Design, Synthesis, and Characterization of a Fluorescence Polarization Pan-BET Bromodomain Probe

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S Supporting Information

ABSTRACT: Several chemical probes have been developed for use in fluorescence polarization screening assays to aid in drug discovery for the bromodomain and extra-terminal domain (BET) proteins. However, few of those have been characterized in the literature. We have designed, synthesized, and thoroughly characterized a novel fluorescence polarization pan-BET chemical probe suitable for high-throughput screening, structure–activity relationships, and hit-to-lead potency and selectivity assays to identify and characterize BET bromodomain inhibitors.

KEYWORDS: bromodomain and extra-terminal domain, BET, pan-BET probe, fluorescence polarization

Bromodomain (BRD)-containing proteins are highly conserved epigenetic regulators that recognize and bind to acetylated lysine (KAc) residues of histones. BRDs recruit proteins to macromolecular complexes essential for chromatin remodeling and transcriptional control.¹ The highly diverse BRD family members share a conserved structural motif comprised of a left-handed bundle of four alpha helices (α Z, α A, α B, α C) that are linked by two variable loop regions (ZA and BC loops) flanking a deep central hydrophobic cavity that recognizes sequences containing ϵ -N-acetylated lysine residues.² Each of the BRD and extra-terminal domain (BET) family members, BRD2, BRD3, BRD4, and BRDT, contain two N- and C-terminal KAc binding domains (designated as BD1 and BD2, respectively), which play important roles in regulating the transcription of growth-promoting genes and cell cycle regulators.³ While BRD2, 3, and 4 are ubiquitously expressed, BRDT is exclusively found in the testis.⁴ Mutagenesis studies have shown that loss of BRDT-BD1 (BRDT-1) results in abnormal spermatids and complete sterility in mice⁵ making BRDT-1 an attractive target for developing nonhormonal male

contraceptives.⁶ The greatest challenge for BRD drug discovery efforts is identifying compounds that are selective for individual BRD proteins due to the high homology of KAc binding sites within BRD families.⁷ In addition, the discovery of potent inhibitors with high selectivity for BD1 over BD2 (or BD2 over BD1) has been challenging because, although the BET BRD loop regions are variable, the BD1 and BD2 substrate binding pockets show high sequence similarity.⁷

Several chemical probes for use in fluorescence polarization (FP) assays have been developed to aid in drug discovery programs for BET proteins. Previously reported probes for BET BRDs either have low binding affinities or have low polarization signal windows, and most of them are not well characterized. For example, BODIPY-conjugated BI6727 (BI-BODIPY) is reported to have a K_D of 50–110 nM for BRD4-BD1 (BRD4-1)^{8,9} and only a 40 mA polarization signal window, while the

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Alexafluor488-IBET¹⁰ and FITC-JQ1^{11–13} probes remain uncharacterized. This prompted us to develop a novel FP small molecule chemical probe utilizing the high affinity pan-BET inhibitor SG3-179 (Figures 1 and 3; compound 5 in Ember et al.¹⁴). SG3-179–BODIPY probe 16 was selected for further characterization as described below.

Figure 1. Molecular docking predicts that compound 16 will maintain high affinity for BRDT-1. Compound 16 orients similarly to the parent compound SG3-179 and maintains the key interaction with Asn109 of BRDT-1 (PDB: 4FLP). The fluorophore is solvent exposed without the possibility of any steric hindrance with the protein.

A general challenge for probe development is to avoid a significant loss of binding affinity caused by steric hindrance of the attached fluorophore. To achieve this goal, we docked SG3-179 to a representative BET-family protein BRDT-1 (PDB: 4FLP) to reveal the binding mode of SG3-179 for BRDT-1 (Figure 1). The predicted binding mode is similar to the later released crystal structure SG3-179 in complex with BRD4-1 (PDB: 5F63).¹⁴ The diaminopyrimidine core of SG3-179 forms a direct hydrogen bond with Asn 109 in the KAc binding site of BRDT-1, and the water exposed piperidine moiety is far away from this key interaction, which therefore provided a reasonable position to connect to a fluorophore. We chose BODIPY as the fluorophore due to its long fluorescence lifetime (5–6 ns)¹⁵ and because tetra-methyl BODIPY is an inexpensive fluorophore among BODIPY dyes and numerous chemistries involving tetra-methyl BODIPY are available for rapid linker incorporation.^{16–18} Although a longer linker length may alleviate steric hindrance, local motion of fluorophores attached by flexible linkers can decrease the polarization of the bound probe (“propeller effect”).¹⁹ In order to identify the optimal balance between steric hindrance and unrestrained fluorophore motion, linker length and flexibility were explored.

The synthetic route for SG3-179 based probes 16–18 is shown in Schemes 1 and 2. The synthesis of the KAc binding site ligand employed procedures similar to the methods reported for the preparation of SG3-179 (Scheme 1).^{20,21} Protection of commercially available 4-chloro-3-nitroaniline with Boc anhydride gave 1, followed by reduction of the nitro group with hydrazine to provide aniline 2. Reaction of 2 with *tert*-butylsulfinyl chloride furnished *tert*-butylsulfinyl 3 that was further oxidized with mCPBA to sulfonamide 4. Deprotection of 4 with TFA produced aniline 5, which was reacted with 2,4-dichloro-5-methylpyrimidine to afford chloropyrimidine 6. Intermediate 7 was obtained by coupling 4-amino-2-fluorobenzoic acid and *tert*-butyl 4-aminopiperidine-1-carboxylate, using

Scheme 1^a

^aReagents: (a) (Boc)₂O, THF, reflux, 28 h, 58%; (b) Pd/C, hydrazine monohydrate, MeOH, reflux, 10 h, 64%; (c) 2-methylpropane-2-sulfinyl chloride, pyridine, DCM, rt, 20 h, 77%; (d) mCPBA, DCM, rt, overnight, 84%; (e) TFA, DCM, rt, 2 h, 78%; (f) 2,4-dichloro-5-methylpyrimidine, DIPEA, isopropanol, 120 °C, 2 d, 36%; (g) *tert*-butyl 4-aminopiperidine-1-carboxylate, HATU, DIPEA, DMF, rt, 12 h, 95%; (h) compound 6, Pd₂(dba)₃, (±)-2,2′-Bis-(diphenylphosphino)-1,1′-binaphthalene (BINAP), NaOtBu, 1,4-dioxane, 110 °C, 5 h, 90%; (i) TFA, DCM, 12 h, 91%.

Scheme 2^a

^aReagents: (a) DMF, POCl₃, 0 °C, 2–3 h, then 50 °C, 3 h, 89%; (b) NaClO₂, H₂O, NaH₂PO₄, rt, 15 h, 30%; (c) malonic acid, pyridine, DMF, 90 °C, 5 h, 28%; (d) methyl acrylate, 20 mol % of Pd(OAc)₂, *tert*-BuOOBz, DMSO, 35 °C, 5 d, 57%; (e) Pd/C, H₂, MeCN, MeOH, rt, 12 h, 80%; (f) NaOH, DCM/MeOH/H₂O, rt, 10 h, then neutralize, 98%; (g) intermediate 11, 12, or 15, HOBt, EDC, DIPEA, DCM, rt, 6 h, 72–93%.

1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) in the presence of *N,N*-diisopropylethylamine (DIEPA) in dry DMF. Connection of fragments 6 and 7 by a Buchwald–Hartwig coupling reaction yielded 8, which was then Boc deprotected with TFA to give the KAc binding site ligand 9.

The synthesis of the fluorophore moiety commenced with commercially available tetramethyl-BODIPY (Scheme 2) by reaction with DMF in the presence of POCl₃ to produce aldehyde 10,¹⁷ which was then oxidized to carboxylic acid 11. Acrylic acid 12 was synthesized from aldehyde 10 by a Knoevenagel condensation.¹⁸ A Heck-type reaction between tetramethyl-BODIPY and methyl acrylate catalyzed by Pd(0) yielded methyl acrylate 13.¹⁶ The double bond in 13 was

reduced with H_2 to give methyl propionate **14**, which was hydrolyzed to yield acid **15**. Fluorophores **11**, **12**, and **15** were linked to ligand **9** (HOBt and EDC) to produce BODIPY based FP probes **16–18**.

In comparison to compound **16**, which has sharp UV absorption and fluorescence excitation and emission peaks similar to BODIPY, the peaks for compounds **17** and particularly **18** are broadened and red-shifted (Figure S1). Although it is not clear why compound **17** has a red-shifted emission spectrum, the extended π -conjugated system present in compound **18** is expected to produce a higher LUMO level and narrowed HOMO–LUMO gap,²² which likely explains the red-shifted emission spectrum.

The three probes (**16–18**) were then tested against BRDT-1 in a FP assay to assess probe affinity and signal window (Figure S2). Whereas probes **16** and **18** have similar affinities, probe **16** had the highest FP signal window ($B_{\max} > 100$ mP) at the standard BODIPY excitation and emission wavelengths and was therefore selected for further characterization. K_D values for all the BET family proteins (BRDT-1, BRDT-2, BRDT-T, BRD2-1, BRD2-2, BRD3-1, BRD3-2, BRD4-1, BRD4-2, and BRD4-T, where -T designates the BD1 and BD2 tandem BRD protein), and two representative non-BET family proteins (BRD7 and BRD9) were determined with FP probe **16** (Table 1, Figures 2 and S3).

Table 1. Affinity of Pan-BET FP Probe 16 for BRD Proteins^a

	K_D , nM		
	BD1	BD2	tandem
BRDT	17 ± 2	10 ± 2	5.5 ± 0.6
BRD4	17 ± 1	9.6 ± 0.4	10 ± 1
BRD3	23 ± 1	7.5 ± 0.5	
BRD2	56 ± 4	12 ± 1	
BRD7	12 000 ± 2000		
BRD9	7600 ± 1300		

^aValues are mean + SEM in nM, where $n \geq 3$ independent experiments.

Figure 2. Pan-BET probe **16** binds with high affinity to BET BRDs, but only weakly to other BRD proteins. Error bars represent $\pm$ SEM where $n \geq 3$.

FP probe **16** exhibited low nanomolar affinities for all of the BET family proteins (6–56 nM) but had K_D values in the low micromolar range for the two non-BET family proteins (8–12 μ M) (Figure 2). The lower affinity of probe **16** for BRD7 and BRD9 was expected based upon previously reported SG3-179 BROMOScan data.¹⁴ Therefore, compound **16** is a pan-BET probe but is unlikely to be a useful tool for non-BET BRDs. Probe **16** has a slight preference for BD2 over BD1 BRDs, ranging from 1.7- to 4.7-fold higher affinity for BD2 depending

on the BET family member. In contrast to the BRD4-T protein, the pan-BET probe **16** bound BRDT-T with 2- to 3-fold higher affinity than the individual BRD2 BRDs. Although it has been argued that the two BRDs of BRDT function independently due to the long flexible linker connecting them,²³ the higher affinity of the probe for BRDT-T suggests that there may be cooperativity between the tandem BRDs or that the 123 aa linker may stabilize one or both BRDs.

Ten representative BET BRD inhibitors with varying scaffolds and selectivities were chosen for competition studies to establish that pan-BET probe **16** provides the expected affinity and selectivity values for the BET BRDs. The structures of the reference compounds SG3-179, ABBV-075, TG101209, (+)-JQ1, (–)-JQ1, CAS 2098312-12-8, bromosporine, BI2536, MS436, and RVX-208 are illustrated in Figure 3. IC_{50}

Figure 3. Structures of the 10 reference BET inhibitors with diverse chemical scaffolds selected to validate the FP assay. CAS 2096312-12-8 refers to compound **3s** from Ayoub et al.⁹

values were generated for all ten compounds against the BET-family proteins, and K_i values were calculated using the equations of Nikolovska-Coleska et al.²⁴ (Figures 4 and S4, Table 2). In some cases, K_i values could not be determined because IC_{50} values were too low relative to the protein concentration (30–130 nM) used in the assay. In these cases, the apparent IC_{50} values likely underestimate the true potency of the inhibitors. As protein concentrations could not be lowered in order to maintain a sufficient signal window, an alternate method must be employed to obtain accurate affinity estimates for these very potent inhibitors due to this “floor effect”. Additionally, K_i values are not reported for BRDT-T and BRD4-T as the K_i calculation equation cannot be used for proteins with >1 ligand binding site.²⁴

Parent compound SG3-179 has been reported to have an $IC_{50} \approx 20$ nM for both BRDT-1 and BRD4-1 using an AlphaScreen assay.¹⁴ SG3-179 FP K_i values are ~ 15 nM for these BRDs. SG3-179 FP K_i values for BD1 domains of the four BET BRDs correlate well with the K_D values obtained for the SG3-179-BODIPY probe **16**. However, SG3-179 has 3- to 5-fold lower affinity for the BD2 domains of BRDT, BRD2, and BRD4 based on SG3-179 K_i values relative to probe **16** K_D values, perhaps due to a contribution of the fluorophore to the binding of probe **16** to these proteins.

Figure 4. Competitive displacement of pan-BET probe **16** by 10 reference BET inhibitors at (a) BRDT-BD1 and (b) BRDT-BD2. Error bars represent $\pm$ SEM where $n \geq 3$. Z' value for BRDT-1 = 0.78 ± 0.04 (mean $\pm$ SEM).

ABBV-075, also known as mivebresib, is a potent small molecule BET inhibitor presently in a Phase I clinical trial for patients with advanced hematologic malignancies and solid tumors.²⁵ ABBV-075 has previously been reported to be selective for tandem BRDT, BRD2, and BRD4 proteins ($K_i = 1$ –2 nM) over the tandem BRD3 protein ($K_i = 12.2$ nM) in TR-FRET assays.²⁵ When tested in the FP assay reported here, the IC_{50} values ranged from 9 to 32 nM for all of the BRD proteins. These IC_{50} values likely underestimate the true affinity of ABBV-075, and K_i values could not be calculated due to the “floor effect” described above, consistent with the high affinity values reported using the TR-FRET assay.²⁵

The prototypic BET inhibitor, (+)-JQ1, is a thieno-triazolo-1,4-diazepine with reported AlphaScreen and TR-FRET IC_{50} values ranging from 18–91 nM for BRD4-1,^{14,26,29,30} 14–33 nM for BRD4-2 and BRD3-1,^{29,30} and 100–150 nM for BRDT-1 and BRD2-1.^{14,26} The FP assay provides similar IC_{50} values, particularly for BRDT-1, although it indicates a higher potency of (+)-JQ1 for BRD2-1, but a lower potency at BRD3-1 and BRD4-1. (–)-JQ1, the inactive enantiomer of (+)-JQ1, is considerably less potent with reported IC_{50} values of 8354 and >10 000 nM for BRD4-1 and 52 120 nM for BRD4-2.^{29,30} We report similar findings in our FP assay in which (–)-JQ1 had IC_{50} values >10 000 nM for all BET BRDs.

The dihydropyridopyrimidine compound (CAS registry number: 2098312-12-8) is a potent BET inhibitor that has been shown to have submicromolar affinity (BRDT-1, $K_i = 200$ nM; BRD4-1, $K_i = 110$ nM) in a FP assay using BI-BODIPY.⁹ Three- to 6-fold higher affinity values were obtained using the SG3-179-BODIPY probe **16** for BRDT-1 and BRD4-1, respectively. The prior report by Ayoub et al.⁹ carried out the experiment at a 5-fold higher DMSO concentration (0.5% vs 0.1%), which is known to affect BRD binding interactions.²⁸ Additionally, 2098312-12-8 had higher affinity for BD1 and BD2 of both BRD2 and BRD3, which exceeded the sensitivity found in the literature, K_D values for bromosporine using ITC have been reported to be in the 40–100 nM range for BRDT-1 and for both BD1 and BD2 of BRD2, BRD3, and BRD4, whereas BRDT-2 had a significantly higher K_D .

Bromosporine is a promiscuous BRD inhibitor that was developed to target the conserved KAc recognition site of BRD-containing proteins. While IC_{50} and K_i information could not be found in the literature, K_D values for bromosporine against all of the BET proteins using ITC have been reported to be in the 40–100 nM range for BRD2, BRD3, BRD4, and BRDT, forms BD1 and BD2, excluding BRDT-2 which had a significantly higher K_D value ($K_D = 172$ nM).²⁷ Similarly, K_i values obtained in the FP assay for BRD4-1, BRD4-2, and BRDT-1 ranged from 35–90

nM, and bromosporine had lower affinity for BRDT-2 ($K_i = 310$ nM). In contrast, bromosporine had higher affinities for BD1 and BD2 of both BRD2 and BRD3 in the FP assay that exceeded the sensitivity of the assay.

BI2536 is a potent and selective inhibitor of PLK1 ($IC_{50} = 0.83$ nM) and has antitumor activity against relapsed or refractory acute myeloid lymphoma (AML) and nonsmall cell lung cancer in phase I/II clinical trials. It binds to the KAc recognition site of BRD4 through an elaborate network of hydrogen bonding and van der Waals interactions.²⁶ BI2536 was shown to be selective for BRD4-1 over BRDT-1 with IC_{50} values of 25 and 260 nM, respectively, in an AlphaScreen assay.²⁶ Similarly, in the FP assay, BI2536 had higher affinity for BRD4-1 ($K_i = 15$ nM) compared to BRDT-1 ($K_i = 71$ nM). We also found that BI2536 had high affinity for BRD2-2 ($K_i = 9.3$ nM).

TG101209, a dual BET BRD and JAK2 kinase inhibitor, has been reported in the literature to have lower affinity for BRD2-1 ($IC_{50} = 680$ nM) relative to BRD3-1, BRD4-1, and BRDT-1 ($IC_{50} = 130$ –290 nM).²⁶ The K_i values determined in the FP assay for these BRDs revealed a similar profile in which TG101209 was 2–5 times less active against BRD2-1 ($K_i = 700$ nM) than the other BRD BD1s ($K_i = 120$ –320 nM).

The BD1-selective diazobenzene-based inhibitor MS436 has been reported to have a $K_i < 85$ nM for BRD4-1 and had >4-fold lower affinity for BD2.³¹ In our assay, MS436 showed similar activity with a BRD4-1 K_i of 130 nM and 4-fold lower affinity for BRD4-2. MS436 had ~2- to 4-fold lower affinity for the other BET BRDs and showed lower selectivity for their BD2 domains.

RVX-208 has been evaluated in several clinical trials for the treatment of atherosclerosis and associated cardiovascular disease.³² RVX-208 has been reported to be a BD2-selective inhibitor of BET proteins with K_D values measured by ITC for BD1 that are 8.5- to 23-fold higher than those determined for BD2, with highest selectivity for BRD2 and BRD3.³² In the FP assay, a similar trend was observed where RVX-208 is 3–17 times selective for BD2 over BD1 depending on the BRD protein, with highest BD2 selectivity in BRD3.

Several compounds, including SG3-179, ABBV-075, (+)-JQ1, TG101209, MS436, and RVX-208 inhibited the binding of the pan-BET probe **16** to the BRDT-T and BRD4-T proteins with IC_{50} values close to their respective BD1 and BD2 domains, as expected assuming no effect of the 123 aa linker on BRD affinity. In contrast, CAS 2098312-12-8, bromosporine, and BI2536 had considerably lower IC_{50} values for the tandem BRDT and BRD4 BRDs compared to the individual BD1 and BD2 domains, up to >80-fold higher potency for BRD4-T over BRD4-2 in the case of bromosporine. These latter inhibitors apparently bind to BRDs in the tandem protein in a cooperative manner or, alternatively,

Table 2. Inhibitory Potency (IC_{50}) and Affinity (K_i) of Representative Inhibitors for BET Family BRDs Determined Using pan-BET FP Probe 16^a

compd	BRDT-1	BRD2-1	BRD3-1	BRD4-1	BRDT-2	BRD2-2	BRD3-2	BRD4-2	BRDT-T	BRD4-T
SG3-179	IC_{50} 140 ± 10	114 ± 1	200 ± 20	250 ± 16	237 ± 8	250 ± 25	90 ± 10	220 ± 20	307 ± 7	137 ± 7
ABBV-075 (+)-JQ1	K_i 15 ± 2	42 ± 1	20 ± 3	17 ± 1	50 ± 2	41 ± 6	6 ± 2	27 ± 341		
	IC_{50} 12 ± 1	9.0 ± 0.5	17 ± 2	19 ± 2	11 ± 1	14.3 ± 0.6	7.7 ± 0.7	13 ± 1	32 ± 3	11.5 ± 0.3
	IC_{50} 130 ± 10	60 ± 7	110 ± 10	120 ± 10	85 ± 7	110 ± 10	60 ± 7	97 ± 4	170 ± 12	70 ± 5
CAS 2098312-12-8	K_i 13 ± 2				15 ± 1	14 ± 2		8.6 ± 0.6		
	IC_{50} 380 ± 10	7.4 ± 0.2	21 ± 2	260 ± 10	130 ± 10	12.5 ± 0.9	8.3 ± 0.7	180 ± 16	120 ± 16	15 ± 1
	K_i 60 ± 2			18 ± 2	27 ± 3			21 ± 3		
bromosporine	IC_{50} 390 ± 9		43 ± 5	410 ± 40	1300 ± 300	52 ± 7	27 ± 2	700 ± 100	39 ± 1	8.5 ± 0.2
	K_i 62 ± 2	18 ± 2		35 ± 5	310 ± 40			90 ± 20		
	IC_{50} 441 ± 7	9.0 ± 0.9	34 ± 2	240 ± 20	1050 ± 80	86 ± 7	25 ± 1	360 ± 10	68 ± 3	12.4 ± 0.7
BI2536	K_i 71 ± 1			15 ± 2	250 ± 20	9.3 ± 1.3		49 ± 2		
	IC_{50} 830 ± 60	1390 ± 40	1800 ± 100	1100 ± 90	2400 ± 100	3900 ± 200	1120 ± 10	2000 ± 80	2300 ± 100	740 ± 10
	K_i 140 ± 10	700 ± 20	320 ± 20	120 ± 10	560 ± 30	760 ± 40	146 ± 1	300 ± 10		
MS436	IC_{50} 2400 ± 100	620 ± 20	1300 ± 80	1200 ± 100	4700 ± 500	2700 ± 400	1550 ± 70	3400 ± 100	5700 ± 300	1200 ± 100
	K_i 450 ± 20	300 ± 10	220 ± 20	130 ± 10	1200 ± 100	540 ± 80	205 ± 9	536 ± 5		
	IC_{50} 43000 ± 1600	13000 ± 600	43000 ± 3000	11000 ± 200	4800 ± 500	4000 ± 200	3300 ± 100	3000 ± 400	26000 ± 3000	9000 ± 1000
RVX-208	K_i 8200 ± 300	6700 ± 300	7800 ± 500	1300 ± 30	1200 ± 100	800 ± 50	450 ± 10	450 ± 60		
	IC_{50} $>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$	$>10\ 000$
	IC_{50} $>10\ 000$									

^aValues are mean $\pm$ SEM in nM, where $n \geq 3$. K_i values are included for BRDs containing a single binding domain (BD1 or BD2) if potency values are greater than the lower sensitivity limit of the assay ($IC_{50} \geq 1.5 \times$ BRD concentration).

the amino acid linker in the tandem proteins induces conformational changes in the BD1 and/or BD2 domains that increase the affinity of these inhibitors, as observed for probe **16** at BRDT-T (Table 1).

In conclusion, the novel pan-BET probe **16** has high affinity for BD1, BD2, and tandem constructs of BET family members and provides a high FP signal window sufficiently robust for HTS ($Z' = 0.78$, Figure 4). It can be used to establish the SAR for inhibitor series, determine the selectivity of inhibitors for BET family members and their respective BD1 and BD2 KAc recognition sites, and for HTS to identify novel BET BRD inhibitor scaffolds.

■ ASSOCIATED CONTENT

Supporting Information

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Materials and methods; UV absorbance and fluorescence spectra of probes **16–18**; saturation binding curves and a table of K_D values of probes **16–18** for BRDT-1; saturation binding curves with probe **16** for BRDT-2, BRDT-T, BRD2-1, BRD2-2, BRD3-1, BRD3-2, BRD4-2, and BRD4-T; competitive displacement of probe **16** by 10 representative BET inhibitors against BRDT-T, BRD4-1, BRD4-2, BRD4-T, BRD3-1, BRD3-2, BRD2-1, and BRD2-2; references; NMR spectra (PDF)

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Notes

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■ ABBREVIATIONS

BD1, binding domain 1; BD2, binding domain 2; BET, bromodomain and extra-terminal domain; BRD, bromodomain; BRD2, bromodomain containing protein 2; BRD3, bromodomain containing protein 3; BRD4, bromodomain containing

protein 4; BRDT, testes specific bromodomain containing protein; FP, fluorescence polarization; KAc, acetylated lysine

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