenuated by compound **68** intervention. Inhibition of compound **68** in the activation of NRLP3 inflammasome was also confirmed by immunofluorescence data (Figure 8C). Increased fluorescence intensity in NLRP3 and ASC staining as well as the co-localization of NLRP3 and ASC in merged data were

significantly decreased by compound **68** (Figure 8C). Moreover, MSU-induced elevation of IL-1β concentrations was ameliorated by compound **68** treatment (Figure 8D), suggesting that compound **68** with high selectivity for the first bromodomain could improve MSU-induced inflammatory cascade and pyroptosis via inhibiting BRD4/NF-κB/NLRP3 signaling pathways.

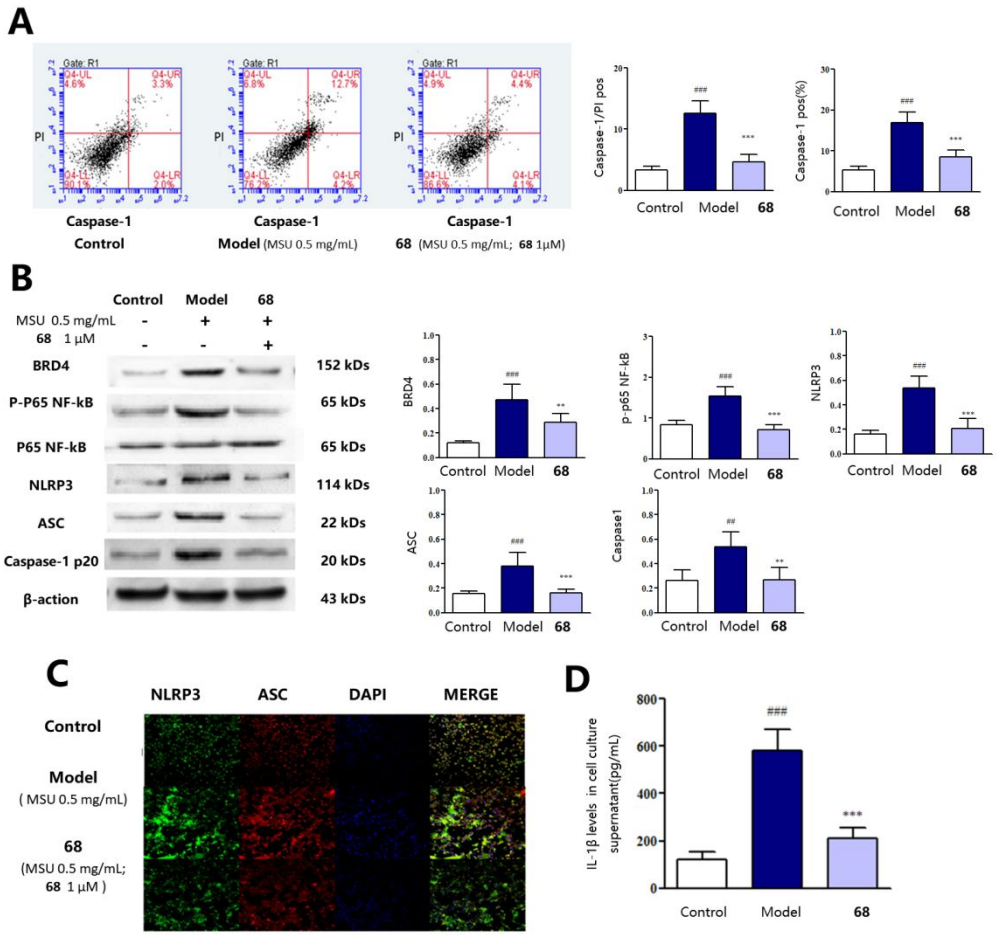


Figure 8. Compound **68** inhibits MSU-induced pyroptosis in THP-1 cells. After PMA treatment, THP-1 cells were incubated with the absence or presence of serum free medium containing **68** (1 μM) for 1 h, followed by the stimulation with MSU (500 μg/mL) for 12 h. The rate of pyroptotic cell death was examined with active PI and Caspase-1 double staining by flow cytometry (A). The expression of BRD4 and NRLP3 inflammasome activation was measured by western blotting and protein quantitative analysis normalized to β-action (B). Representative confocal microscopy photographs of THP-1 cells with immunofluorescence changes are presented (C). The culture supernatants level of IL-1β was detected using the ELISA kit (D). The data was presented as

means ± SDs. Compared with Control group: $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. Compared with Model group: *P<0.05, **P<0.01, ***P<0.001. Each group (n=4). Independent experiments were performed in triplicate.

Compound 68 improved MSU-induced acute gouty arthritis

With the promising In vitro results, we further investigated the pharmaceutical effect of compound **68** in rat. As shown in Figure 9C, after treatment with compound **68** via intra-articular injection (ia) at a dose of 1 mg/kg, the challenged joints were subjected to circumference detection at 0, 2, 4, 8, 12, 24 h after MSU crystals induction at 4 mg/kg intra-articular injection (ia). As shown in Figure 9A, joint circumference in the model group increased approximately 1.28-fold at 2, 4, 8 h time points and enlarged to 1.52-fold at 12 h point than that of control group, while treatment with compound **68** restored the joint circumference to normal level, suggesting the powerful defense against swelling pathology brought by MSU-induced acute gouty arthritis. These results were supported by histopathological analysis of H&E-stained synovial tissue specimens, which revealed that rats receiving compound **68** exhibited suppressed synovial hyperplasia as well as severe neutrophil infiltration compared with the model group (Figure 9B). In addition, an attenuation of MSU-induced raise in the synovial IL-1β level was observed after the treatment of compound **68**, which was consistent with in vitro study (Figure 9D). The in vivo study suggested that compound **68** has a good therapeutic effect on MSU-induced acute gouty arthritis.

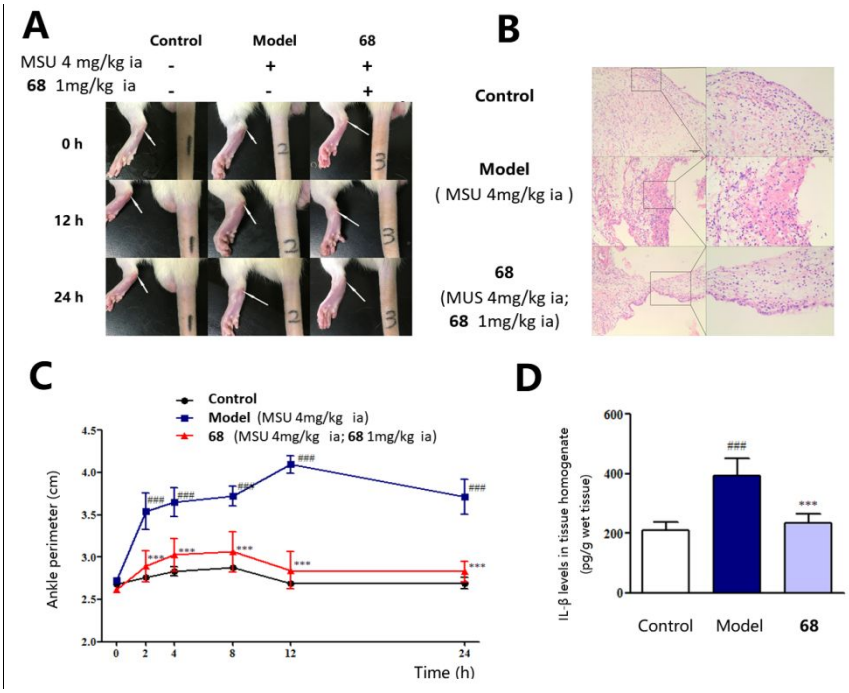


Figure 9. The effect of BRD4 inhibitor on MSU-induced acute gouty arthritis (4 mg/kg, intra-articular injection) in vivo. Representative photographs to show the swelling of joints are presented (A). Representative photographs of histopathologic changes in synovium are presented (B).The injected ankle joint circumference of each rat was determined at 0, 2, 4, 8, 12, 24 h after MSU stimulation (C). The IL-1β level in synovium homogenate was detected by the ELISA kit (D). The data was presented as means ± SDs. Compared with Control group: $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. Compared with Model group: *P<0.05, **P<0.01, ***P<0.001. Data in panels represent means ± SDs from 8 animals. Independent experiments were performed in triplicate.

Compound 68 suppressed pyroptosis of macrophages in rat synovial tissues through regulating BRD4/NF-κB/NLRP3 signaling pathway

To further validate the in vivo biological mechanisms, we investigated whether compound **68** suppresses pyroptosis of macrophages in rat synovial tissue through regulating BRD4/NF-kB/NLRP3 signaling pathway. To determine the rate of pyroptotic cell death in synovial tissue, macrophages were classified based on positive staining for CD68. As shown in Figure 10A,

there was a remarkable elevation in the number of PI and Caspase-1 double-positive macrophages obtained from rat synovium of model rats in contrast with those of normal group, while this increased number was pulled down closer to normal level by the intervention of compound **68**. Consistent with in vitro results, western blotting analysis revealed a significant increase in BRD4 up-regulation, NF-κB p65 phosphorylation and NLRP3 inflammasome activation after MSU exposure in synovium specimens, which could be restored by compound **68** treatment (Figure 10B). Besides, immunofluorescence assay of synovial samples showed that compound **68** treatment could inhibit the enhanced fluorescence intensity of synovial NLRP3, ASC and their co-localization induced by MSU injection (Figure 10C). This study confirmed that compound **68** was able to improve MSU-induced acute gouty arthritis through regulating BRD4/NF-κB/NLRP3 signaling pathways.

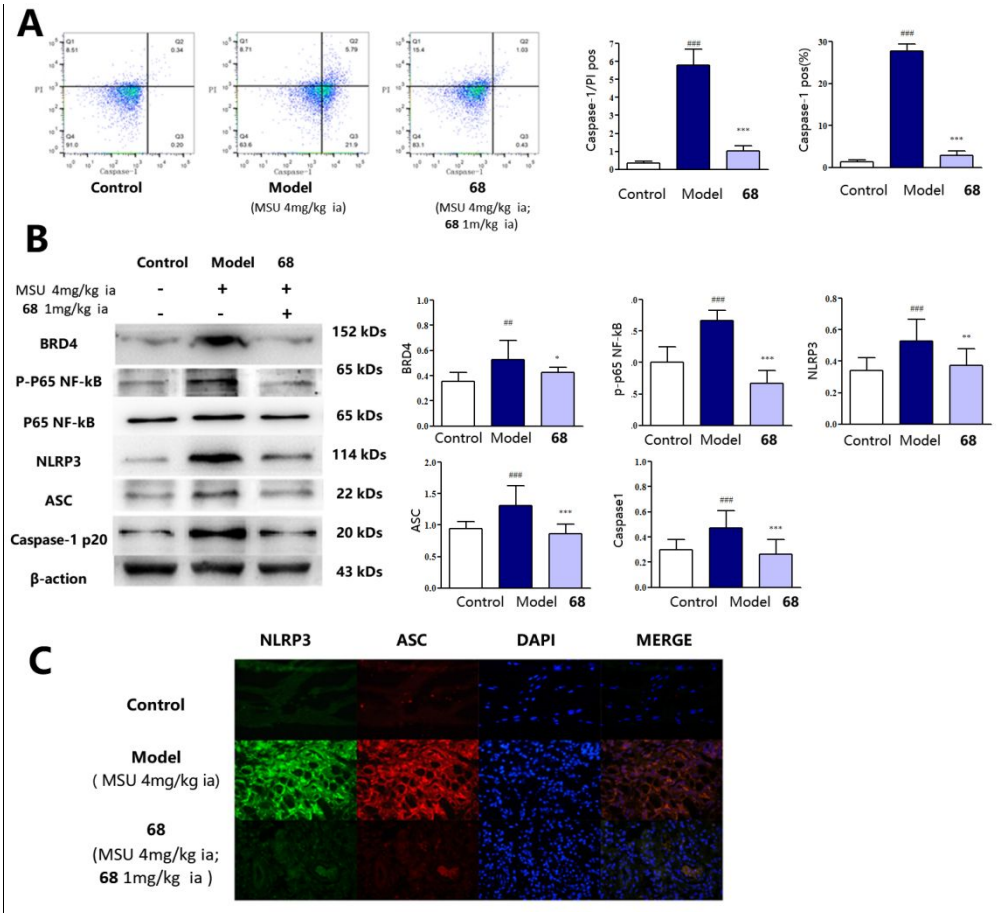


Figure 10. Compound **68** regulates the pyroptosis death in synovial tissue. Pyroptosis assay was performed in the macrophages obtained from synovium using active PI and Caspase-1 double staining by flow cytometry (A). The protein expression of BRD4 and NLRP3 inflammasome pathway in synovium after compound **68** treatment at 1 mg/kg intra-articular injection (ia) by western blotting and protein quantitative analysis normalized to β-action (B). NLPR3 inflammasome activation in synovial tissues was evaluated with immunofluorescence staining (C). The data was presented as means ± SDs. Compared with Control group: $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. Compared with Model group: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Data in panels represent means ± SDs from 4 animals. Independent experiments were performed in triplicate.

CONCLUSIONS

In this study, a promising BD1-selective BET inhibitorwith a benzo[*cd*]indol-2(1*H*)-one core was obtained by virtual screening. Starting from this scaffold, we designed and synthesized a series of derivatives, which demonstrated high BRD4 BD1 potency, significant selectivity over the BRD4 BD2 and excellent selectivity for all other bromodomains and activities. Among them, Compound **68** (LT052) showed nanomolar BRD4 BD1 potency and 138-fold selectivity over BRD4 BD2. Our detailed high-resolution crystal structural analysis of the BRD4 BD1 bound to the lead benzo[*cd*]indol-2(1*H*)-one ligands reveals that the steric hindrance effect of the amino residues in the BC loop region of BRD4 BD2 have an important contribution to the selectivity of BD1, especially His437 (Figure 4 and Figure 5).

Compound **68** (inhibition rate: 101.89±1.01%) displayed better activity in reducing NO production in RAW264.7 cells than JQ1 (inhibition rate: 47.56±0.55%) and other compounds in the same series (Table 4). Besides, **68** displayed good activity inhibiting NF-κB transcriptional

activity in HUVECs. Due to the inhibitory activity of pan-BET inhibitors on multiple Bromodomains, pan-BET inhibitors exhibit various toxicities and side effects such as thrombocytopenia, fatigue and nausea in clinical trials[46]. Among them, some toxicity and side effects have a dose-dependent limitation of drug treatment[47]. Moreover, the selectivity for BRD4 BD1 was more closely related to their anti-fibrosis activity, whereas, the selective BD2 inhibitor (RVX-208) did not indicate any of this activity[48]. Furthermore, NUE20798 with potent and benign safety against BET BD1 in fibrosis diseases and atopic dermatitis has been developed and without the observation of the typical adverse toxicity seen with non-selective BET inhibition[49]. Encouraged by the results, NUE20798 hopes to launch human clinical trials. It provides a value therapeutic strategy for drug development in the inhibition of the single bromodomain of the intra-BET family.

Further studies revealed that compound **68** with BRD4 / NF-κB / NLRP3 signaling pathway inhibits MSU-induced apoptosis of THP-1 cells and improves MSU-induced acute gouty arthritis. In the rat animal model, Compound **68** inhibits the pyrophosphorylation of macrophages in rat synovial tissue by modulating this signaling pathway, thereby achieving treatment of acute gout arthritis. Currently, there are few specific drugs for the treatment of acute gout arthritis, and more toxic side effects[50, 51]. We explored a new therapeutic mechanism for the treatment of acute gout joints by selectively inhibiting BET BD1, which has clinical therapeutic significance. Meanwhile, the identification of the selective BET BD1-bromodomain inhibitors may facilitate studies on the biological role of the BET BD1-bromodomain and bromodomain-selective treatment of acute gout sources.

EXPERMENTAL SECTION

Protein Inhibition Assay

The differential scanning fluorimetry (DSF) was tested on a Mx3005p Real Time PCR machine (Stratagene) device. The BRD4 protein was added to a buffer solution containing 10 nM HEPES, 500 nM NaCl, pH 7.5, and finally formulated into a 2 μM/20 μL solution in a 96-well plate. The test compound was added to the solution at a concentration of 10 μM while adding the fluorescent probe SYPRO Orange at a ratio of 1:1000. The excitation and emission wavelengths of SYPRO Orange are set at 465 nm and 590 nm, respectively. The temperature is raised from 25 °C to 96 °C by 3 °C per minute and fluorescence readings are taken at each interval. Then, according to the theoretical conversion formula between fluorescence intensity and temperature change, the temperature difference between the protein before and after binding of the ligand is calculated by mathematical statistics, as a method for evaluating the binding ability.

The AlphaScreen technology assay[52] is used the binding assay of the readers, which was performed by Reaction Biology Corp. (Malvern PA) as described previously. The biotinylated peptide binding to the reader domain of His-tagged protein is monitored by the singlet oxygen transfer from the Streptavidin-coated donor beads to the AlphaScreen Ni-chelate acceptor beads. *Reagent*: Reaction buffer for BRD4s: 50 mM Hepes, pH7.5, 100 mM NaCl, 0.05% CHAPS, 0.1 % BSA, and 1% DMSO final. Reaction buffer for TAF1-2: 20 mM Tris, pH 8, 25 mM NaCl, 0.05% Tween20, 3 mM Bmercaptethanol, and 0.25% DMSO final (the final DMSO concentration may different depending on compound stock and test concentrations). Bromodomain BRD4-1: RBC Cat# RD-11-140 Recombinant Human Bromodomain containing protein 4, bromodomain 1 (aa

44-170; Genbank Accesstion # NM_058243), expressed in E. coli with N-terminal His-tag. MW=17.8 kDa. BRD4-2: RBC Cat# RD-11-141 Recombinant Human Bromodomain containing protein 4, bromodomain 2 (aa 349-460; Genbank Accesstion # NM_058243), expressed in E. coli with N-terminal His-tag. MW=15.7 kDa. bromodomain 2 (aa 1502-1635; Genbank Accesstion # NM_138923), expressed in E. coli with N-terminal His-tag. MW=18.4 kDa. Ligand (C-term-Biotin) Histone H4 peptide (1-21) K5/8/12/16Ac-Biotin Detection beads: PerkinElmer Donor beads: Streptavidin-coated donor beads Acceptor beads: AlphaScreen Ni acceptor beads *Reaction Procedure:* 1. Deliver 2.5X BRD in wells of reaction plate except No BRD control wells. Add buffer instead. 2. Deliver compounds in 100% DMSO into the BRD mixture by Acoustic technology (Echo550; nanoliter range). Spin down and pre-incubation for 30 min. 3. Deliver 5X Ligand. Spin and shake. 4. Incubate for 30 min at room temperature with gentle shaking. IC_{50} values and curve fits were obtained by GraphPad Prism 7.0 Software.

BROMOscan™ is a novel industry leading platform for identifying small molecule bromodomain inhibitors, which was performed by DiscoverX Corp. as described previously (www.eurofinsdiscoveryservices.com)[53]. Assay Process shown 5 steps: 1. Assemble Assay Components:*E. coli* or mammalian cell-expressed bromodomain labeled with DNA tag for qPCR readout Known bromodomain ligand immobilized on a solid support Test compound or control (solvent only); 2. Equilibrate; 3. Wash solid support to remove unbound bromodomain; 4. Quantify bromodomain captured on solid support (qPCR); 5. Compare captured bromodomain levels in test compound and control samples. The compound(s) were screened at the concentration(s) requested, and results for primary screen binding interactions are reported as "% Ctrl". An 11-point 3-fold serial dilution of each test compound was prepared in 100% DMSO at 1000x final test concentration. All

compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The K_d values and curve fits were obtained by GraphPad Prism 7.0 Software.

Griess reagents method for detecting NO in RAW264.7 cells

DMEM medium was cultured in RAW264.7. The cell concentration was 5*104 cells/mL, and seeded in a 96-well plate at 200 uL per well, cultured at 37 degrees, 5% CO_2 for 24 hours, and the medium was discarded. 197 μL of medium (no serum) was added: blank control group, LPS model group, and test drug group. Subsequently, 2 μL of the medium was added to the blank control group. The LPS model group was spiked with 2 μL of DMSO at a concentration of 1 μM in the test group and 3 replicate wells per sample. After dosing, incubate for 2 h in a 37-degree, 5% CO_2 incubator, then add 1 uL of PBS-configured LPS (200 ug/mL) to the LPS control group and each test group to a final concentration of 1 ug/mL. After continuing to culture for 22 h, the cells were centrifuged, and the supernatant was weighed to determine the NO content by Griess reagents. Mean and STDEV are shown. Inhibition rate = (model group NO concentration – Compound group NO concentration) / (model group NO concentration– control group NO concentration) × 100%.

The dual luciferase reporter assay for detecting NF-kB activity in HUVEC cell lines

To confirm the alteration of NF-kB activity by inhibitors in HUVEC cell lines, we performed a dual luciferase reporter assay. The p-NF-kB luc vector (Clontech, Palo Alto, CA) contains the firefly luciferase gene and four copies of the NF-kB consensus sequence. The phRL-null vector (Promega) contains a renilla luciferase gene, and the transfection efficiency was normalized by cotransfection with this vector. The p-NF-kB luc and the phRL-null vectors were cotransfected in

a 30:1 ratio using SuperFect (Qiagen, Valencia, CA) according to the manufacturer of instructions. After 12 h transfection, the medium was treated with compounds and positive inhibitors (except the control group) for 0.5 h followed by treatment with TNF-A (50 ng/mL). Another 24 h later, luciferase activity was measured by luminometer (Lumat LB 9507; EG&G Berthold Tokyo, Japan) using the dual luciferase reporter assay system (Promega).

Metabolic Stability in Liver Microsomes

The metabolic stability of compound **68** in RLM (rat liver microsomes), DLM (dog liver microsomes), CLM (monkey liver microsomes) and HLM (human liver microsomes) were tested following the same procedures by 3D BioOptima Co. Ltd. Add 10 µL compound **68** or control working solution/well to all plates (T0, T5, T15, T30, T60, NCF60) except matrix blank. Then add 80 µL/well to every plate by Apricot, incubate the mixture of microsome solution and compound at 37°C for about 10 min. Add 10 µL 100 mM potassium phosphate buffer/well to NCF60, incubate at 37°C for 1h. After pre-warming, add 10 µL/well to every plate by Apricot to start reaction. Reactions were terminated at 5, 15, 30 and 60 min of incubation with a chilled mixture of Tolbutamide and Labetalol (1:1). The mixture was vortexed for 5 min, centrifuged at 4000 rpm for 20 min at 4°C, and the supernatants were analyzed by LC−MS/MS. The data was analysis by the first order kinetics to calculate $t_{1/2}$ and Cl. Drug elimination rate constant k (min^{-1}), elimination half-life $T_{1/2}$ (min), and in vitro intrinsic clearance CLint, in vitro ($\mu L \cdot min^{-1} \cdot mg^{-1}$ protein) were calculated according to the following equations: k = - slope, $T_{1/2}$ = 0.693/k, C_{Lint}, in vitro = $k/C_{protein}$, where $C_{protein}$ ($mg \cdot mL^{-1}$) is the microsomal protein concentration in the incubation.

Molecular Modeling

The X-ray crystal structure of BRD4(1) binding with (+)JQ1(**1**) (PDB code: 3MXF) and BRD4(2) (PDB code: 5uf0) were retrieved from the Protein Data Bank[54]. Compounds **6** and **15** were prepared using the protein preparation wizard in Maestro with standard settings. Grids of BRD4(1) and BRD4(2) were generated using Glide, version 10.2, following the standard procedure recommended by Schrodinger. The conformational ensembles were docked flexibly using Glide with standard settings in both standard and extra precision mode. Only poses with low energy conformations and good hydrogen bond geometries were considered. Molecular-protein docking results and small molecule-protein co-crystal structures were shown to use the Pymol. At the same time, the overlap of BRD4(1) and BRD4(2) were the align module of the Pymol software. The numbering of the amino residues of the protein and the editing of the figure were performed using HprSnap7.

X-ray Crystallography

Protein Crystallization. Crystallization was performed by using the sitting drop vapor diffusion method. One μL of protein solution containing 7 mg/ml N-terminal bromodomain of BRD4, 5 mM inhibitors and 10 mM methyl 2-oxo-2,3-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-6-carboxylate in 20 mM Tris, pH 8.0, 50 mM NaCl, was mixed with 1 μL of well solution containing 4.0 M Sodium Formate. The crystallization drop was incubated against 50 μL of well solution at 295 K. The best rod-like crystals of maximally 200 × 50 × 50 μM appeared after 12 hours and reached final size after 24 hours.

Data Collection and Processing. Crystals were transferred to a cryoprotectant solution comprising 4.0 M Sordium Formate and 20% Glycerol then flash frozen in liquid nitrogen. X-ray data of each protein-inhibtor complex were collected from a single crystal at 100 K at BL17U1 of Shanghai Synchrotron Radiation Facility. Images were processed with Mosflm[55]. The crystals belong to space group $P2_12_12_1$, with one molecule per asymmetric unit (Table S5).

Phasing, Model Building, and Refinement. The structures of protein-inhibitor complexes were solved by molecular replacement implemented in Phaser[56]. Coordinates of N-terminal bromodomain of BRD4 (PDB ID code 4PCE) were used as the original search model. For each protein-inhibtor complex, the initial electron density difference map clearly showed extra density indentified as the inhibitor in the binding pocket of BRD4. The structure models of the inhibitors were manually fit in the extra electron density using COOT[57]. The built models were refined by the program PHENIX[58]. Figures of protein structures were created with Pymol (http://www.pymol.org).

Cell Culture

THP-1 cell line was obtained from American Type Culture Collection (Manassas, VA, USA). The THP-1 cells were cultured at 37 °C under 5 % CO_2 in RPMI1640 medium supplemented with 10% fetal bovine serum (Gibco, USA), 100 IU/ml penicillin, and 100 IU/ml streptomycin. After phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich, St. Louis, MO, USA) treatment, the THP-1 cells were incubated with the absence or presence of serum free medium containing inhibitors (1 μM) for 1 h, followed by the stimulation with MSU (0.5 mg/mL) for 12 h. Subsequently, the culture supernatants were collected for IL-1β detection and the cell lysates were utilized for protein

extraction.

Animals models

24 Male Adult Sprague-Dawley rats (250-280 g) purchased from Central Institute for Experimental Animals of Zhejiang were allowed one week to adapt to the laboratory environment before used for experiments. All animal studies were performed in accordance with the Animal Ethics Committee of China Pharmaceutical University. Acute gouty arthritis animal models were established by MSU crystals injection (1.0 mg suspended in 50 μl sterile saline, 4 mg/kg intra-articular injection). Rats were randomly assigned into three groups (n = 8 per group): (1) Normal group, (2) Model group, (3) **68**(LT052) group. Rats received intra-articular injection of vehicle (0.1% DMSO) or **68**(LT052) (1 mg/kg) 1 h before treatment of MSU. The injected ankle joint circumference of each rat was determined at 0, 2, 4, 8, 12, 24 h after MSU stimulation. Then animals were sacrificed, serum and synovial tissues were collected for further detection.

Measurements of IL-1β

The levels of IL-1β in THP-1 cell supernatants and rat synovial tissue homogenates were detected using ELISA Kits (R&D, Minneapolis, USA) followed the manufacturer's instructions.

Pyroptosis assay

Pyroptosis was determined following the instruction of FLICA® 660 in vitro Caspase-1 Detection Kit (Immuno Chemistry Technologies, Bloomington, USA) by flow cytometry, while propidium iodide (PI) was used to mark cells with membrane pores (Beyotime Biotechnology, Nanjing, CN). To determine the rate of pyroptotic cell death in synovial tissue, macrophages were

classified using CD68 staining.

Immunofluorescence

The fixed synovial tissues from rats were embedded, decalcified and sectioned, while THP-1 cells were fixed with 4% Paraformaldehyde Fix Solution at room temperature after experiments. Immunofluorescence staining was conducted using primary antibodies at 4 °C overnight. Subsequently, the samples were incubated with different polyclonal secondary antibody at a 1:200 dilution for the primary antibodies detection. After being washed in PBS, the samples were incubated for 1 h at room temperature with DAPI followed by observation using a confocal laser scanning microscope (LSM 700, Zeiss, CA, USA). The antibodies were listed in Table S6.

Western blot

The protein extractions of THP-1 cells and rat synovial tissues were prepared for western blot analyses of BRD4, p-p65 NF-κB, p65 NF-κB, NLRP3, ASC, Caspase-1 p20 and β-actin. Western blot analysis was performed as in our previous study. The levels of target proteins were determined using a gel imaging system (ChemiScope 2850, Clinx Science Instruments Co., Ltd., Shanghai, China) and normalized to that of the reference band. Primary antibodies were listed in Table S7.

Hematoxylin-eosin (H&E) staining

The synovium tissue samples were collected immediately, fixed in the 4% paraformaldehyde solution for 48 h and then embedded with paraffin. After that, coronal sections having penumbral cortex of 5 μm thickness was dewaxed according to the standard protocol. Then the histopathological evaluation was performed by two pathologists in blinded manners.

General chemical procedure:

Unless otherwise specified, reagents were purchased from commercial suppliers and used without further purification. Melting points were determined by X-4 digital display micro-melting point apparatus (Beijing Tech Instrument Co., Ltd.); NMR spectra were recorded on Bruker AVANCE AV-600 spectrometer (400 or 300 MHz for 1H, 200 or 150 MHz for ^{13}C); Mass spectra were obtained on the Agilent 1100 LC / MSD mass spectrometer (Agilent, USA) and Q-tofmicro MS (micromass company). All reactions were monitored by TLC (Merck Kieselgel GF254) and spots were visualized with UV light or iodine. The purity of biologically evaluated compounds was ≥ 95% as determined by HPLC.

General Procedure A for Synthesis of Compound 9, 12-34.

A mixture of benzo[*cd*]indol-2(1*H*)-one (1 equiv) in anhydrous DMF was stirred at 0 °C. The solution was added 60% NaH (1.5 equiv) in 4 batches and stirring for 30 minutes. Appropriate iodide (1.5 equiv) was added dropwise to the mixture, and the whole was stirred at room temperature for 3 h and then was poured into ice water. This mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), and evaporated to afford **7a-7b** as yellow solid (75%-90% yield).

To a suspension of compounds **7a** (500 mg, 2.73 mmol, 1 equiv) in $ClSO_2OH$ (27.32 mmol, 10 equiv) was stirred at 0 °C for 2 h. The reaction mixture was slowly added dropwise to ice water and extracted with AcOEt. Concentration in vacuo and purification by silica gel column chromatography afforded yellow intermediate **8** (0.56 g, 2.0 mmol, yield 74%). To a solution of compound **8** (150 mg, 0.53 mmol) and aniline (65 mg, 0.69 mmol) in DCM (10 mL) was added

pyridine (0.5 mL). The mixture was stirred at rt for 2 h. The reaction mixture was concentrated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (DCM/PE) yield the title compound **9** (96 mg, 0.28 mmol, yield 54%) as yellow solid.

To a solution of benzo[*cd*]indol-2(1*H*)-one or compounds **7a-7b** (1 equiv) in AcOH was added 69% HNO_3 (1.5 equiv) heat to 50 °C - 65 °C. The mixture reaction was stirred for 4 h. When TLC analysis showed complete conversion of the starting material, the reaction mixture was poured into water filtrated through Cellit and the filtrate was concentrated in vacuum. The crude product was purified by silica gel column chromatography (PE/DCM) yield the title compound as yellow solid.

To a solution of compounds **10a-10c** (1 equiv) in THF/MeOH (1:1) was added Pd/C (10 % mol). The mixture reaction was flushed thrice with argon then thrice with hydrogen. The flask was charged with hydrogen gas, and reaction mixture was left to stir for 5 h. The suspension was filtered through Celite, and rinsed with EtOAc. The filtrate was evaporated and purified by column chromatography (SiO2: 45% EtOAc/petroleum ether) yield the Intermediate compounds **11a-11c** as red solid.

A reaction mixture of compound **11a** (1 equiv), Halogen (1.5 equiv) and K_2CO_3 (3 equiv) in anhydrous DMF was stirred at 80 °C for 8 h. After the TLC detection reaction was completed, it was extracted with H_2O and EA. The layer organic was concentrated in vacuum. The crude products were purified by silica gel column chromatography (PE/DCM) yield the title compounds **12-13** as light yellow solid.

To a solution of compound **11a-11c** (1 equiv) and proper sulfonyl chloride (1.5 equivl) in DCM (10 mL) was added pyridine (0.5 mL). The mixture was stirred at rt. for 2 h. The reaction mixture was concentrated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (DCM/PE) yield the title compounds **15-25** and **27-34** (yield 20% - 70%) as yellow solid. The synthesis method of **26** described here is not described as similar to the preparation of **7a**.

General Procedure A for Synthesis of Intermediate Compound

1-Methylbenzo[*cd*]indol-2(1*H*)-one (7a). Compound **7a** was prepared according to general procedure A on 41.2 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound (6.48 g, 35.4 mmol, yield 86%). $[M+H]^+$: 184.1. ^1H NMR (300 MHz, DMSO-d_6) δ 8.16 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 7.0 Hz, 1H), 7.79 (t, J = 7.5 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.54 (t, J = 7.7 Hz, 1H), 7.13 (d, J = 6.9 Hz, 1H), 3.38 (s, 3H).

1-Ethylbenzo[*cd*]indol-2(1*H*)-one (7b). Compound **7b** was prepared according to general procedure A on 23.5 mmol scale. Purification by column chromatography (15% EA/PE) yielded the intermediate compound (3.9 g, 19.7 mmol, yield 84 %). $[M+H]^+$: 198.2. ^1H NMR (300 MHz, Chloroform-d) δ 7.99 (d, J = 7.0 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.47 – 7.34 (m, 2H), 6.82 (d, J = 6.7 Hz, 1H), 3.92 (q, J = 7.2 Hz, 2H), 1.34 (t, J = 7.2 Hz, 3H).

1-Methyl-2-oxo-1,2-dihydrobenzo[*cd*]indole-6-sulfonyl chloride (8). Compound **8** was prepared according to general procedure A on 2.7 mmol scale. Purification by column chromatography (50 % PE/DCM) yielded the intermediate compound (0.56 g, 2.0 mmol, yield 74%). $[M+H]^+$: 282.2. ^1H NMR (300 MHz, Chloroform-d) δ 8.72 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 7.1 Hz,

1H), 7.96 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 3.49 (s, 3H).

1-Methyl-2-oxo-*N*-phenyl-1,2-dihydrobenzo[*cd*]indole-6-sulfonamide (9). Compound **9** was prepared according to general procedure A on 0.53 mmol scale. Purification by column chromatography (DCM) yielded the title compound (96 mg, 0.28 mmol, yield 54%). Yellow solid; m.p 219-220 °C. HPLC analysis: retention time = 6.047 min; peak area, 97.71%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.59 (s, 1H), 8.70 (d, *J* = 8.4 Hz, 1H), 8.15 (dd, *J* = 11.1, 7.5 Hz, 2H), 7.93 (t, *J* = 7.3 Hz, 2H), 7.17 (dd, *J* = 19.1, 7.6 Hz, 3H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 1H), 3.34 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.4 , 144.6 , 137.9 , 133.8 , 130.9 , 129.5 , 128.2 , 126.4 , 125.5 , 125.1 , 124.2 , 124.1 , 119.7 , 104.2 , 26.6 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{18}H_{15}N_2O_3S^{+}$: 339.0798, found: 339.0803.

6-Nitrobenzo[*cd*]indol-2(1*H*)-one (10a). Compound **10a** was prepared according to general procedure A on 5.9 mmol scale. Purification by column chromatography (70 % DCM/PE) yielded the intermediate compound (0.93 g, 4.5 mmol, yield 74%). $[M-H]^{-}$: 213.0. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.86 (d, *J* = 8.4 Hz, 1H), 8.61 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 7.0 Hz, 1H), 8.05 (dd, *J* = 8.4, 7.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H).

1-Methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one (10b). Compound **10a** was prepared according to general procedure A on 21.8 mmol scale. Purification by column chromatography (60 % DCM/PE) yielded the intermediate compound (3.58 g, 15.7 mmol, yield 72%). $[M+H]^{+}$: 229.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 – 8.68 (m, 1H), 8.56 (d, *J* = 8.2 Hz, 1H), 8.08 (d, *J* = 7.0 Hz, 1H), 8.00 – 7.88 (m, 1H), 7.20 (dd, *J* = 8.1, 1.1 Hz, 1H), 3.35 (s, 3H).

1-Ethyl-6-nitrobenzo[cd]indol-2(1H)-one (10c). Compound **10c** was prepared according to general procedure A on 5.1 mmol scale. Purification by column chromatography (50 % DCM/PE) yielded the intermediate compound (0.85 g, 3.5 mmol, yield 69%). $[M+H]^+$: 243.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (dd, *J* = 8.5, 0.6 Hz, 1H), 8.60 (d, *J* = 8.1 Hz, 1H), 8.18 – 8.09 (m, 1H), 7.99 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 3.94 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H).

6-(Benzylamino)-1-methylbenzo[*cd*]indol-2(1*H*)-one (12). Compound **12** was prepared according to general procedure A on 0.53 mmol scale. Purification by column chromatography (DCM) yielded the title compound (96 mg, 0.28 mmol, yield 54%). Yellow solid; m.p 135-136 °C. HPLC analysis: retention time = 4.867 min; peak area, 96.24%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.53 (d, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 7.0 Hz, 1H), 7.72 (t, *J* = 7.6 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.26 – 7.11 (m, 2H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.20 (d, *J* = 7.7 Hz, 1H), 4.48 (d, *J* = 5.8 Hz, 2H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 156.8 , 138.0 , 135.4 , 130.5 , 129.1 (2C), 128.4 , 127.9 , 126.8 , 126.4 , 125.9 , 124.7 (2C), 120.4 , 113.0 , 106.0 , 56.3 , 26.5 . HRMS-EI *m/z* $[M+H]^+$ calcd for $C_{19}H_{17}N_2O^+$: 289.1335, found: 289.1340.

1-Methyl-6-((1-phenylethyl)amino)benzo[*cd*]indol-2(1*H*)-one (13). Compound **13** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (41 mg, 0.18 mmol, yield 36%). Yellow solid; m.p 145-146 °C. HPLC analysis: retention time = 4.924 min; peak area, 96.00%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.70 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 6.9 Hz, 1H), 7.73 (dd, *J* = 8.3, 7.0 Hz, 1H), 7.48 – 7.39 (m, 2H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.23 – 7.11 (m, 1H), 6.81 – 6.72 (m, 2H), 6.07 (d, *J* = 7.8 Hz, 1H), 4.64 (t, *J* = 6.7 Hz, 1H), 3.25 (s, 3H), 1.57 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 146.0 ,

140.4 , 129.1 , 128.8 (2C), 127.4 , 127.1 , 126.9 , 126.7 , 126.3 (2C), 125.1 , 124.1 , 121.5 , 108.0 , 104.9 , 52.9 , 26.4 , 25.0 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{20}H_{18}N_2O^+$: 302.1419, found: 303.1487.

***N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzamide (14).** Compound **14** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (63 mg, 0.21 mmol, yield 41%). Yellow solid; m.p 207-208 °C. HPLC analysis: retention time = 6.102 min; peak area, 96.83%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.21 (s, 1H), 8.15 – 8.00 (m, 3H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.73 – 7.51 (m, 4H), 7.18 (q, *J* = 6.5 Hz, 1H), 3.39 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.5 , 166.7 , 137.9 , 134.8 , 132.1 , 129.4 , 129.0 , 128.9 (2C), 128.3 (2C), 126.6 , 125.7 , 125.1 , 125.0 , 124.6 , 106.0 , 26.7 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{19}H_{15}N_2O_2^+$: 303.1128, found: 303.1132.

***N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (15).** Compound **15** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (63 mg, 0.22 mmol, yield 41%). Yellow solid; m.p 185-186 °C. HPLC analysis: retention time = 5.864 min; peak area, 99.23%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.00 (d, *J* = 6.8 Hz, 1H), 7.72 – 7.66 (m, 3H), 7.62 – 7.55 (m, 1H), 7.54 – 7.45 (m, 2H), 7.16 (d, *J* = 7.4 Hz, 1H), 7.05 (d, *J* = 7.5 Hz, 1H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 139.7 , 138.5 , 133.2 , 129.8 , 129.5 (2C), 129.2 , 128.2 , 127.5 , 127.2 (2C), 126.4 , 126.3 , 126.2 , 124.7 , 105.9 , 26.6 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{18}H_{15}N_2O_3S^+$: 339.0798, found: 339.0802.

***N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)thiophene-2-sulfonamide (16).** Compound **15** was prepared according to general procedure A on 0.51 mmol scale. Purification by column

chromatography (DCM) yielded the title compound (108 mg, 0.32 mmol, yield 62%). Yellow solid; m.p 183-184 °C. HPLC analysis: retention time = 5.638 min; peak area, 98.76%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.44 (s, 1H), 8.04 (dd, *J* = 15.9, 7.6 Hz, 2H), 7.85 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.77 – 7.65 (m, 1H), 7.39 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 7.07 – 7.01 (m, 1H), 3.34 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 140.1 , 138.7 , 133.7 , 132.9 , 129.30 , 128.1 (2C), 127.3 , 126.7 , 126.4 , 126.3 , 124.9 , 124.7 , 105.9 , 26.5 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{16}H_{13}N_2O_3S_2^{+}$: 345.0362, found: 345.0360.

***N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)pyridine-3-sulfonamide (17).** Compound **17** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (89 mg, 0.26 mmol, yield 52%). Yellow solid; m.p 212-214 °C. HPLC analysis: retention time = 4.003 min; peak area, 96.27%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.47 (s, 1H), 8.79 (d, *J* = 1.8 Hz, 1H), 8.75 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.06 (d, *J* = 8.5 Hz, 1H), 8.04 – 7.98 (m, 2H), 7.71 (dd, *J* = 8.3, 7.0 Hz, 1H), 7.54 (dd, *J* = 8.1, 4.8 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 1H), 3.33 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 153.8 , 147.59 , 138.9 , 136.0 , 135.3 , 129.4 , 128.1 , 127.0 , 126.8 , 126.5 , 126.2 , 125.0 , 124.9 , 124.6 , 106.0 , 26.6 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{17}H_{14}N_3O_3S^{+}$: 340.0750, found: 340.0757.

***N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)quinoline-8-sulfonamide (18).** Compound **18** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (117 mg, 0.30 mmol, yield 52%). Yellow solid; m.p 274-275 °C. HPLC analysis: retention time = 6.062 min; peak area, 97.97%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.22 (dd, *J* = 4.3, 1.8 Hz, 1H), 8.54 (dd, *J* = 8.4, 1.8 Hz, 1H), 8.29 – 8.11

(m, 3H), 7.92 (d, *J* = 6.9 Hz, 1H), 7.77 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.67 – 7.52 (m, 2H), 7.02 (d, *J* = 7.7 Hz, 1H), 6.89 (d, *J* = 7.7 Hz, 1H), 3.24 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2 , 152.0 , 143.35 , 138.1 , 137.5 , 135.7 , 134.6 , 132.3 , 128.89 , 128.83 , 128.6 , 128.0 , 126.3 , 126.09 , 126.04 , 124.8 , 124.6 , 124.5 , 123.1 , 105.8 , 26.5 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{21}H_{16}N_3O_3S^+$: 390.0907, found: 390.0914.

4-Chloro-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (19). Compound **19** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (89 mg, 0.24 mmol, yield 47%). Yellow solid; m.p 221-222 °C. HPLC analysis: retention time = 5.873 min; peak area, 95.02%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.04 (dd, *J* = 13.7, 7.6 Hz, 2H), 7.72 (dd, *J* = 8.4, 6.9 Hz, 1H), 7.67 – 7.51 (m, 4H), 7.10 (q, *J* = 7.6 Hz, 2H), 3.33 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 138.8 , 138.7 , 138.1 , 129.7 (2C), 129.3 , 129.2 (2C), 128.2 , 127.2 , 126.6 , 126.5 , 126.3 , 125.1 , 124.8 , 105.9 , 26.6 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{18}H_{14}ClN_2O_3S^+$: 373.0408, found: 373.0406.

4-Chloro-*N*-(2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (20). Compound **20** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (82 mg, 0.23 mmol, yield 45%). Yellow solid; m.p 182-184 °C. HPLC analysis: retention time = 5.707 min; peak area, 96.61%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.79 (s, 1H), 10.16 (s, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 6.9 Hz, 1H), 7.70 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 7.5 Hz, 1H), 3.38 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1 , 138.7 , 138.0 ,

137.3 , 129.7 (2C) , 129.3 , 129.2 (2C) , 128.1 , 127.3 , 126.9 , 126.8 , 126.5 , 126.4 , 124.5 , 106.6 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{17}H_{12}ClN_2O_3S^{+}$: 359.0252, found: 359.0245.

4-Chloro-*N*-(1-ethyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (21).

Compound **21** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (86 mg, 0.23 mmol, yield 44%). Yellow solid; m.p 241-242 °C. HPLC analysis: retention time = 6.208 min; peak area, 98.22%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.05 (dd, *J* = 21.0, 7.6 Hz, 2H), 7.75 – 7.71 (m, 1H), 7.70 – 7.65 (m, 2H), 7.59 – 7.52 (m, 2H), 7.19 – 7.06 (m, 2H), 3.86 (q, *J* = 7.1 Hz, 2H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.9 , 138.7 , 138.1 , 137.7 , 129.7 , 129.3 , 129.2 , 128.2 , 127.1 , 126.6 , 126.5 , 126.4 , 125.1 , 124.9 , 106.1 , 34.9 , 14.2 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{19}H_{16}ClN_2O_3S^{+}$: 387.0565, found: 387.0560.

4-Methyl-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (22).

Compound **22** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (100 mg, 0.28 mmol, yield 56%). Yellow solid; m.p 237-238 °C. HPLC analysis: retention time = 5.647 min; peak area, 97.95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.79 (s, 1H), 10.16 (s, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 6.9 Hz, 1H), 7.70 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 7.5 Hz, 1H), 3.38 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1 , 143.5 , 137.0 , 129.9 (2C), 129.2 , 128.3 , 127.3 (2C), 127.0 , 126.7 , 126.3 , 126.3 , 124.5 , 106.6 , 21.4 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{19}H_{17}N_2O_3S^{+}$: 353.0954, found: 353.0957.

2-Chloro-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (23).

Compound **23** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (125 mg, 0.33 mmol, yield 56%). Yellow solid; m.p 229-230 °C. HPLC analysis: retention time =5.699 min; peak area, 97.45%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.28 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 6.9 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.62 – 7.53 (m, 1H), 7.43 – 7.35 (m, 1H), 7.21 (d, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 3.30 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2 , 138.5 , 137.1 , 134.9 , 132.2 , 131.8 , 131.3 , 129.3 , 128.1 , 128.0 , 126.9 , 126.4 , 126.2 , 125.7 , 124.96 , 124.90 , 105.8 , 26.5 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{18}H_{14}ClN_2O_3S^{+}$: 373.0408, found: 373.0408.

2-Methyl-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (24).

Compound **24** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (102 mg, 0.29 mmol, yield 57%). Yellow solid; m.p 190-192 °C. HPLC analysis: retention time = 6.084 min; peak area, 97.79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.15 (d, *J* = 8.3 Hz, 1H), 8.00 (d, *J* = 6.9 Hz, 1H), 7.77 – 7.63 (m, 2H), 7.45 (td, *J* = 7.5, 1.4 Hz, 1H), 7.34 (d, *J* = 9.5 Hz, 1H), 7.24 (td, *J* = 7.4, 1.3 Hz, 1H), 7.13 (d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 3.31 (s, 3H), 2.59 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 138.4 , 138.0 , 137.2 , 133.3 , 132.9 , 129.6 , 129.3 , 128.1 , 127.4 , 126.7 , 126.5 , 126.2 , 125.7 , 124.9 , 124.8 , 105.9 , 26.6 , 20.5 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{19}H_{17}N_2O_3S$: 353.0954, found: 353.0957.

2-Methoxy-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (25).

Compound **25** was prepared according to general procedure A on 1.53 mmol scale. Purification

by column chromatography (DCM) yielded the title compound (327 mg, 0.88 mmol, yield 56%). Yellow solid; m.p 187-188 °C. HPLC analysis: retention time = 5.852 min; peak area, 95.15%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.31 (d, *J* = 8.3 Hz, 1H), 8.00 (d, *J* = 6.9 Hz, 1H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.52 (dd, *J* = 20.1, 7.8 Hz, 2H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.91 (t, *J* = 7.6 Hz, 1H), 3.90 (s, 3H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2 , 156.8 , 137.9 , 135.4 , 130.6 , 129.0 , 128.3 , 127.9 , 126.9 , 126.4 , 125.9 , 124.8 , 124.7 , 120.4 , 112.9 , 105.9 , 56.3 , 26.4 , HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{19}H_{17}N_2O_4S^{+}$: 369.0904, found: 369.0909.

2-Methoxy-*N*-methyl-N-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (26). Compound **26** was prepared according to general procedure A on 0.27 mmol scale. Purification by column chromatography (DCM) yielded the title compound (64 mg, 0.17 mmol, yield 56%). Yellow solid; m.p 223-224 °C. HPLC analysis: retention time = 5.975 min; peak area, 97.61%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.11 (d, *J* = 8.2 Hz, 1H), 8.05 (d, *J* = 6.9 Hz, 1H), 7.82 (dd, *J* = 8.3, 7.0 Hz, 1H), 7.64 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.53 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 1H), 7.04 – 6.92 (m, 2H), 3.95 (s, 3H), 3.42 (s, 3H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.6 , 157.2 , 140.0 , 135.6 , 132.3 , 131.3 , 129.8 , 129.6 , 128.5 , 128.1 , 126.8 , 126.6 , 125.3 , 124.9 , 120.5 , 113.5 , 105.8 , 56.5 , 41.2 , 26.6 . HRMS-EI *m/z* [M+H] + calcd for $C_{20}H_{19}N_2O_4S^{+}$: 383.1060, found: 383.1063.

***N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)-2-(trifluoromethoxy)benzenesulfonamide (27).** Compound **27** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (79 mg, 0.19 mmol,

yield 37%). Yellow solid; m.p 189-190 °C. HPLC analysis: retention time = 5.655 min; peak area, 95.39%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 6.9 Hz, 1H), 7.87 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.80 – 7.69 (m, 2H), 7.54 – 7.43 (m, 2H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 3.33 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 145.6 , 138.7 , 135.65 , 132.0 , 131.4 , 129.2 , 127.9 , 127.7 , 127.0 , 126.5 , 126.3 , 126.2 , 125.0 , 124.7 , 121.4 , 121.1 , 105.7 , 26.4 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{19}H_{14}F_3N_2O_4S^+$: 423.0621, found: 423.0622.

2-Ethoxy-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (28).

Compound **28** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (70 mg, 0.18 mmol, yield 36%). Yellow solid; m.p 180-182 °C. HPLC analysis: retention time = 6.024 min; peak area, 96.37%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.85 (s, 1H), 8.29 (d, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 6.9 Hz, 1H), 7.74 (dd, *J* = 8.3, 7.0 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.49 (dd, *J* = 8.8, 7.5 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.91 (t, *J* = 7.6 Hz, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.30 (s, 4H), 1.32 (t, *J* = 6.9 Hz, 3H) . ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 156.2 , 137.9 , 135.3 , 130.8 , 129.0 , 128.4 , 128.0 , 127.1 , 126.4 , 125.9 , 124.8 , 124.7 , 124.7 , 120.2 , 113.8 , 106.0 , 64.7 , 26.5 , 14.6 . HRMS-EI *m/z* [M+H] + calcd for $C_{20}H_{19}N_2O_4S^+$: 383.1060, found: 383.1057.

2,6-Dichloro-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (29).

Compound **29** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (82 mg, 0.20 mmol, yield 40%). Yellow solid; m.p 244-245 °C. HPLC analysis: retention time = 5.599 min; peak area, 97.99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.27 (d, *J* = 8.2 Hz, 1H), 8.04 (d, *J* = 6.9 Hz, 1H), 7.79 (dd,

J = 8.2, 7.1 Hz, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.57 (s, 1H), 7.50 (dd, *J* = 9.3, 6.5 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 7.6 Hz, 1H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 138.7 , 134.9 (2C), 134.8 , 134.3 , 132.2 (2C), 129.5 , 128.0 , 126.5 (2C), 126.1 , 125.4 , 125.0 , 124.9 , 105.8 , 26.5 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{18}H_{13}Cl_2N_2O_3S^+$: 407.0018, found: 407.0013.

3-Chloro-2-fluoro-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (30).

Compound **30** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (62 mg, 0.16 mmol, yield 33%). Yellow solid; m.p 213-214 °C. HPLC analysis: retention time = 6.190 min; peak area, 95.26%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 8.04 (d, *J* = 6.9 Hz, 1H), 7.90 – 7.67 (m, 3H), 7.28 (td, *J* = 8.7, 2.5 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 3.31 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 138.9 , 136.1 , 129.5 , 129.4 , 129.2 , 129.1 , 127.7 , 126.9 , 126.5 , 126.4 , 126.3 , 126.2 , 124.9 , 122.0 , 121.7 , 105.9 , 26.5 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{18}H_{13}ClFN_2O_3S^+$: 391.0314, found: 391.0316.

2,6-Difluoro-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (31).

Compound **31** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (72 mg, 0.19 mmol, yield 56%). Yellow solid; m.p 238-239 °C. HPLC analysis: retention time = 5.697 min; peak area, 98.58%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.18 (d, *J* = 8.3 Hz, 1H), 8.04 (d, *J* = 6.9 Hz, 1H), 7.83 – 7.72 (m, 1H), 7.66 (dd, *J* = 14.5, 8.4 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 7.21 (t, *J* = 9.1 Hz, 2H), 7.09 (d, *J* = 7.6 Hz, 1H), 3.33 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 (2C), 161.0 , 157.6 , 138.9 , 136.3 , 129.5 , 127.8 , 126.5 , 126.4 , 126.1 (2C), 124.9 , 124.9 , 114.0 , 113.7 , 105.9 , 26.5 .

HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{18}H_{12}F_2N_2O_3S^{+}$: 375.0609, found: 375.0618.

4-Fluoro-2-methyl-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (32). Compound **32** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (66 mg, 0.17 mmol, yield 35%). Yellow solid; m.p 214-215 °C. HPLC analysis: retention time = 2.254 min; peak area, 96.97%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.15 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 6.9 Hz, 1H), 7.82 – 7.66 (m, 2H), 7.25 (dd, *J* = 9.8, 2.7 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.08 (dt, *J* = 8.5, 4.6 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 3.33 (s, 3H), 2.61 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 141.1 (d, *J* = 9.4 Hz), 134.4 , 134.4 , 132.8 , 132.6 , 129.3 , 128.0 , 127.2 , 126.5 , 126.1 , 124.8 , 119.5 (d, *J* = 22.3 Hz), 113.5 (d, *J* = 21.8 Hz), 105.9 , 26.5 , 20.5 , 20.5 . HRMS-EI m/z [M+H] + calcd for $C_{19}H_{16}FN_2O_3S^{+}$: 371.0860, found: 371.0864.

2-Chloro-4-fluoro-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (33). Compound **33** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (63 mg, 0.16 mmol, yield 56%). Yellow solid; m.p 228-229 °C. HPLC analysis: retention time = 6.577 min; peak area, 97.37%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.13 (d, *J* = 8.3 Hz, 1H), 8.04 (d, *J* = 6.9 Hz, 1H), 7.88 – 7.80 (m, 1H), 7.80 – 7.73 (m, 1H), 7.64 – 7.53 (m, 1H), 7.27 (dd, *J* = 15.5, 7.8 Hz, 2H), 7.08 (d, *J* = 7.6 Hz, 1H), 3.40 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 138.8 , 134.2 , 134.0 , 133.9 , 133.2 , 129.4 , 128.1 , 126.7 , 126.5 , 126.4 , 126.2 , 125.0 , 124.9 , 119.8 (d, *J* = 25.9 Hz), 115.3 (d, *J* = 21.9 Hz), 105.9 , 26.6 . HRMS-EI *m/z* [M+H] + calcd for $C_{18}H_{13}ClFN_2O_3S^{+}$: 391.0314, found: 391.0313.

4-Chloro-2-methoxy-*N*-(1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (34). Compound **34** was prepared according to general procedure A on 0.51 mmol scale. Purification by column chromatography (DCM) yielded the title compound (47 mg, 0.12 mmol, yield 24%). Yellow solid; m.p 152-154 °C. HPLC analysis: retention time = 5.900 min; peak area, 98.79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.32 (d, *J* = 8.3 Hz, 1H), 8.01 (d, *J* = 6.9 Hz, 1H), 7.79 (dd, *J* = 8.4, 7.0 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.32 – 7.21 (m, 2H), 6.99 (dd, *J* = 8.5, 2.0 Hz, 2H), 5.76 (d, *J* = 0.9 Hz, 1H), 3.95 (s, 3H), 3.31 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 157.5 , 139.7 , 138.2 , 131.9 , 129.2 , 128.3 , 127.6 , 126.4 , 126.1 , 126.0 , 125.1 , 124.9 , 124.7 , 120.5 , 113.5 , 105.9 , 57.0 , 55.3 , 26.5 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{19}H_{16}ClN_2O_4S^{+}$: 403.0514, found: 403.0516.

General Procedure B for Synthesis of Compound 45-70.

To a solution of benzo[*cd*]indol-2(1*H*)-one (20 g, 111.11 mmol, 1 equiv) in anhydrous DMF (400 mL) was stirred at 0 °C. The solution was added 60% NaH (6.67, 166.67 mmol, 1.5 equiv) in 3 batches and stirring for 30 minutes. Methyl iodide (10.31 mL, 166.67 mmol, 1.5 equiv) was added dropwise to the mixture, and the whole was stirred at room temperature for 3 h and then was poured into ice water. This mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine for 4 times, dried ($MgSO_4$), and evaporated to afford **35** as red solid (17.12 g, 87.78 mmol, yield 79%).

To a solution of **35** (8 g, 41.03 mmol, 1 equiv.) palladium acetate (91 mg, 0.41 mmol, 1 mol-%), XPhos (585 mg, 1.23 mmol, 3 mol-%), K_2CO_3 (8.5 g, 61.55 mmol, 1.5 equiv.) and *tert*-butyl carbamate (5.75 g, 49.20 mmol, 1.2 equiv.) in anhydrous 1,4-dioxane (150 mL) was

stirred at 100 °C for 8 h. After cooling the reaction mixture, the product was poured into CH_2Cl_2 (500 mL), filtered, and washed with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by flash column chromatography (EtOAc/petroleum ether, 1:4) to afford **36** as a red solid (27.90 g, 101.11 mmol,yield 91%).

To a solution of **36** (4.0 g, 14.49 mmol) in CH_2Cl_2 (100 mL) was slowly added a solution of tert-butyl (triphenylphosphoranylidene)acetate (5.56 g, 14.49 mmol, 1 equiv.) in CH_2Cl_2 (40 mL) by using constant pressure funnel. The solution was stirred for 2 h and then concentrated. The crude mixture was purified by flash column chromatography (DCM/petroleum ether, 1:5) to give **37** as a 3:1 mixture of isomers (4.45 g, 12.17 mmol,yield 84%).

To a solution of **37** (8.0 g, 21.39 mmol) in TFA (20 mL) was heated at 70 °C for 8 h. The solution was cooled and concentrated. The crude mixture was purified by recrystallization to give **38** as a yellow solid (3.42 g, 17.11 mmol, yield 80%). A solution of compound **38** (6.0 g, 30 mmol) in $POCl_3$ (50 mL) was heated at 90 °C for 2 h. The solution was cooled and then carefully added by pipette to a stirred solution of ice/water (500 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL). The combined extracts were washed with water (200 mL) and brine (200 mL), dried, filtered, and concentrated. The product was loaded onto silica gel and purified by column chromatography (CH_2Cl_2) to give **39** as a yellow solid (5.43 g, 24.9 mmol, yield 83%). To mixture of HNO_3 and H_2SO_4 (40 ml) was added a solution of **39** in H_2SO_4 (10 ml) under -15 °C to -10 °C. The mixture was stirred at -10 °C for 7 h, poured into ice-cold water (300 mL), filtered, and washed with hot water to give **40** as a yellow solid (4.91 g, 18.67 mmol, yield 75%).

To a solution of compound **40** (1.0 g, 3.80 mmol, 1 equiv) and $KHCO_3$ (1.14 g, 11.40 mmol, 3

equiv) in THF/MeOH (1:1) was added 10 % Pd/C (200 mg, 10 % mmol). The mixture reaction was flushed thrice with argon then thrice with hydrogen. The flask was charged with hydrogen gas, and reaction mixture was left to stir for overnight. The suspension was filtered and concentrated to give the rude product **43** for the further reaction. To a solution of the intermediate **43** (1 equiv) and proper phenylsulfonyl chloride (1.5 equiv) in CH_2Cl_2 was added pyridine (0.5 mL) and stirred at room temperature for overnight.The mixture was concentrated and purified by silica gel column chromatography to afford the target compound **45-48** as yellow solid (30%-60% yield).

To a solution of compounds **40** (200 mg, 1 equiv) and appropriate amine (2 equiv) in anhydrous 1,4-dioxine (15 mL) was heated at 100 °C to 130 °C for 6-20 h. When TLC analysis showed complete, the reaction was concentrated and purified by silica gel column chromatography to afford the title compound **41a-41l** as yellow solid (40%-95% yield).

To mixture of **40** (1 equiv), $Pd(PPh_3)_4$ (0.05 equiv), K_2CO_3 (3 equiv), H_2O (10 equiv) and proper boric acid or borate (1.5 equiv) was dissolved in 1,4-dioxane. The reaction system was exchanged by N_2, then, heated to 80 °C for 7 h. When TLC analysis showed complete conversion of the starting material, the mixture was extracted with AcOEt and water. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), concentrated and purified by silica gel column chromatography to afford the title compound **41m-41r** as yellow solid (45%-90% yield).

To a solution of compounds **40**, **41a-41l** (1 equiv) in THF/MeOH (1:1) was added Pd/C (10 % mol). The mixture reaction was flushed thrice with argon then thrice with hydrogen. The flask was charged with hydrogen gas, and reaction mixture was left to stir for 5 h. The suspension was filtered through Celite, and rinsed with EtOAc. The filtrate was evaporated to afford the rude product **43**, **44a-44l** to the further reactions. To a solution of the intermediate **43**, **44a-44l** (1

equiv) and proper phenylsulfonyl chloride (1.5 equiv) in CH_2Cl_2 was added pyridine (15 equiv) and stirred at room temperature for overnight. When TLC analysis showed complete conversion of the starting material, the mixture was concentrated and purified by silica gel column chromatography to afford the title compound **49-71** as yellow solid (30%-70% yield).

General Procedure B for Synthesis of Intermediate Compound

4-Chloro-1-methylindoline-2,3-dione (35). Compound **35** was prepared according to general procedure B on 111.11 mmol scale. Purification by column chromatography (80% DCM/PE) yielded the intermediate compound (17.12 g, 87.78 mmol, yield 79%). $[M+H]^+$: 196.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63 (t, J = 8.0 Hz, 1H), 7.11 (t, J = 7.5 Hz, 2H), 3.14 (s, 3H).

***Tert*-butyl (1-methyl-2,3-dioxoindolin-4-yl)carbamate (36).** Compound **36** was prepared according to general procedure B on 41.03 mmol scale. Purification by column chromatography (80% DCM/PE) yielded the intermediate compound (10.34 g, 37.34 mmol, yield 79%). $[M+Na]^+$: 299.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.61 (d, J = 7.7 Hz, 2H), 6.74 (d, J = 6.8 Hz, 1H), 3.12 (s, 3H), 1.50 (s, 9H).

***Tert*-butyl 2-(4-((*tert*-butoxycarbonyl)amino)-1-methyl-2-oxoindolin-3-ylidene)acetate (37).** Compound **37** was prepared according to general procedure B on 14.49 mmol scale. Purification by column chromatography (80% DCM/PE) yielded the intermediate compound (4.45 g, 12.17 mmol, yield 84%). $[M+H]^+$: 375.2. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 9.72 (s, 1H), 7.77 (dd, J = 8.6, 0.7 Hz, 1H), 7.39 – 7.27 (m, 1H), 6.93 (s, 1H), 6.46 (dd, J = 7.6, 0.9 Hz, 1H), 3.21 (s, 3H), 1.59 (s, 9H), 1.51 (s, 9H).

1-Methylpyrrolo[4,3,2-*de*]quinoline-2,4(1*H*,5*H*)-dione (38). Compound **38** was prepared

according to general procedure B on 21.39 mmol scale. Purification by recrystallization (80% EtOH/H_20) yielded the intermediate compound (3.42 g, 17.11 mmol, yield 80%). $[M+H]^+$: 201.07. ^1H NMR (300 MHz, Chloroform-*d*) δ 7.56 – 7.46 (m, 1H), 7.17 (s, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.68 (d, *J* = 7.5 Hz, 1H), 3.36 (s, 3H).

4-Chloro-1-methylpyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (39). Compound **39** was prepared according to general procedure B on 17.11 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (5.43 g, 24.9 mmol, yield 83%). $[M+H]^+$: 219. 3. ^1H NMR (300 MHz, Chloroform-*d*) δ 7.83 (s, 1H), 7.70 – 7.61 (m, 2H), 6.92 (dd, *J* = 6.2, 1.4 Hz, 1H), 3.43 (s, 3H).

4-Chloro-1-methyl-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (40). Compound **40** was prepared according to general procedure B on 24.9 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (4.91 g, 18.67 mmol, yield 75%). $[M+H]^+$: 264.2. ^1H NMR (300 MHz, DMSO-d_6) δ 8.65 (d, *J* = 8.0 Hz, 1H), 8.24 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 3.38 (s, 3H).

4-(Tert-butylamino)-1-methyl-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41a). Compound **41a** was prepared according to general procedure B on 1.14 mmol scale. Purification by recrystallization (60% EA/PE) yielded the intermediate compound as a yellow solid (147 mg, 0.49 mmol, 43%). $[M+H]^+$: 301.1. ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, *J* = 8.1 Hz, 1H), 7.81 (s, 1H), 7.28 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 3.30 (s, 3H), 1.51 (s, 9H).

4-(Cyclopropylamino)-1-methyl-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41b). Compound **41b** was prepared according to general procedure B on 1.14 mmol scale. Purification by

recrystallization (50% EA/PE) yielded the intermediate compound as a yellow solid (220 mg, 0.78 mmol, yield 68%). $[M+H]^+$: 285.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, *J* = 8.2 Hz, 2H), 7.21 (s, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 3.31 (s, 3H), 3.25 – 2.74 (m, 1H), 0.87 - 0.79 (m, 2H), 0.61 - 0.52 (m, 2H).

1-Methyl-4-(((1-methylpiperidin-4-yl)methyl)amino)-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41c). Compound **41c** was prepared according to general procedure B on 1.14 mmol scale. Purification by recrystallization (50% EA/PE) yielded the intermediate compound as a yellow solid (227 mg, 0.64 mmol, yield 56%). $[M+H]^+$: 356.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (dd, *J* = 8.1, 1.4 Hz, 1H), 8.15 (t, *J* = 5.5 Hz, 1H), 7.25 (s, 1H), 6.90 (d, *J* = 6.4 Hz, 1H), 3.42 - 3.36 (t, *J* = 5.9 Hz, 4H), 3.30 (s, 3H), 2.76 (d, *J* = 11.3 Hz, 2H), 2.13 (s, 3H), 1.87 – 1.67 (m, 4H), 1.65 - 1.60 (s, 1H).

1-Methyl-6-nitro-4-(((tetrahydro-2H-pyran-4-yl)methyl)amino)pyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41d). Compound **41d** was prepared according to general procedure B on 1.14 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (281 mg, 0.82 mmol, yield 72%). $[M+H]^+$: 343.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 – 8.06 (m, 2H), 7.24 (d, *J* = 5.9 Hz, 1H), 6.89 (s, 1H), 3.87 (s, 2H), 3.34 – 3.18 (m, 7H), 2.09 – 1.80 (m, 2H), 1.78 – 1.53 (m, 4H), 1.46 – 0.96 (m, 6H).

1-Methyl-6-nitro-4-((2-(piperidin-1-yl)ethyl)amino)pyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41e). Compound **41e** was prepared according to general procedure B on 1.14 mmol scale. Purification by recrystallization (60% EA/PE) yielded the intermediate compound as a yellow solid (219 mg, 0.62 mmol, yield 54 %). $[M+K]^+$: 356.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.63 (s, 1H), 8.39 (d, *J* = 8.1 Hz, 1H), 7.31 (s, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 3.88 (d, *J* = 6.2 Hz, 2H), 3.57 (s, 2H),

3.38 (s, 2H), 3.32 (s, 3H), 2.97 (s, 2H), 1.86 – 1.26 (m, 6H).

1-Methyl-4-((2-(4-methylpiperazin-1-yl)ethyl)amino)-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41f). Compound **41f** was prepared according to general procedure B on 1.14 mmol scale. Purification by recrystallization (EA) yielded the intermediate compound as a yellow solid (262mg, 0.71 mmol, yield 62%). $[M+Na]^+$: 393.3. ^1H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, *J* = 8.1 Hz, 1H), 7.80 (s, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 3.89 (t, *J* = 4.9 Hz, 3H), 3.32 (s, 3H), 2.44 (t, *J* = 5.0 Hz, 3H), 2.24 (s, 3H).

(R)-1-methyl-6-nitro-4-((1-(pyridin-2-yl)ethyl)amino)pyrrolo[4,3,2-de]quinolin-2(1*H*)-one (41g). Compound **41g** was prepared according to general procedure B on 1.14 mmol scale. Purification by recrystallization (EA) yielded the intermediate compound as a yellow solid (171 mg, 0.49 mmol, yield 43 %). $[M+H]^+$: 350.3. ^1H NMR (300 MHz, DMSO-d_6) δ 8.83 – 8.59 (m, 2H), 8.52 – 8.26 (m, 2H), 7.87 (s, 1H), 7.35 (s, 1H), 7.24 (s, 1H), 7.00 – 6.70 (m, 1H), 3.38-3.30 (m, 1H), 3.28 (s, 3H), 1.60 (s, 3H).

1-Methyl-4-morpholino-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41h). Compound **41h** was prepared according to general procedure B on 1.14 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (208 mg, 0.66 mmol, yield 58 %). $[M+H]^+$: 315.2. ^1H NMR (300 MHz, DMSO-d_6) δ 8.42 (d, *J* = 8.1 Hz, 1H), 7.82 (s, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 3.88 (t, *J* = 4.7 Hz, 4H), 3.74 (t, *J* = 4.8 Hz, 4H), 3.33 (s, 4H).

1-Methyl-4-(4-methylpiperazin-1-yl)-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41i). Compound **41i** was prepared according to general procedure B on 1.14 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (269

mg, 0.82 mmol, yield 72 %). $[M+H]^+$: 328.3. ^1H NMR (300 MHz, DMSO-d_6) δ 8.40 (d, *J* = 8.1 Hz, 1H), 7.80 (s, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 3.89 (t, *J* = 5.1 Hz, 4H), 3.32 (s, 3H), 2.44 (d, *J* = 5.0 Hz, 4H), 2.24 (s, 3H).

4-(4-(Hydroxymethyl)piperazin-1-yl)-1-methyl-6-nitropyrrolo[4,3,2-*de*]quinolin-2(1*H*)-one (41j). Compound **41j** was prepared according to general procedure B on 1.14 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (191 mg, 0.56 mmol, yield 49 %). $[M+H]^+$: 343.3. ^1H NMR (300 MHz, DMSO-d_6) δ 8.35 (d, *J* = 8.1 Hz, 1H), 7.74 (s, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 4.75 (d, *J* = 13.4 Hz, 2H), 4.52 (s, 1H), 3.30 (s, 3H), 3.05 (t, *J* = 12.7 Hz, 2H), 1.79 (d, *J* = 13.4 Hz, 4H), 1.24 – 1.10 (m, 2H).

Ethyl2-(4-(1-methyl-6-nitro-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-4-yl)piperazin-1-yl)acetate (41k). Compound **41k** was prepared according to general procedure B on 1.14 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (241 mg, 0.60 mmol, yield 53 %). $[M+H]^+$: 399.3. ^1H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, *J* = 8.1 Hz, 1H), 7.81 (s, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 4.76 (d, *J* = 7.0 Hz, 2H), 4.07 (q, *J* = 7.0 Hz, 2H), 3.32 (s, 3H), 3.09 (t, *J* = 12.6 Hz, 2H), 2.27 (d, *J* = 6.9 Hz, 2H), 2.06 (s, 1H), 1.79 (d, *J* = 12.5 Hz, 2H), 1.27 – 1.16 (m, 5H).

***Tert*-butyl-4-(1-methyl-6-nitro-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-4-yl)piperazine-1-carboxylate (41l).** Compound **41l** was prepared according to general procedure B on 1.14 mmol scale. Purification by column chromatography (DCM) yielded the intermediate compound as a yellow solid (212 mg, 0.51 mmol, yield 45%). $[M-Boc]^+$: 314.3. ^1H NMR (300 MHz, Chloroform-*d*) δ 8.42 (d, *J* = 8.0 Hz, 1H), 7.44 (s, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 3.92 (d, *J* = 5.7 Hz, 4H), 3.63 (dd, *J* =

6.5, 4.0 Hz, 4H), 3.40 (s, 3H), 1.50 (s, 9H).

General Procedure B for Synthesis of Title Compound 45-70

2-Chloro-4-fluoro-*N*-(1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (45). Compound **45** was prepared according to general procedure B on 0.50 mmol scale. Purification by column chromatography (DCM) yielded the title compound **45** (74 mg, 0.19 mmol, yield 38%). Yellow solid; m.p 177-178 °C. HPLC analysis: retention time = 5.843 min; peak area, 100.00%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.57 (s, 1H), 9.10 (d, *J* = 4.4 Hz, 1H), 7.97 (q, *J* = 5.3 Hz, 2H), 7.66 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.29 (td, *J* = 8.5, 2.6 Hz, 1H), 7.13 (d, *J* = 7.6 Hz, 1H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.7 , 153.3 , 140.5 , 138.6 , 134.3 , 128.1 , 127.1 , 120.1 , 119.8 , 119.5 , 119.1 , 118.5 , 115.1 , 114.8 , 106.9 , 27.1 , 26.9 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{17}H_{12}ClFN_3O_3S^{+}$: 392.0266, found: 392.0269.

4-Chloro-*N*-(1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (46). Compound **46** was prepared according to general procedure B on 0.50 mmol scale. Purification by column chromatography (DCM) yielded the title compound **46** (59 mg, 0.16 mmol, yield 32%). Yellow solid; m.p 213-214 °C. HPLC analysis: retention time = 5.902 min; peak area, 98.39%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.70 (t, *J* = 8.2 Hz, 4H), 7.58 – 7.49 (m, 6H), 7.37 (s, 1H), 7.19 (d, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 7.7 Hz, 1H), 3.93 (s, 3H), 3.31 (d, *J* = 7.8 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 153.2 , 140.2 , 139.3 , 138.3 , 137.9 , 134.2 , 129.5 (2C), 129.2 (2C), 128.4 , 126.5 , 119.1 , 118.4 , 107.0 , 26.9 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{17}H_{13}ClN_3O_3S^{+}$: 374.0361, found: 374.0363.

4-Methyl-*N*-(1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide

(47). Compound **47** was prepared according to general procedure B on 0.50 mmol scale. Purification by column chromatography (DCM) yielded the title compound **47** (91 mg, 0.26 mmol, yield 52%). Yellow solid; m.p 194-195 °C. HPLC analysis: retention time = 5.877 min; peak area, 96.16%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.42 (s, 1H), 9.12 (d, *J* = 4.4 Hz, 1H), 7.96 (d, *J* = 4.4 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.7 Hz, 1H), 3.31 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 153.1 , 143.5 , 139.9 , 137.7 , 137.6 , 134.3 , 129.8 , 129.0 , 127.3 , 124.6 , 118.4 , 107.1 , 26.9 , 21.3 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{18}H_{16}N_3O_3S^{+}$: 354.0907, found: 354.0905.

2-Methoxy-*N*-(1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (48). Compound **48** was prepared according to general procedure B on 0.50 mmol scale. Purification by column chromatography (DCM) yielded the title compound **48** (79 mg, 0.215 mmol, yield 43%). Yellow solid; m.p 229-230 °C. HPLC analysis: retention time = 5.838 min; peak area, 95.18%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.50 (s, 1H), 9.17 (d, *J* = 4.4 Hz, 1H), 7.99 (d, *J* = 4.4 Hz, 1H), 7.71 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.59 – 7.44 (m, 2H), 7.16 – 7.03 (m, 2H), 6.96 (td, *J* = 7.6, 1.0 Hz, 1H), 3.77 (s, 3H), 3.30 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 153.1 , 143.5 , 139.9 , 137.7 , 137.6 , 134.3 , 129.8 (2C), 129.0 , 127.3 (2C), 124.6 , 118.4 , 107.1 , 26.9 , 21.3 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{18}H_{16}N_3O4S^{+}$: 370.0856, found: 370.0856.

4-Chloro-*N*-(4-chloro-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (49). Compound **49** was prepared according to general procedure B on 0.43 mmol scale. Purification by column chromatography (50% EA/PE) yielded the title compound (73 mg, 0.18 mmol, yield 42%). Yellow solid; m.p 197-198 °C. HPLC analysis: retention time = 4.537 min; peak

area, 95.20%. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.93 (s, 1H), 7.78 (t, *J* = 4.3 Hz, 4H), 7.34 (d, *J* = 8.3 Hz, 2H), 6.83 (d, *J* = 7.7 Hz, 1H), 3.38 (s, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 165.1 , 153.1 , 139.8 , 137.6 , 137.3 , 137.1 , 136.8 , 129.3 (2C), 128.8 (2C), 127.4 , 121.9 , 120.2 , 118.2 , 106.4 , 26.9 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{17}H_{12}Cl_2N_3O_3S^{+}$: 407.9971, found: 407.9969.

***N*-(4-chloro-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxybenzenesulfonamide (50).** Compound **50** was prepared according to general procedure B on 0.43 mmol scale. Purification by column chromatography (50% EA/PE) yielded the title compound (54 mg, 0.13 mmol, yield 31%). Yellow solid; m.p 214-215 °C. HPLC analysis: retention time = 6.046 min; peak area, 95.02%. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.57 (s, 1H), 7.87 – 7.74 (m, 3H), 7.41 (dd, *J* = 8.3, 7.4 Hz, 1H), 6.90 (dd, *J* = 8.2, 6.8 Hz, 2H), 6.78 (d, *J* = 7.8 Hz, 1H), 4.05 (s, 3H), 3.34 (s, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 165.2 , 156.4 , 152.6 , 137.6 , 137.4 , 136.2 , 135.0 , 130.6 , 128.4 , 125.9 , 121.6 , 120.0 , 119.8 , 118.1 , 111.7 , 106.7 , 56.4 , 26.8 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{18}H_{15}ClN_3O_4S^{+}$: 404.0466, found: 404.0474.

***N*-(4-(tert-butylamino)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxybenzenesulfonamide (51).** Compound **51** was prepared according to general procedure B on 0.37 mmol scale. Purification by column chromatography (50% EA/PE) yielded the title compound (57mg, 0.13 mmol, yield 35%). Yellow solid; m.p 252-254 °C. HPLC analysis: retention time = 4.768 min; peak area, 95.63%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.88 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.57 (dd, *J* = 9.0, 7.3 Hz, 1H), 7.42 (s, 1H), 7.21 (t, *J* = 3.8 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.07 (t, *J* = 7.6 Hz, 1H), 6.59 (d, *J* = 7.7 Hz, 1H), 5.76 (s, 1H), 3.68 (s, 3H), 3.19 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4 , 159.5 , 156.8 , 136.5 , 135.7 , 134.8 , 130.8 , 126.7 ,

126.6 , 120.6 , 119.2 , 113.7 , 111.9 , 111.8 , 101.7 , 56.8 , 55.3 , 51.9 , 28.7 (3C) , 26.8 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{22}H_{25}N_4O_4S^{+}$: 441.1591, found: 441.1594.

***N*-(4-(cyclopropylamino)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxy benzenesulfonamide (52).** Compound **52** was prepared according to general procedure B on 0.39 mmol scale. Purification by column chromatography (50% EA/PE) yielded the title compound (60 mg, 0.14 mmol, yield 36%). Yellow solid; m.p 217-218 °C. HPLC analysis: retention time = 2.508 min; peak area, 96.68%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (s, 1H), 7.94 (d, *J* = 3.6 Hz, 1H), 7.85 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.54 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.20 (s, 1H), 7.13 – 7.01 (m, 2H), 6.59 (d, *J* = 7.8 Hz, 1H), 3.79 (s, 3H), 3.19 (s, 3H), 2.99-2.94 (m, 1H), 0.86 – 0.83 (m, 2H), 0.60 – 0.49 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.3 , 161.2 , 156.6 , 136.9 , 136.3 , 135.7 (2C), 130.7 (2C), 126.6 , 126.2 , 120.5 , 118.3 , 113.3 , 112.7 , 102.0 , 56.7 , 26.8 , 24.7 , 14.4 , 7.1 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{21}H_{21}N_4O_4S^{+}$: 425.1278, found: 425.1280.

2-Methoxy-*N*-(1-methyl-4-(((1-methylpiperidin-4-yl)methyl)amino)-2-oxo-1,2-dihydropyrrolo[4,3 ,2-*de*]quinolin-6-yl)benzenesulfonamide (53). Compound **53** was prepared according to general procedure B on 0.31 mmol scale. Purification by column chromatography (70 % EA/PE) yielded the title compound (65 mg, 0.13 mmol, yield 43%). Yellow solid; m.p 230-232 °C. HPLC analysis: retention time = 8.380 min; peak area, 95.78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.72 (t, *J* = 5.3 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 7.7 Hz, 1H), 7.19 (s, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 1H), 3.75 (s, 3H), 3.19 (s, 3H), 2.90 (d, *J* = 11.1 Hz, 2H), 2.26 (s, 3H), 2.03 (t, *J* = 11.8 Hz, 2H), 1.94 – 1.49 (m, 4H), 1.47 – 1.13 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4 , 160.2 , 156.7 , 137.2 , 136.5 , 135.6 (2C), 135.1 ,

130.5 , 126.8 , 126.5 , 120.5 (2C), 119.9 , 113.4 , 101.8 , 56.7 , 55.2 , 47.1 , 46.0 , 34.8 , 29.9 , 26.8 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{25}H_{30}N_5O_4S^{+}$: 496.2013, found: 496.2020.

2-Methoxy-*N*-(1-methyl-2-oxo-4-(((tetrahydro-2*H*-pyran-4-yl)methyl)amino)-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (54). Compound **54** was prepared according to general procedure B on 0.32 mmol scale. Purification by column chromatography (70 % EA/PE) yielded the title compound (54 mg, 0.11 mmol, yield 35%). Yellow solid; m.p 289-290 °C. HPLC analysis: retention time = 5.951 min; peak area, 95.30%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.83 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.70 (t, *J* = 5.4 Hz, 1H), 7.62 – 7.49 (m, 1H), 7.32 (d, *J* = 7.7 Hz, 1H), 7.19 (s, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 1H), 3.96 – 3.84 (m, 2H), 3.75 (s, 3H), 3.43 - 3.37 (m, 2H), 3.30 (dd, *J* = 11.6, 2.0 Hz, 2H), 3.19 (s, 3H), 1.95 – 1.80 (m, 1H), 1.70 (dd, *J* = 12.4, 3.1 Hz, 2H), 1.36 - 1.27 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4 , 160.2 , 156.7 (2C), 137.3 , 136.5 , 135.6 , 135.2 , 130.5 , 126.9 , 126.5 , 120.5 , 120.1 , 113.4 , 112.3 , 101.8 , 67.3 (2C), 56.7 , 47.4 , 34.9 , 31.1 (2C), 26.8 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for$C_{24}H_{27}N_4O_5S^{+}$: 483.1697, found: 483.1702.

2-Methoxy-*N*-(1-methyl-2-oxo-4-((2-(piperidin-1-yl)ethyl)amino)-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (55). Compound **55** was prepared according to general procedure B on 0.31 mmol scale. Purification by column chromatography (70 % EA/PE) yielded the title compound (55 mg, 0.11 mmol, yield 36%). Yellow solid; m.p 220-221 °C. HPLC analysis: retention time = 6.393 min; peak area, 96.875%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (s, 0H), 7.82 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.69 (s, 1H), 7.54 (dd, *J* = 8.8, 7.4 Hz, 1H), 7.30 (d, *J* = 7.7 Hz, 1H), 7.22 (s, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.03 (td, *J* = 7.6, 1.0 Hz, 1H), 6.61 (d, *J* = 7.7 Hz, 1H), 3.75 (s, 3H), 3.69

– 3.54 (m, 2H), 3.19 (s, 3H), 2.87 – 2.26 (m, 6H), 1.70 – 1.29 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4 , 159.9 , 156.7 , 137.3 , 136.5 , 135.5 (2C) , 135.2 , 130.5 , 126.9 , 126.7 , 120.5 (2C) , 113.4 , 112.1, 101.9 , 56.7 (4C) , 54.4 , 39.5 , 26.8 (3C) .HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{25}H_{30}N_5O_4S^+$: 496.2013, found: 496.2010.

2-Methoxy-*N*-(1-methyl-4-((2-(4-methylpiperazin-1-yl)ethyl)amino)-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (56). Compound **56** was prepared according to general procedure B on 0.29 mmol scale. Purification by column chromatography (EA) yielded the title compound (55 mg, 0.11 mmol, yield 37%). Yellow solid; m.p 117-118 °C. HPLC analysis: retention time = 6.501 min; peak area, 96.108%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (s, 1H), 7.83 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.61 (s, 1H), 7.54 (dd, *J* = 8.9, 7.4Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.22 (s, 1H), 7.13 – 7.09 (m, 1H), 7.03 (td, *J* = 7.6, 1.0 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 1H), 3.76 (s, 3H), 3.56 (t, *J* = 6.1 Hz, 2H), 3.19 (s, 3H), 2.57 (t, *J* = 6.6 Hz, 2H), 2.51 – 2.25 (m, 8H), 2.16 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.4 , 160.0 , 156.7 (2C), 137.2 , 136.5 , 135.6 , 135.2 , 130.5 , 126.7 , 126.6 , 120.5 , 119.8 , 113.3 , 112.4 , 101.9 , 56.9 , 56.7 (2C), 55.1 (2C), 53.3 , 46.2 , 40.9-39.1 (1C). 26.8 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{25}H_{31}N_6O_4S^+$: 511.2122, found: 511.2117.

(*R*)-2-methoxy-*N*-(1-methyl-2-oxo-4-((1-(pyridin-2-yl)ethyl)amino)-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (57). Compound **57** was prepared according to general procedure B on 0.31 mmol scale. Purification by column chromatography (50 % EA/PE) yielded the title compound (71 mg, 0.14 mmol, yield 46%). Yellow solid; m.p 241-242 °C. HPLC analysis: retention time = 4.065 min; peak area, 97.71%. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.60 (d, *J* = 4.2 Hz, 1H), 8.25 (s, 1H), 7.99 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.73 (t, *J* = 7.7 Hz, 1H), 7.51 – 7.35 (m, 3H),

7.26 – 7.17 (m, 1H), 7.11 (s, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.60 (d, *J* = 6.9 Hz, 1H), 6.39 (d, *J* = 7.8 Hz, 1H), 5.40 (p, *J* = 6.8 Hz, 1H), 3.79 (s, 3H), 3.25 (s, 3H), 1.65 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.5 , 160.2 , 156.8 (2C), 137.3 , 136.7 , 136.3 , 135.4 (2C), 130.4 (2C), 127.2 (2C), 126.9 (2C), 121.9 , 120.4 (2C), 113.3 , 107.2 , 102.7 , 66.4 , 56.6 , 45.9 , 26.9 .HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{25}H_{24}N_5O_4S^+$: 490.1544, found: 490.1532.

2-Methoxy-*N*-(1-methyl-4-morpholino-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (58). Compound **58** was prepared according to general procedure B on 0.35 mmol scale. Purification by column chromatography (DCM) yielded the title compound (72 mg, 0.16 mmol, yield 45%). Yellow solid; m.p 247-248 °C. HPLC analysis: retention time = 7.919 min; peak area, 95.52%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.03 (d, *J* = 2.3 Hz, 1H), 7.78 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 7.53 (ddd, *J* = 8.8, 7.5, 1.8 Hz, 1H), 7.39 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.10 (dd, *J* = 8.5, 0.9 Hz, 1H), 7.00 (td, *J* = 7.6, 1.0 Hz, 1H), 6.68 (dd, *J* = 7.7, 3.1 Hz, 1H), 3.74 (s, 8H), 3.72 (s, 3H), 3.22 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 160.2 , 156.8 , 137.3 , 136.7 , 136.3 , 135.4 , 130.4 , 127.2 , 126.9 , 121.8 , 120.4 , 113.3 , 112.9 , 107.2 , 102.7 , 66.4 (2C) , 56.6 , 45.9 (2C) , 26.91 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{22}H_{23}N_4O_5S^+$: 455.1384, found: 455.138.

4-Chloro-*N*-(1-methyl-4-morpholino-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (59). Compound **59** was prepared according to general procedure B on 0.35 mmol scale. Purification by column chromatography (70 % EA/PE) yielded the title compound (69 mg, 0.15 mmol, yield 43%). Yellow solid; m.p 223-225 °C. HPLC analysis: retention time = 4.882 min; peak area, 98.26%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.08 (s, 1H), 7.70 (s, 1H), 7.67 (s, 1H), 7.61 (s, 1H), 7.50 (s, 1H), 7.47 (s, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 6.81 (d, *J* = 7.7 Hz, 1H), 3.95 - 3.69 (m, 4H),

3.27 (s, 3H), 2.80 - 2.55 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.8 , 159.7 , 139.7 , 138.9 , 138.4 , 137.7 , 136.2 , 129.4 , 129.0 , 128.3 , 128.1 , 127.9 , 125.7 , 113.0 , 107.2 , 102.9 , 54.2 (2C) , 44.6 (2C), 26.9 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{21}H_{20}ClN_4O_4S^+$: 459.0888, found: 459.0892.

2-Methoxy-*N*-(1-methyl-4-(4-methylpiperazin-1-yl)-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (60). Compound **60** was prepared according to general procedure B on 0.34 mmol scale. Purification by column chromatography (90 % EA/PE) yielded the title compound (57 mg, 0.12 mmol, yield 36%). Yellow solid; m.p 227-248 °C. HPLC analysis: retention time = 5.148 min; peak area, 97.19%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98 (s, 1H), 7.78 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.63 (s, 1H), 7.52 (ddd, *J* = 8.8, 7.4, 1.8 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.67 (d, *J* = 7.7 Hz, 1H), 3.77 (t, *J* = 4.8 Hz, 4H), 3.73 (s, 3H), 3.22 (s, 3H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.25 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 160.1 , 156.8 , 136.7 , 136.3 , 135.4 (2C) , 130.3 , 127.2 , 126.8 , 121.8 , 120.4 (2C) , 113.3 , 112.8 , 102.6 , 56.6 , 54.9 (2C) , 46.1 , 45.4 (2C) , 26.9 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{23}H_{26}N_5O_4S^+$: 468.1700, found: 468.1708.

4-Chloro-*N*-(1-methyl-4-(4-methylpiperazin-1-yl)-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (61). Compound **61** was prepared according to general procedure B on 0.34 mmol scale. Purification by column chromatography (80 % EA/PE) yielded the title compound (48 mg, 0.10 mmol, yield 30%). Yellow solid; m.p 247-248 °C. HPLC analysis: retention time = 4.331 min; peak area, 97.97%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.03 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.55 (s, 1H), 7.50 (s, 1H), 7.48 (d, *J* = 1.9 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 6.79 (d, *J* = 7.7 Hz, 1H), 3.76 - 3.62 (m, 8H), 3.26 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.8 , 160.0 , 139.7 ,

139.0 , 138.4 , 137.7 , 136.1 , 129.4 (2C) , 129.0 (2C) , 128.5 , 125.7 , 113.1 , 106.9 , 102.8 , 66.5 (2C) , 45.8 (3C) , 26.9 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{22}H_{23}ClN_5O_3S^+$: 472.1205, found: 472.1206.

***N*-(4-(4-(hydroxymethyl)piperidin-1-yl)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxybenzenesulfonamide (62).** Compound **62** was prepared according to general procedure B on 0.32 mmol scale. Purification by column chromatography (80 % EA/PE) yielded the title compound (48 mg, 0.10 mmol, yield 31%). Yellow solid; m.p 191-192 °C. HPLC analysis: retention time = 4.297 min; peak area, 96.22%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.27 (s, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.63 (s, 1H), 7.52 (dd, *J* = 8.9, 7.5 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.16 – 6.95 (m, 2H), 6.64 (d, *J* = 7.7 Hz, 1H), 4.70 (d, *J* = 13.2 Hz, 2H), 4.02 (s, 2H), 3.73 (s, 3H), 3.34 (s, 1H), 3.21 (s, 3H), 2.99 (t, *J* = 12.1 Hz, 2H), 2.04-1.95 (m, 1H), 1.91-1.80 (m, 2H), 1.33 – 1.12 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 162.6 , 159.8 , 156.8 , 137.4 , 136.6 , 136.3 , 135.4 , 130.4 , 127.1 , 126.7 , 121.3 , 120.4 , 113.3 , 107.5 , 102.3 , 67.7 , 56.6 , 45.4 (2C) , 35.6 , 28.4 , 26.8 (2C) . HRMS-EI m/z [M+H] $^+$ calcd for $C_{24}H_{27}N_4O_5S$+: 483.1697, found: 483.1698.

Ethyl2-(1-(6-((2-methoxyphenyl)sulfonamido)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-4-yl)piperidin-4-yl)acetate (63). Compound **63** was prepared according to general procedure B on 0.27 mmol scale. Purification by column chromatography (60 % EA/PE) yielded the title compound (50 mg, 0.09 mmol, yield 34%). Yellow solid; m.p 195-196 °C. HPLC analysis: retention time = 3.833 min; peak area, 97.88%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 7.79 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.63 (s, 1H), 7.52 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.10 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.00 (td, *J* = 7.6, 1.0 Hz, 1H), 6.65 (d, *J* = 7.7 Hz, 1H), 4.09 (q, *J* = 7.1 Hz, 2H),

3.72 (s, 3H), 3.21 (s, 3H), 3.04 – 2.91 (m, 2H), 2.51 (p, *J* = 1.8 Hz, 2H), 2.28 (d, *J* = 7.0 Hz, 2H), 2.04 (dd, *J* = 7.3, 3.6 Hz, 1H), 1.78 (d, *J* = 12.8 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.3 (2C) , 166.6 , 159.9 , 156.8 , 137.4 , 136.6 , 136.3 , 135.5 , 130.4 , 127.1 , 126.7 , 121.2 , 120.4 , 113.3 , 107.5 , 102.3 , 60.2 , 56.6 , 45.7 (2C) , 40.7 , 33.3 , 31.6 (2C) , 26.9 , 14.6 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{27}H_{31}N_4O_6S^+$: 539.1959, found: 539.1957.

***Tert*-butyl4-(6-((2-methoxyphenyl)sulfonamido)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-4-yl)piperazine-1-carboxylate(64).** Compound **64** was prepared according to general procedure B on 0.26 mmol scale. Purification by column chromatography (50 % EA/PE) yielded the title compound (77 mg, 0.14 mmol, yield 53%). Yellow solid; m.p 204-205 °C. HPLC analysis: retention time = 4.523 min; peak area, 98.21%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.07 (s, 1H), 7.79 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.63 (s, 1H), 7.52 (dd *J* = 8.3, 7.4 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.14 – 6.94 (m, 2H), 6.67 (d, *J* = 7.7 Hz, 1H), 3.85 – 3.75 (m, 4H), 3.51 – 3.41 (m, 4H), 3.34 (s, 2H), 3.22 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.6 , 159.8 , 156.8 , 154.4 , 137.3 , 136.67 , 136.40 , 135.4 , 130.4 , 127.2 , 126.9 , 121.7 , 120.4 , 113.3 , 112.8 , 107.4 , 102.7 , 79.5 , 56.6 (3C) , 45.2 (2C) , 28.5 (3C) , 26.9 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{27}H_{32}N_5O_6S^+$: 554.2068, found: 554.2061.

***N*-(4-(furan-2-yl)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxybenzene sulfonamide (65).** Compound **65** was prepared according to general procedure B on 0.38 mmol scale. Purification by column chromatography (50 % EA/PE) yielded the title compound (74 mg, 0.17 mmol, yield 45%). Yellow solid; m.p 251-252 °C. HPLC analysis: retention time = 4.423 min; peak area, 96.72%. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.78 (s, 1H), 8.31 (s, 1H), 7.97 (dd, *J* = 7.8,

1.7 Hz, 1H), 7.75 – 7.63 (m, 2H), 7.45 – 7.33 (m, 2H), 7.02 – 6.90 (m, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 6.72 – 6.62 (m, 2H), 3.81 (s, 3H), 3.35 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.1 , 156.6 , 153.2 , 151.2 , 146.2 , 137.9 , 136.7 , 135.7 , 135.6 , 130.5 , 128.8 , 126.4 , 120.9 , 120.4 , 117.6 , 114.7 , 113.38 , 113.27 , 113.2 , 106.7 , 56.6 , 27.0 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{22}H_{18}N_3O_5S^+$: 436.0962, found: 436.0958.

***N*-(4-(furan-3-yl)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxybenzene sulfonamide (66).** Compound **66** was prepared according to general procedure B on 0.38 mmol scale. Purification by column chromatography (DCM) yielded the title compound (92 mg, 0.21 mmol, yield 56%). Yellow solid; m.p 248-250 °C. HPLC analysis: retention time = 6.069 min; peak area, 95.52%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.86 – 8.81 (m, 1H), 8.44 (s, 1H), 7.88 – 7.78 (m, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.52 – 7.43 (m, 2H), 7.06 – 6.97 (m, 3H), 3.63 (s, 3H), 3.29 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.7 , 154.4 , 145.1 , 144.9 , 137.8 , 136.5 , 135.6 , 135.5 , 130.6 , 129.0 , 128.0 , 126.6 , 120.5 , 120.4 , 117.6 , 116.6 , 113.2 , 109.9 , 106.4 , 99.9 , 56.5 , 26.9 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{22}H_{18}N_3O_5S^+$: 436.0962, found: 436.0968.

***N*-(4-(1*H*-imidazol-1-yl)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)-2-methoxyb enzenesulfonamide (67).** Compound **67** was prepared according to general procedure B on 0.38 mmol scale. Purification by column chromatography (DCM) yielded the title compound (64 mg, 0.15 mmol, yield 39%). Yellow solid; m.p 246-247 °C. HPLC analysis: retention time = 5.899 min; peak area, 95.83%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.15 (s, 1H), 9.05 (t, *J* = 1.1 Hz, 1H), 8.52 (s, 1H), 8.48 (t, *J* = 1.4 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.47 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.22 – 7.15 (m, 1H), 7.00 (q, *J* = 7.8 Hz, 3H), 3.61 (s, 3H), 3.29 (s, 3H). ^{13}C NMR (75 MHz,

DMSO-d_6) δ 165.8 , 156.9 , 150.2 , 138.2 , 137.1 , 136.8 , 136.6 , 135.5 , 130.7 , 130.6 , 129.0 , 127.0 , 123.0 , 120.3 , 118.2 , 117.6 , 113.2 , 110.2 , 106.8 , 56.3 , 27.1 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{21}H_{18}N5O_4S^+$: 436.1074, found: 436.1069.

2-Methoxy-*N*-(1-methyl-4-(4-methyl-1*H*-imidazol-1-yl)-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (68). Compound **68** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (DCM) yielded the title compound (51 mg, 0.11 mmol, yield 32%). Yellow solid; m.p 269-270 °C. HPLC analysis: retention time = 4.383 min; peak area, 100.00%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.92 (s, 1H), 8.48 – 8.39 (m, 1H), 8.17 – 8.09 (m, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.54 – 7.42 (m, 1H), 7.01 (dt, *J* = 7.5, 3.3 Hz, 3H), 3.62 (s, 3H), 3.29 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8 , 156.9 , 150.2 , 139.3 , 138.1 , 136.9 , 136.7 , 136.4 , 135.5 , 130.6 , 128.9 , 127.0 , 123.0 , 120.3 , 117.4 , 114.1 , 113.2 , 109.9 , 106.6 , 56.3 , 27.1 , 14.1 . HRMS-EI *m/z* [M+H] $^+$ calcd for $C_{22}H_{20}N_5O_4S^+$: 450.1231, found: 450.1228.

2-Methoxy-*N*-(1-methyl-4-(1-methyl-1*H*-pyrazol-4-yl)-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl)benzenesulfonamide (69). Compound **69** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (80 % EA/PE) yielded the title compound (79 mg, 0.18 mmol, yield 49%). Yellow solid; m.p over 300 °C. HPLC analysis: retention time = 4.479 min; peak area, 95.27%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.74 (s, 1H), 8.41 (s, 1H), 8.31 (s, 1H), 7.90 – 7.77 (m, 1H), 7.50 (dd, *J* = 9.6, 6.9 Hz, 2H), 7.01 (t, *J* = 7.4 Hz, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 3.96 (s, 3H), 3.66 (s, 3H), 3.26 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.5 , 156.6 (2C) , 155.0 , 139.0 , 137.8 , 136.4 , 135.7 , 135.4 , 131.9 , 130.7 , 128.7 , 126.4 ,

123.6 , 120.4 , 120.0 , 117.1 , 116.1 , 113.2 , 105.9 , 56.6 , 26.9 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{22}H_{20}N_5O_4S^{+}$: 450.1231, found: 450.1238.

***N*-(4-(1-cyclopropyl-1*H*-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinolin-6-yl) -2-methoxybenzenesulfonamide (70).** Compound **70** was prepared according to general procedure B on 0.33 mmol scale. Purification by column chromatography (90 % EA/PE) yielded the title compound (78 mg, 0.16 mmol, yield 50%). Yellow solid; m.p 253-255 °C. HPLC analysis: retention time = 6.168 min; peak area, 96.75%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.84 (s, 1H), 8.41 (s, 1H), 8.34 (s, 1H), 7.83 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.51 – 7.42 (m, 1H), 7.07 – 6.95 (m, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 3.85 (t, *J* = 7.4 Hz, 1H), 3.64 (s, 3H), 3.26 (s, 3H), 1.25 – 1.00 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.5 , 156.7 , 154.9 , 139.0 , 137.8 , 136.5 , 135.6 , 135.4 , 131.0 , 130.6 , 128.8 , 126.6 , 123.3 , 120.4 , 120.2 , 117.1 , 116.1 , 113.2 , 105.9 , 56.5 , 33.5 , 26.9 , 6.9 (2C) . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{24}H_{22}N_5O_4S^{+}$: 476.1387, found: 476.1380.

General Procedure C for Synthesis of Compound 77-84.

To a solution of 1,8-Naphthalic anhydride (10.0 g, 50.5 mmol) and silver sulfate (7.9 g, 25.25 mmol, 0.50 equiv) in concentrated sulfuric acid (200 mL) were stirred at room temperature. Bromine (3.2 mL, 63.0 mmol, 1.26 equiv) was added over 30 min, and then heated to 60 °C for 8-10 h, before cooling back to 20 °C. The solid silver bromide by product was filtered off at a water pump to give a clear orange solution. This mixture reaction was added dropwise to ice water mixture (1 L). An off-white solid precipitates was filtered. The filter cake was washed once by displacement with water (50 mL) and twice with cold ethanol (100 mL each) and dried in

vacuo at 60 °C to constant weight to yielded the title compound **71** as white solid (12.54 g, 45.5 mmol, 90% yield).

To a solution of 5-bromo-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione **71** (27.59 g, 100 mmol) and hydroxylamine hydrochloride (6.9 g, 100 mmol) in pyridine (200 mL) was conducted under reflux for 2 h, followed by cooling to 80 °C. Then ptoluenesulfonyl chloride (38.14 g, 200 mmol) was added to the reaction system. After addition, the temperature was raised and the reaction was carried out under reflux for 2 h, followed by cooling. The reaction mixture was poured into 0.50 L of water and stirred to precipitate crystals, which were collected by filtration. The crystals were transferred to a beaker and washed successively with 0.5 L of a $NaHCO_3$ aqueous solution and 0.5 L of water, followed by filtration. The crystals were washed with water and dried to give an intermediate for further reaction. The whole amount of the intermediate, 85 ml of ethanol and100 ml of water were put in a reactor and stirred. Then 325 ml of a 1.4 mol/L aqueous solution of NaOH was added dropwise to the mixture. Thereafter, the mixture was heated to refluxing temperature, at which the reaction was carried out for 3 h while distilling off ethanol. After completion of the reaction, the reaction mixture was cooled to 75 °C, and 60 ml of concentrated HCl was added dropwise. In the meantime, crystals precipitated at 60 °C. After completion of the dropwise addition, the mixture was further cooled. The precipitated crystals were collected by filtration, washed with ion-exchanged water, and dried to give the title compound **72** as white solid (7.17 g, 29.14 mmol, yield 29%).

To a solution of 4-bromo-6-nitrobenzo[*cd*]indol-2(1*H*)-one **72** (7 g, 28.5 mmol) in AcOH (15 mL) was added 69% HNO_3 (2 mL, 42.75 mmol) heat to 50 °C - 65 °C. The mixture reaction was stirred for 4 h. When TLC analysis showed complete conversion of the starting material, the

reaction mixture was poured into water filtrated through Cellit and the filtrate was concentrated in vacuum. The crude product was purified by silica gel column chromatography (PE/DCM) to yielded the title compound **73** as yellow solid (6.9 g, 23.65 mmol, yield 83%).

To a solution of 4-bromo-1-methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one **73** (6.5 g, 22.26 mmol) in anhydrous DMF (100 mL) was stirred at 0 °C. The solution was added 60% NaH (1.34 g, 33.35 mmol) in 3 batches and stirring for 30 minutes. Methyl iodide (2.06 mL, 33.35 mmol) was added dropwise to the mixture, and the whole was stirred at room temperature for 3 h and then was poured into ice water. This mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine for 4 times, dried ($MgSO_4$), concentrated and purified by silica gel column chromatography to afford the title compound **74** as yellow solid (5.52 g, 18.04 mmol, 81% yield).

To mixture of 4-bromo-1-methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one **74** (1 equiv), $Pd(PPh_3)_4$ (0.05 equiv), K_2CO_3 (3 equiv), H_2O (10 equiv) and proper boric acid or borate (1.5 equiv) was dissolved in 1,4-dioxane. The reaction system was exchanged by N_2, then, heated to 80 °C for 7 h. When TLC analysis showed complete conversion of the starting material, the mixture was extracted with AcOEt and water. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), concentrated and purified by silica gel column chromatography to afford the title compound **75a-75g** as yellow solid (50%-80% yield).

To a solution of compounds **75a-75g** (1 equiv) in THF/MeOH (1:1) was added Pd/C (10 % mol). The mixture reaction was flushed thrice with argon then thrice with hydrogen. The flask was charged with hydrogen gas, and reaction mixture was left to stir for 5 h. The suspension was filtered through Celite, and rinsed with EtOAc. The filtrate was evaporated to afford the rude product **76a-76g** as yellow solid (70%-90% yield). To a solution of the intermediate **76a-76g** (1

equiv) and proper phenylsulfonyl chloride (1.5 equiv) in CH_2Cl_2 was added pyridine (15 equiv) and stirred at room temperature for overnight. When TLC analysis showed complete conversion of the starting material, the mixture was concentrated and purified by silica gel column chromatography to afford the target compound **75a-75g** as yellow solid (30%-70% yield).

General Procedure C for Synthesis of Intermediate

5-Bromo-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (71). Compound **71** was prepared according to general procedure B on 50.5 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the Intermediate compound as white solid (12.54 g, 45.5 mmol, 90% yield). $[M+H]^+$: 301.13. ^1H NMR (300 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.57 – 8.44 (m, 3H), 7.93 (q, *J* = 9.4, 8.6 Hz, 1H).

4-Bromobenzo[*cd*]indol-2(1*H*)-one (72). Compound **72** was prepared according to general procedure B on 100 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the Intermediate compound as white solid (7.17 g, 29.14 mmol, yield 29%). $[M+H]^+$: 247.9. ^1H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.44 (s, 1H), 8.10 (s, 1H), 7.58 (d, *J* = 3.5 Hz, 2H), 7.01 (dd, *J* = 5.9, 1.8 Hz, 1H).

4-Bromo-6-nitrobenzo[*cd*]indol-2(1*H*)-one (73). Compound **73** was prepared according to general procedure B on 28.5 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the Intermediate compound as light yellow solid (6.9 g, 23.65 mmol, yield 83%). $[M+H]^+$: 291.0. ^1H NMR (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.67 (d, *J* = 8.1 Hz, 1H), 8.31 (s, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 3.38 (s, 3H).

4-Bromo-1-methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one (74). Compound **74** was prepared according

to general procedure B on 22.6 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the Intermediate compound as light yellow solid (5.52 g, 18.04 mmol, yield 81%). $[M+H]^+$: 306.9. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.67 (d, *J* = 8.1 Hz, 1H), 8.31 (s, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 3.38 (s, 3H).

1-Methyl-4-(1-methyl-1*H*-pyrazol-4-yl)-6-nitrobenzo[*cd*]indol-2(1*H*)-one (75a). Compound **75a** was prepared according to general procedure B on 0.82 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the Intermediate compound as white solid (197 mg, 23.65 mmol, yield 86%). $[M+H]^+$: 309.2. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.50 (s, 1H), 8.95 (d, *J* = 1.3 Hz, 1H), 8.61 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 1.3 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H).

4-(1-Ethyl-1H-pyrazol-4-yl)-1-methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one (75b). Compound **75b** was prepared according to general procedure B on 0.82 mmol scale. Purification by column chromatography (10% EA/DCM) yielded the Intermediate compound as white solid (207 mg, 0.62 mmol, yield 75%). $[M+H]^+$: 337.1.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, *J* = 1.2 Hz, 1H), 8.57 (d, *J* = 8.1 Hz, 1H), 8.52 (s, 1H), 8.34 (d, *J* = 1.1 Hz, 1H), 8.12 (s, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 4.13 (t, *J* = 7.0 Hz, 2H), 3.35 (s, 3H), 1.87 (h, *J* = 7.2 Hz, 2H), 0.90 (t, *J* = 7.4 Hz, 3H).

4-(1-Cyclopropyl-1*H*-pyrazol-4-yl)-1-methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one (75c). Compound **75c** was prepared according to general procedure B on 0.82 mmol scale. Purification by column chromatography (10% EA/DCM) yielded the Intermediate compound as white solid (225 mg, 0.67mmol, yield 82%). $[M+H]^+$: 335.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.61 (d, *J* = 7.8 Hz, 2H), 8.41 (s, 1H), 8.11 (s, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 3.80 (s, 1H), 3.38 (s, 3H), 1.16 - 1.09 (m, 2H), 1.08 - 1.00 (m, 2H).

1-Methyl-4-(5-methylfuran-2-yl)-6-nitrobenzo[*cd*]indol-2(1*H*)-one (75d). Compound **75D** was prepared according to general procedure B on 0.82 mmol scale. Purification by column chromatography (DCM) yielded the Intermediate compound as white solid (159 mg, 0.52mmol, yield 63%). $[M+H]^+$: 309.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.65 (d, *J* = 8.2 Hz, 1H), 8.47 (s, 1H), 7.30 (d, *J* = 21.2 Hz, 1H), 6.48 – 6.26 (m, 1H), 5.76 (s, 1H), 3.39 (s, 3H), 2.43 (s, 3H).

4-(1-Benzyl-1H-pyrazol-4-yl)-1-methyl-6-nitrobenzo[*cd*]indol-2(1*H*)-one (75e).Compound **75e** was prepared according to general procedure B on 0.82 mmol scale. Purification by column chromatography (DCM) yielded the Intermediate compound as light yellow solid (258 mg, 0.67 mmol, yield 82%). $[M+H]^+$: 385.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.87 (d, *J* = 1.1 Hz, 1H), 8.68 (s, 1H), 8.61 (d, *J* = 8.1 Hz, 1H), 8.42 (d, *J* = 1.1 Hz, 1H), 8.20 (s, 1H), 7.35 (qd, *J* = 6.9, 2.4 Hz, 5H), 7.20 (d, *J* = 8.1 Hz, 1H), 5.40 (s, 2H), 3.37 (s, 3H).

General Procedure C for Synthesis of Target Compound 77-83

2-Methoxy-*N*-(1-methyl-4-(1-methyl-1*H*-pyrazol-4-yl)-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (77). Compound **77** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (84 mg, 0.19 mmol, yield 52%). Yellow solid; m.p 285-288 °C. HPLC analysis: retention time = 5.893 min; peak area, 97.36%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.59 (d, *J* = 1.1 Hz, 1H), 8.33 (s, 1H), 8.26 (d, *J* = 1.0 Hz, 1H), 8.05 (s, 1H), 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.49 (ddd, *J* = 8.8, 7.4, 1.8 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 7.01 – 6.93 (m, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 3.81 (s, 3H), 3.36 (s, 1H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 156.83 , 137.4 , 136.9 , 135.5 , 134.0 , 130.8 , 129.1 , 128.1 , 127.3 , 126.8 , 125.3 , 123.2 , 122.9 , 122.8 ,

122.5 , 122.3 , 120.4 , 113.1 , 105.4 , 56.5 , 39.3 , 26.6 . HRMS-EI *m/z* [M+H] $^{+}$ calcd for $C_{23}H_{21}N_4O_4S^{+}$: 449.1278, found: 449.1274.

4-Chloro-*N*-(1-methyl-4-(1-methyl-1*H*-pyrazol-4-yl)-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (78). Compound **7** was prepared according to general procedure B on 0.50 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (78 mg, 0.17 mmol, yield 48%). Yellow solid; m.p 212-213 °C. HPLC analysis: retention time = 6.047 min; peak area, 97.28%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.25 (d, *J* = 2.1 Hz, 2H), 8.13 – 8.06 (m, 1H), 7.92 (s, 1H), 7.73 – 7.63 (m, 2H), 7.60 – 7.49 (m, 2H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 3.93 (s, 3H), 3.33 (s, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.4 , 138.6 , 138.5 , 138.2 , 136.8 , 134.2 , 129.7 (2C) , 129.2 (2C) , 129.0 , 127.4 , 127.0 , 126.8 , 126.2 , 123.4 , 122.6 , 122.5 , 121.9 , 105.4 , 39.3 , 26.7 . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{22}H_{18}ClN_4O_3S^{+}$: 453.0783, found: 453.0777.

2-Methoxy-*N*-(1-methyl-4-(5-methylfuran-2-yl)-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (79). Compound **79** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the title compound (98 mg, 0.22 mmol, yield 61%). Yellow solid; m.p over 300 °C. HPLC analysis: retention time = 5.781 min; peak area, 98.08%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.49 (s, 1H), 8.31 (s, 1H), 7.56 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.22 – 7.13 (m, 2H), 6.98 (d, *J* = 7.7 Hz, 1H), 6.95 – 6.84 (m, 1H), 6.30 (dd, *J* = 3.2, 1.2 Hz, 1H), 3.97 (s, 3H), 3.30 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.1 , 156.8 , 153.2 , 151.5 , 137.9 , 135.4 , 131.6 , 130.5 , 128.0 , 127.0 , 125.9 , 125.5 , 123.4 , 120.8 , 120.7 , 120.3 , 113.0 , 109.5 , 109.2 , 105.9 , 99.9 ,

56.5 , 26.6 , 14.0 . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{24}H_{21}N_2O_5S^{+}$:449.1166, found: 449.1167.

_N_-(4-(furan-3-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[_cd_]indol-6-yl)-2-methoxybenzenesulfonamide (80). Compound **80** was prepared according to general procedure B on 0.38 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the title compound (74 mg, 0.17 mmol, yield 45%). Yellow solid; m.p 273-275 °C. HPLC analysis: retention time = 5.358 min; peak area, 98.95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.61 (s, 1H), 8.44 (s, 1H), 8.34 (s, 1H), 7.87 (s, 1H), 7.77 – 7.62 (m, 1H), 7.53 – 7.43 (m, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.18 – 7.06 (m, 2H), 6.96 (d, J = 7.9 Hz, 2H), 3.80 (s, 3H), 3.29 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 145.2 , 141.0 , 138.2 , 133.4 , 129.7 , 129.2 , 127.4 , 127.1 , 126.9 , 126.0 , 125.9 , 123.8 , 123.5 , 122.9 , 109.0 , 105.8 , 26.7 . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{23}H_{19}N_2O_5S^{+}$: 435.1009, found: 435.1007.

4-Chloro-_N_-(4-(furan-3-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[_cd_]indol-6-yl)benzenesulfonamide (81). Compound **81** was prepared according to general procedure B on 0.38 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the title compound (78 mg, 0.18 mmol, yield 47%). Yellow solid; m.p 270-272 °C. HPLC analysis: retention time = 6.301 min; peak area, 98.44%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.35 (d, J = 14.6 Hz, 2H), 8.09 (s, 1H), 7.85 (s, 1H), 7.67 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 7.6 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 1.8 Hz, 1H), 3.34 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 145.2 , 141.04 , 138.6 , 138.5 , 138.2 , 133.4 , 129.7 (2C), 129.2 (2C), 127.4 , 127.1 , 126.9 , 126.0 , 125.9 , 123.8 , 123.5 , 122.9 , 109.0 , 105.8 , 26.7 . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{22}H_{16}ClN_2O_4S^{+}$: 439.0514, found: 439.0516.

2-Methoxy-_N_-(1-methyl-2-oxo-4-(1-propyl-1_H_-pyrazol-4-yl)-1,2-dihydrobenzo[_cd_]indol-6-yl)ben

zenesulfonamide (82). Compound **82** was prepared according to general procedure B on 0.33 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (94 mg, 0.20 mmol, yield 62%). Yellow solid; m.p 264-265 °C. HPLC analysis: retention time = 4.614 min; peak area, 97.54%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.17 (s, 0H), 8.53 (s, 1H), 8.38 (s, 1H), 8.29 (s, 1H), 8.05 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.31 – 7.19 (m, 2H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.95 (d, *J* = 7.7 Hz, 1H), 4.14 (t, *J* = 6.7 Hz, 2H), 3.81 (s, 3H), 3.30 (s, 3H), 1.87 (q, *J* = 7.2 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 157.5 , 139.9 , 137.6 , 136.8 , 134.2 , 132.2 , 128.3 , 127.8 , 127.4 , 125.9 , 125.4 , 123.36 , 123.32 , 122.78 , 122.72 , 121.9 , 120.6 , 113.7 , 105.4 , 57.1 , 53.6 , 26.6 , 23.6 , 11.4 . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{25}H_{25}N_4O_4S^{+}$: 477.1591, found: 477.1592.

4-Chloro-2-methoxy-*N*-(1-methyl-2-oxo-4-(1-propyl-1*H*-pyrazol-4-yl)-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (83). Compound **83** was prepared according to general procedure B on 0.33 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (92 mg, 0.19 mmol, yield 57%). Yellow solid; m.p 271-273 °C. HPLC analysis: retention time = 6.200 min; peak area, 97.51%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.57 – 8.50 (m, 1H), 8.38 (s, 1H), 8.30 (d, *J* = 1.0 Hz, 1H), 8.06 (s, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.32 – 7.20 (m, 2H), 7.03 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 3.82 (s, 3H), 3.30 (s, 3H), 1.87 (q, *J* = 7.2 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 157.5 , 139.9 , 137.6 , 136.8 , 134.2 , 132.2 , 128.3 , 127.8 , 127.4 , 125.9 , 125.4 , 123.3 , 122.7 , 122.7 , 121.9 , 120.6 , 113.7 , 105.4 , 57.1 , 53.6 , 26.6 , 23.6 , 11.4 . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{25}H_{24}ClN_4O_4S^{+}$: 511.1201, found: 511.1206.

_N_-(4-(1-cyclopropyl-1_H_-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[_cd_]indol-6-yl)-2-methoxybenzenesulfonamide (84). Compound **84** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (90 mg, 0.19 mmol, yield 53%). Yellow solid; m.p 258-259 °C. HPLC analysis: retention time = 2.613 min; peak area, 97.84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.56 (s, 1H), 8.46 (s, 1H), 8.32 (s, 1H), 8.03 (s, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 1.9 Hz, 1H), 7.03 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 3.84 (dd, *J* = 7.1, 3.7 Hz, 1H), 3.81 (s, 3H), 1.21 – 1.09 (m, 2H), 1.05 (qd, *J* = 7.5, 3.6 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 157.5 , 139.9 , 137.6 , 136.8 , 134.0 , 132.2 , 128.3 , 127.8 , 127.4 , 125.9 , 125.3 , 123.3 , 123.0 , 122.8 , 122.7 , 121.9 , 120.6 , 113.7 , 105.4 , 57.1 , 33.4 , 26.6 , 6.8 (2C) . HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{25}H_{23}N_4O_4S^{+}$: 475.1435, found: 475.1437.

4-Chloro-_N_-(4-(1-cyclopropyl-1_H_-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[_cd_]indol-6-yl)benzenesulfonamide (85). Compound **85** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (95 mg, 0.20 mmol, yield 55%). Yellow solid; m.p 262-264 °C. HPLC analysis: retention time = 6.031 min; peak area, 96.78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.30 (s, 1H), 8.39 (s, 1H), 8.29 (s, 1H), 8.13 (s, 1H), 7.92 (s, 1H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.25 (d, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 3.82 (tt, *J* = 7.4, 3.9 Hz, 1H), 3.33 (s, 3H), 1.15 (q, *J* = 6.2, 4.7 Hz, 2H), 1.06 (dd, *J* = 7.5, 5.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 138.6 , 138.4 , 138.2 , 136.7 , 134.1 , 129.6 (2C), 129.2 (2C), 128.2 , 127.4 , 127.0 , 126.5 , 126.0 , 123.4 , 122.7 , 122.5 , 121.7 , 105.4 , 33.4 , 26.6 , 6.8 (2C). HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{24}H_{20}ClN_4O_3S^{+}$: 479.0939, found: 479.0939.

4-Chloro-*N*-(4-(1-cyclopropyl-1*H*-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)-2-methoxybenzenesulfonamide (86). Compound **86** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (95 mg, 0.19 mmol, yield 52%). Yellow solid; m.p 263-265 °C. HPLC analysis: retention time = 4.191 min; peak area, 97.08%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.56 (s, 1H), 8.46 (s, 1H), 8.32 (s, 1H), 8.03 (s, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.33 – 7.19 (m, 2H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 3.91 - 3.82 (m, 1H) 3.81 (s, 3H), 3.30 (s, 3H), 1.17 – 1.10 (m, 2H), 1.04 (q, *J* = 7.4, 6.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 157.5 , 139.9 , 137.6 , 136.8 , 134.0 , 132.2 , 128.3 , 127.8 , 127.4 , 125.9 , 125.2 , 123.3 , 123.0 , 122.7 , 122.7 , 121.9 , 120.5 , 113.7 , 105.4 , 57.1 , 33.4 , 26.6 , 6.8 (2C). HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{25}H_{22}ClN_4O_4S^{+}$: 509.1045, found: 509.1044.

4-Chloro-*N*-(4-(1-cyclopropyl-1*H*-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)-2-fluorobenzenesulfonamide (87). Compound **87** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (91 mg, 0.18 mmol, yield 51%). Yellow solid; m.p 260-262 °C. HPLC analysis: retention time = 3.745 min; peak area, 98.26%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.42 (s, 1H), 8.30 (d, *J* = 8.9 Hz, 2H), 7.93 (s, 1H), 7.76 – 7.60 (m, 2H), 7.37 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 3.82 (tt, *J* = 7.4, 3.9 Hz, 1H), 3.33 (s, 4H), 1.14 (q, *J* = 6.1, 4.3 Hz, 2H), 1.07 – 0.97 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 139.8 (d, 1C), 138.6 , 136.6 , 134.2 , 132.1 , 128.3 , 127.4 , 126.8 , 126.6 , 126.5 , 126.2 , 126.1 , 125.7 , 123.4 , 122.9 , 122.3 , 121.7 , 118.2 (d, 1C) , 105.4 , 33.4 , 26.6 , 6.8 (2C). HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{24}H_{19}ClFN_4O_3S^{+}$: 497.0845, found: 497.0844.

5-Chloro-*N*-(4-(1-cyclopropyl-1*H*-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)-2-methoxybenzenesulfonamide (88). Compound **88** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (99 mg, 0.19 mmol, yield 54%). Yellow solid; m.p 260-262 °C. HPLC analysis: retention time = 3.880 min; peak area, 96.50%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.52 (s, 1H), 8.45 (s, 1H), 8.31 (s, 1H), 8.01 (s, 1H), 7.64 – 7.51 (m, 2H), 7.30 (d, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 4.03 (q, *J* = 7.1 Hz, 1H), 3.77 (s, 3H), 3.30 (s, 3H), 1.13 (q, *J* = 4.7, 3.8 Hz, 2H), 1.04 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3 , 155.7 , 137.7 , 136.8 , 135.1 , 134.0 , 129.7 , 128.4 , 128.3 , 127.6 , 127.4 , 125.3 , 124.0 , 123.5 , 123.3 , 122.8 , 122.6 , 121.9 , 115.3 , 105.5 , 56.9 , 33.4 , 26.6 , 6.8 (2C). HRMS-EI m/z [M+H] $^+$ calcd for $C_{25}H_{22}ClN_4O_4S^+$: 509.1045, found: 509.1041.

2,4-Dichloro-*N*-(4-(1-cyclopropyl-1*H*-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[*cd*]indol-6-yl)benzenesulfonamide (89). Compound **89** was prepared according to general procedure B on 0.36 mmol scale. Purification by column chromatography (50% EA/DCM) yielded the title compound (103 mg, 0.20 mmol, yield 56%). Yellow solid; m.p over 300 °C. HPLC analysis: retention time = 7.476 min; peak area, 98.01%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.48 – 8.37 (m, 2H), 8.32 (s, 1H), 7.98 (s, 1H), 7.90 – 7.80 (m, 2H), 7.49 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 3.83 (tt, *J* = 7.5, 3.9 Hz, 1H), 3.31 (s, 3H), 1.15 (dq, *J* = 7.8, 4.0 Hz, 2H), 1.04 (dd, *J* = 7.0, 2.1 Hz, 2H). 13C NMR (75 MHz, DMSO-d6) δ 167.3 , 138.9 , 138.4 , 136.6 , 136.1 , 134.2 , 133.3 , 132.5 , 131.7 , 128.3 , 128.3 , 127.4 , 126.5 , 125.9 , 125.4 , 122.9 , 122.3 , 121.8 , 105.4 , 33.4 , 26.6 , 6.8 (2C) . HRMS-EI m/z [M+H] $^+$ calcd for $C_{23}H_{22}N_4O_4S^+$: 513.0549, found: 513.0543 .

_N_-(4-(1-benzyl-1_H_-pyrazol-4-yl)-1-methyl-2-oxo-1,2-dihydrobenzo[_cd_]indol-6-yl)-2-methoxybenzenesulfonamide (90). Compound **90** was prepared according to general procedure B on 0.33 mmol scale. Purification by column chromatography (20% EA/DCM) yielded the title compound (74 mg, 0.14 mmol, yield 43%). Yellow solid; m.p 243-245 °C. HPLC analysis: retention time = 4.944 min; peak area, 95.51%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.55 (s, 1H), 8.42 (s, 1H), 8.29 (s, 1H), 8.12 (s, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.51 - 7.24 (m, 7H), 7.05 (d, J = 8.4 Hz, 1H), 6.93 (dt, J = 8.0, 3.9 Hz, 2H), 5.43 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2 , 156.7 , 137.6 , 137.3 , 135.5 , 133.8 , 130.8 , 129.1 (3C) , 128.4 , 128.2 (3C) , 128.1 , 127.3 , 126.8 , 125.2 , 123.3 , 123.0 (2C) , 122.6 (2C) , 120.4 , 113.0 , 105.5 , 56.4 , 55.7 , 26.6 .. HRMS-EI m/z [M+H] $^{+}$ calcd for $C_{22}H_{18}ClN_4O_3S^{+}$: 525.1591 , found: 525.1584.

ASSOCIATED CONTENT

The following files are available free of charge.

Pharmacokinetics of **68** in liver microsomes; Permeability assay of compounds in PAMPA; NF-κB transcriptional activity of compounds in HUVECs; The concentration of compounds in different medium condition; Data collection and refinement statistics; Antibodies used for immunofluorescence; Antibodies used for Western blot analysis; X-ray electron density maps of **25** and **60**; the Kd curve Images of **68**; The AlphaScreen assay of **68** for BET families and BRPF1b; The uncropped western blot images; NMR spectra; HPLC analysis result data. (.pdf)

Molecular Formula Strings (.CSV)

Molecular modeling information (.pdb)

Authors will release the atomic coordinates and experimental data upon article publication

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ACKNOWLEDGMENT

We are grateful to the National Natural Science Foundation of China (Program No. 81673301) for financial support. This work was financially supported by the "Double First-Class" University project from China Pharmaceutical University (Program No. CPU2018GF02), the Priority Academic Program Development of Jiangsu Higher Education Institutions (Integration of Chinese and Western Medicine) and the China Postdoctoral Science Foundation (2018M632428). We also express our gratitude to Dr Bingxu Zhang (3D BioOptima Co. Ltd.) and Dr Jamie Planck (RBC, Pennsylvania, USA) for their helpful support in biological evaluation.

ABBREVIATIONS

BRD4(1), the first Bromodomain of Bromodomain-containing protein 4; BRD4(2), the second Bromodomain of Bromodomain-containing protein 4;BRD4, Bromodomain-containing protein 4;TFA, trifluoroacetic acid; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NACHT, LRR and PYD domains-containing protein 3; PI, Propidium iodide; MSU, monosodium urate;

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