Electronic Supplementary Material (ESI) for MedChemComm.

Discovery of novel trimethoxy-ring BRD4 bromodomain inhibitors : Alphascreening, crystallography, cell-based assay

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qRT-PCR

MV-4-11 cells were used to test whether DC-BD-03 inhibits the expression of genes c-Myc, CDK6, Bcl-2, which are closely related to the function of BRD4 (Fu, L.-l., et al.). Cells were treated with compounds of different concentrations or DMSO for 6h. Total RNA was extracted with the UNlQ-10 Column total RNA Purification Kit (Sangon Biotech). Using oligo (dT)20 primer and reverse transcriptase, cDNA was created by using the HiScript® II RT SuperMix for PCR (Vazyme). Reverse transcription and quantitative PCR were performed as the protocol that Vazyme supplied, using SYBR-GREEN (Vazyme, Low Rox for QuantStudio 6 Flex applied Biosystems by life technologies) for BCL2 (primer: GTTTCAAATCAGCTATAACTGGAG; reverse: TAATATCAGTCTACTTCCTCTGTG), CDK6 (primer: TCTAACCTCAGTGGTCGTCAC ; reverse: TTCTCCTGGGAGTCCAATCAC), C-Myc (primer: GTGCTCCATGAGGAGACACC; reverse: GCACCTCTTGAGGACCAGTG), and β2-microglobulin (primer: AAGTTGACTTACTGAAGAATGGAG; reverse: ATGCTGCTTACATGTCTCGATC) purchased from Sangon Biotech. Expression levels were normalized to that of β-Actin were calculated using a standard curve and the relative quantization method as described in ABI User.

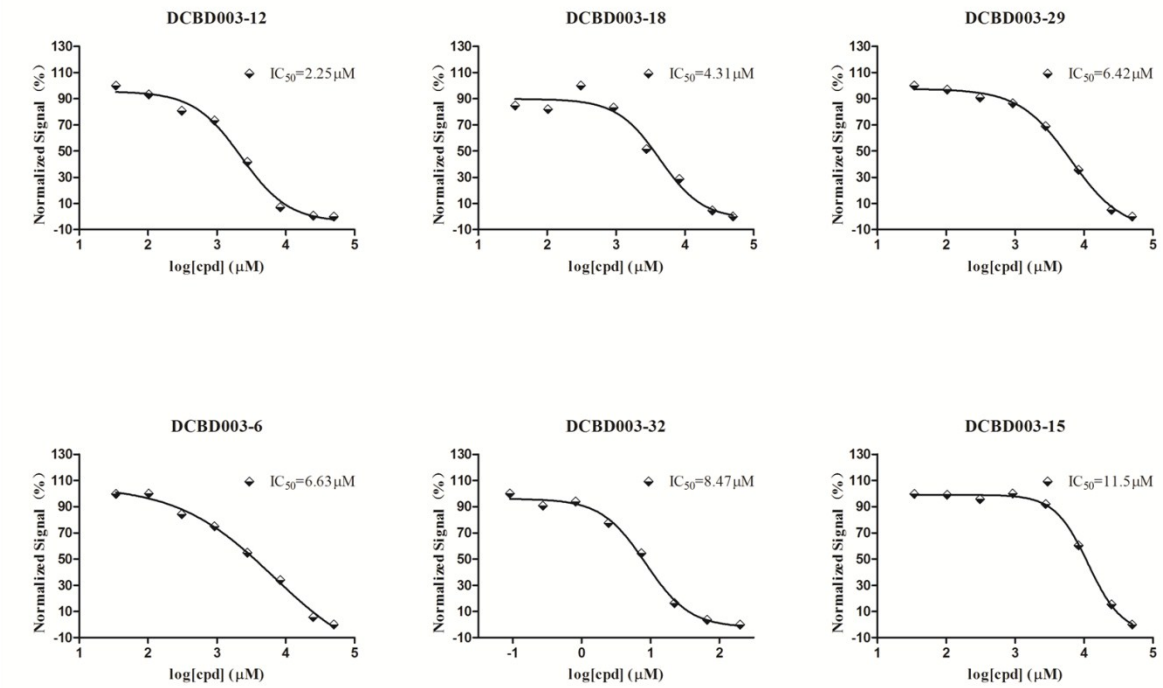


Figure S1. The inhibitory activity of several compounds in Table 1 at different concentrations against the first bromodomain of BRD4. The IC_{50} valueswere calculated and the curveswere plotted using the software GraphPad.

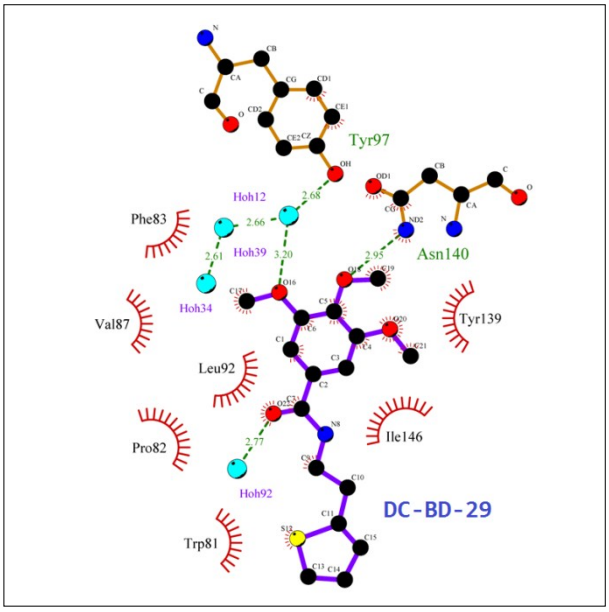


Figure S2. The hydrogen bonds and hydrophobic contacts formed by compound DC-BD-29 with the first bromodomain of BRD4, revealed by the solved crystal structure.

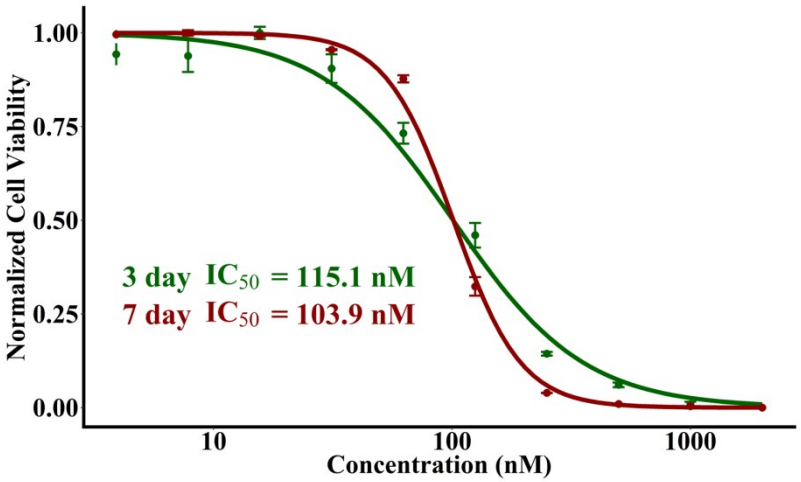


Figure S3. The cellular inhibition of compound I-BET151 against the MV4-11 cells, the IC_{50} values are 115.1 nM for three days after treatment and 103.9 nM for seven days after treatment.

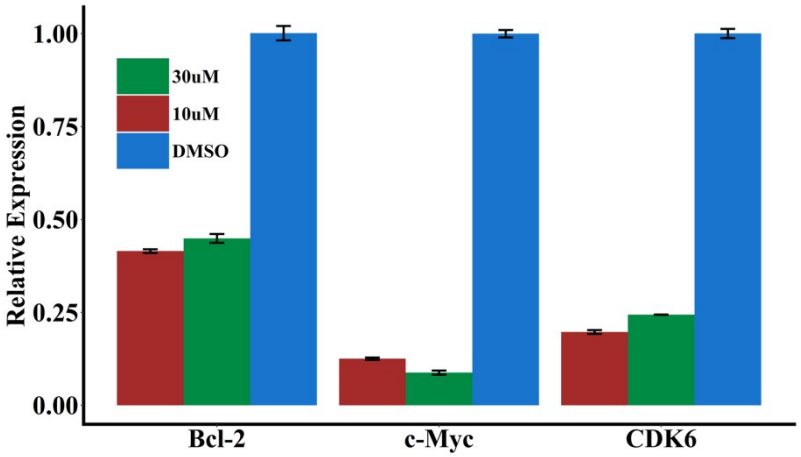


Figure S4. Compound I-BET151 decreased the expression of BRD4 downstream genes Bcl-2, c-Myc, and CDK6, after incubation for 6 hours, at the concentrations of 10 μM and 30 μM.

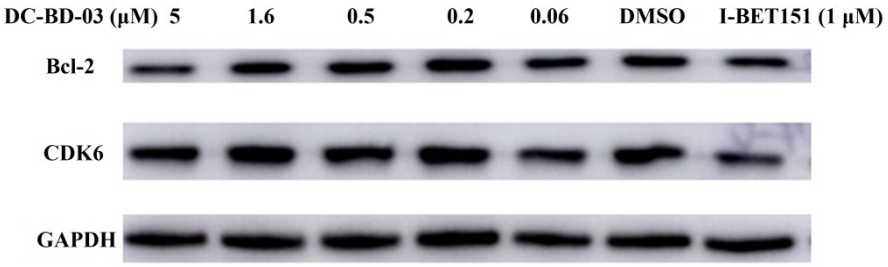
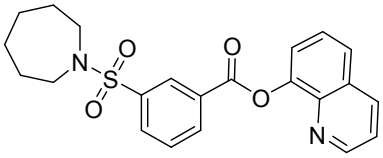
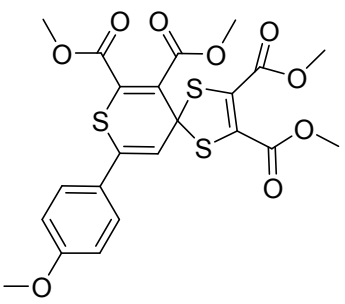
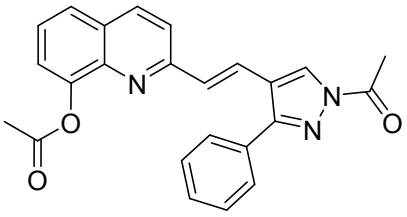
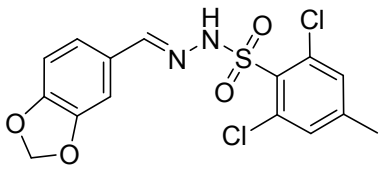
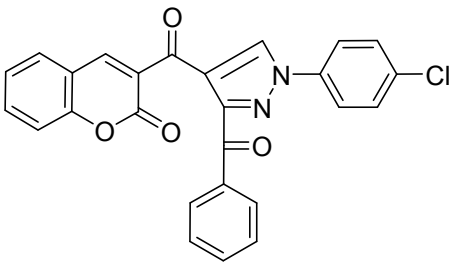
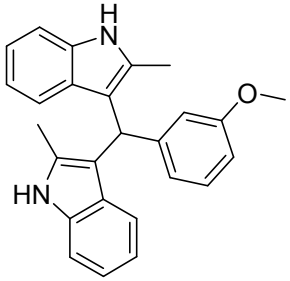
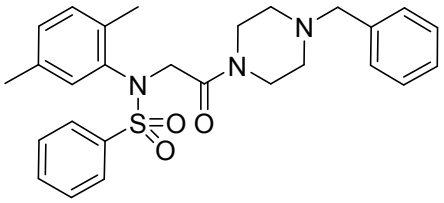
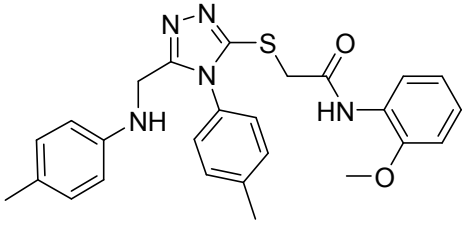
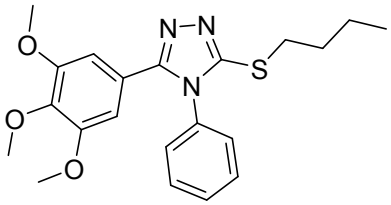
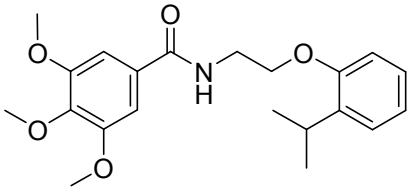
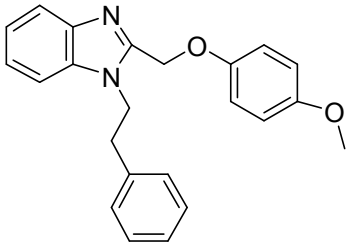
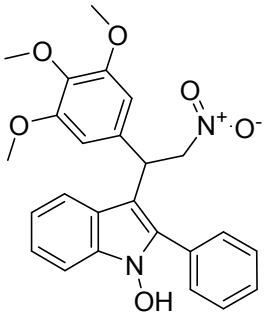
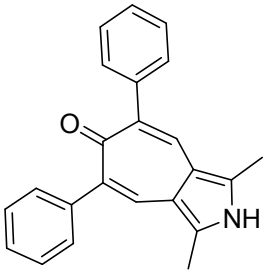
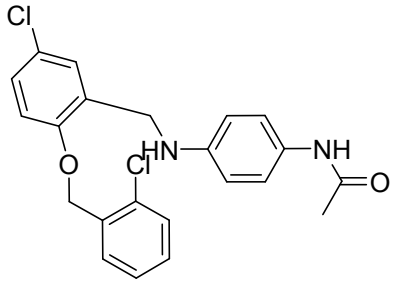
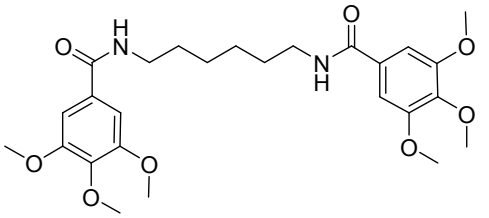
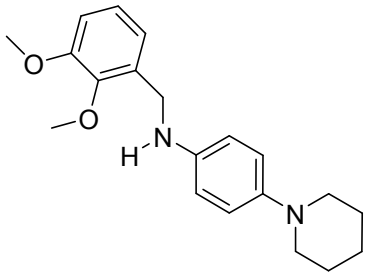
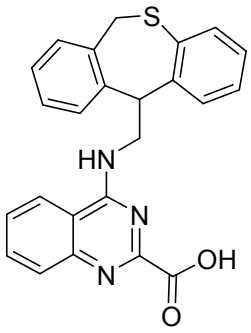
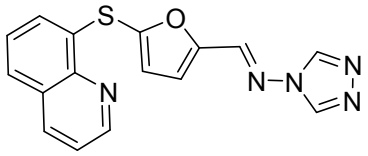
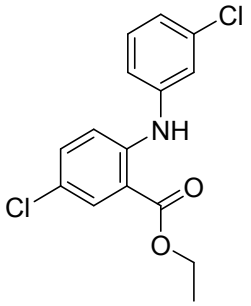
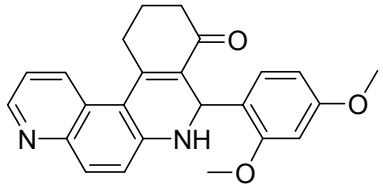


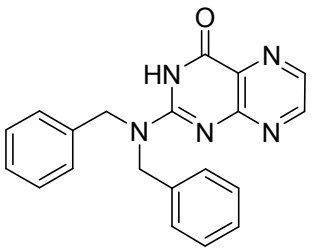
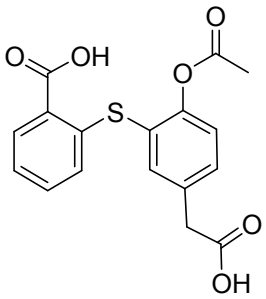
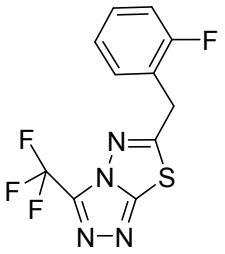
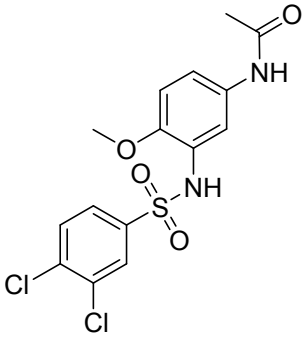
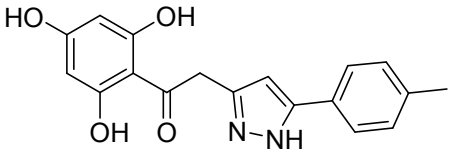
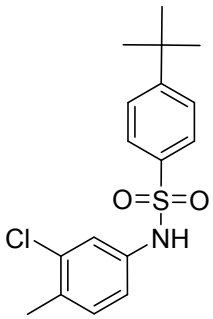
Figure S5. The effect of compound DC-BD-03 against the protein abundance of Bcl-2 and CDK6, at different concentrations, in comparison with the positive control I-BET151.

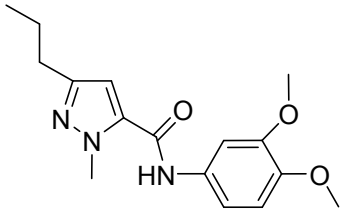
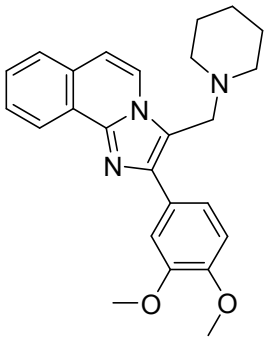
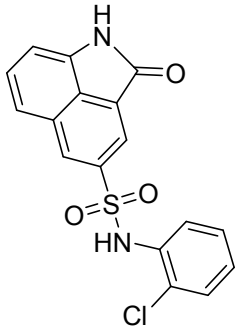
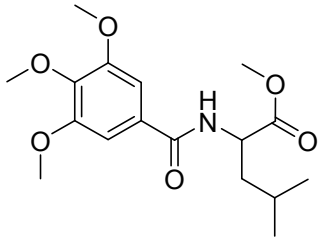
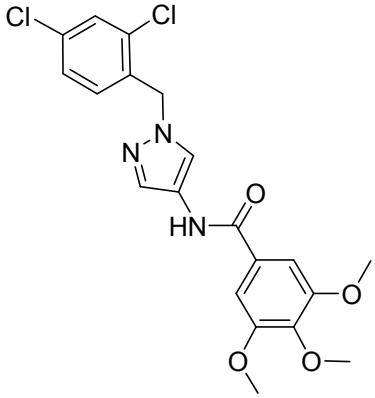
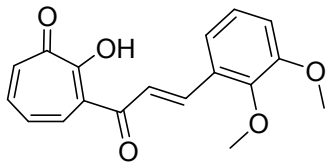
Table S1. The information for top 50 compounds in the first round AlphaScreen assay.

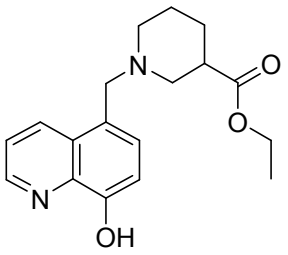
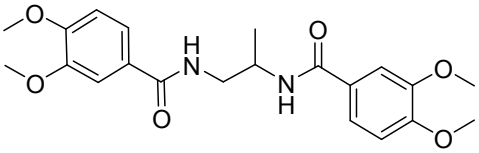
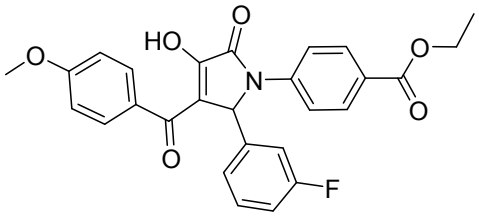
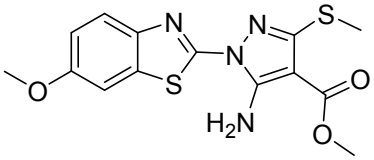
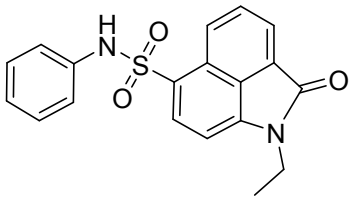
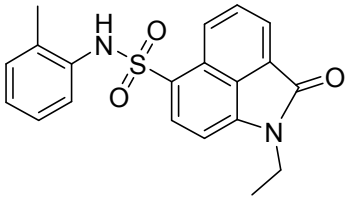
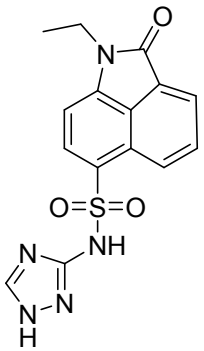
NO.	Structure	50μM Inhibition(%)[a]
1		68.75
2		98.63
3		66.62
4		67.70
5		91.02
6		88.01

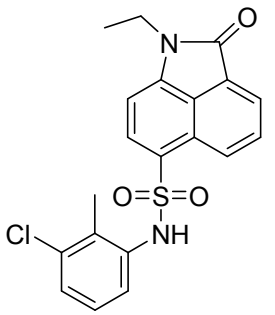
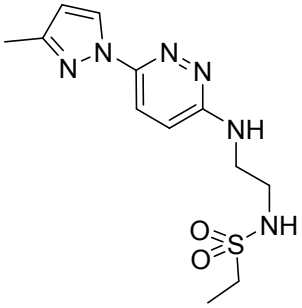
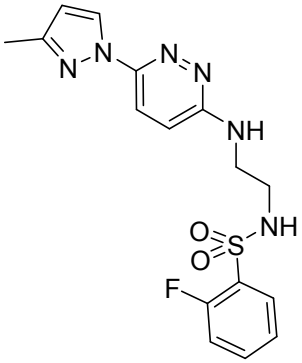
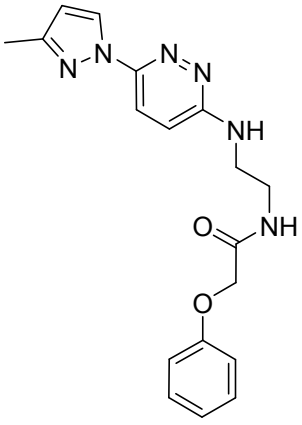
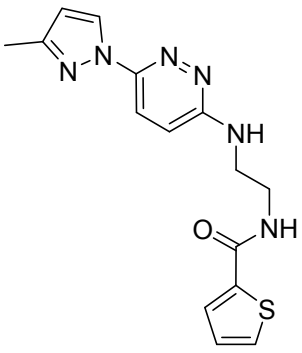
7		85.93
8		68.82
9		75.21
10		74.78
11		98.74
12		65.84
13		63.99

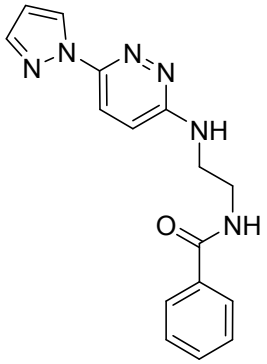
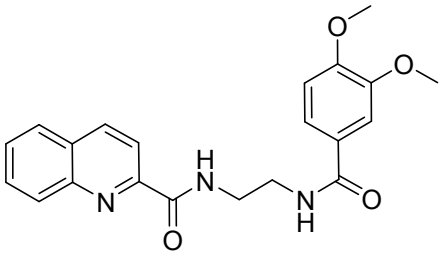
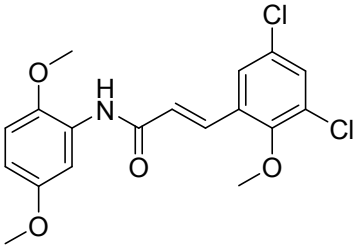
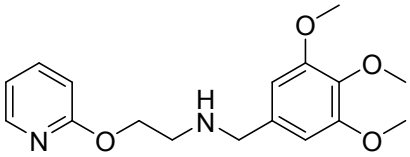
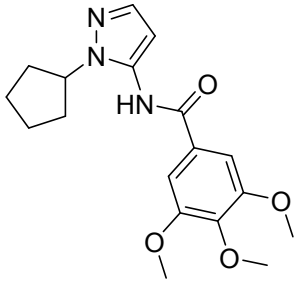
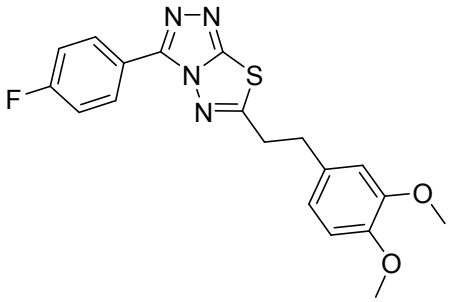
14		63.43
15		61.77
16		82.91
17		101.55
18		101.2
19		94.93
20		78.54

21		60.23
22		66.81
2		61.56
24		84.96
25		91.09
26		60.38

27		65.88
28		86.99
29		68.47
30		68.53
31		74.76
32		95.68

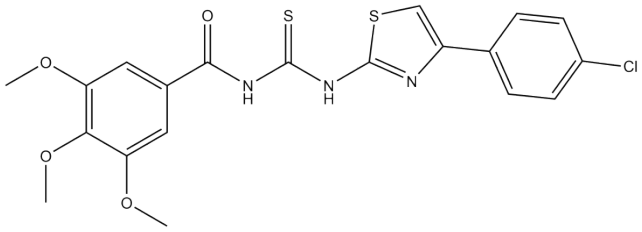
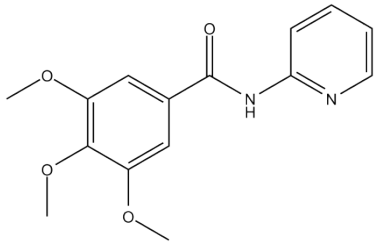
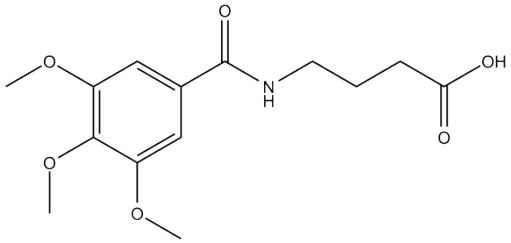
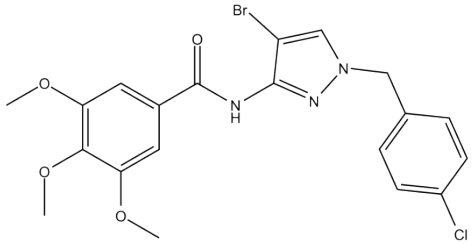
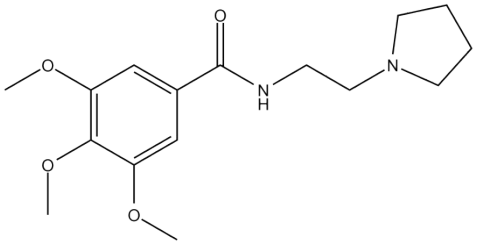
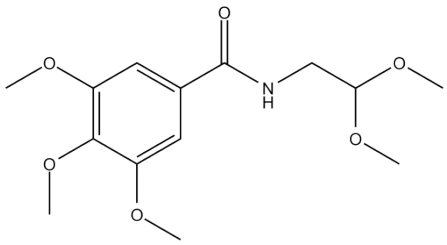
33		100.31
34		191.37
35		70.31
36		64.01
37		60.40
38		91.66
39		78.20

40		65.79
41		84.11
42		78.86
43		75.16
44		62.56

45		92.69
46		64.75
47		64.56
48		84.25
49		80.45
50		65.39

[a]The value of inhibition was calculated from the Alphascreen assay.

Table S2. The structures of DC-BD-03 series of compounds, and their inhibitory activities against the first bromodomain of BRD4, from binding with acetylated lysines. (Continued after Table 1).

Compound ID	Compound Structure	IC_{50} (μM)	Inhibition (50 μM)
DC-BD-10		>50	
DC-BD-13			80%
DC-BD-14		>50	
DC-BD-20			70%
DC-BD-23			80%
DC-BD-28			80%

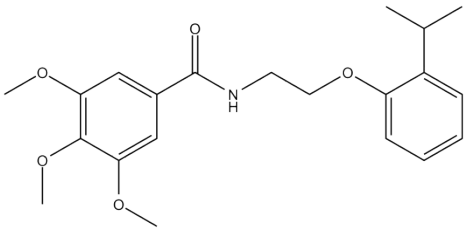
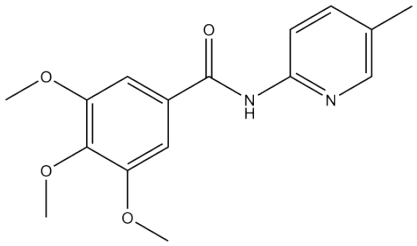
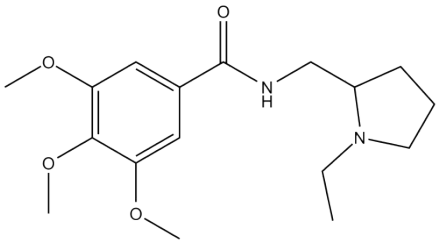
DC-BD-33		>50
DC-BD-34		>50
DC-BD-35		>50

Table S3. The IC50 of compound DC-BD-03 on other BET bromodomains.

Cpd	**IC_{50} [c](uM) on**			
	BRD2(1,2)	**BRD3(1,2)**	**BRD4(2)**	**BRDT(1)**
DC-BD-03	43	19	> 50	> 50

c The value of IC_{50} was calculated from the Alphascreen assay.

Table S4. The inhibition rates of compound DC-BD-03 for different BRD proteins.

Target	Inh% at 100μM	Inh% at 50μM
BRD7	8%	N.I.[b]
BRD9	3%	N.I.
FLAZ	N.I.	N.I.
SMACAR	N.I.	N.I.

[b]N.I. represents no inhibition.

Table S5. X-Ray diffraction data collection and refinement statistics.

Data Set Title	DC-BD-29
Wavelength	0.978
Resolution range	39.73 - 1.591 (1.648 - 1.591)
Space group	P 21 21 21
Unit cell	32.322 47.291 79.466 90 90 90
Total reflections	218990 (20931)
Unique reflections	16972 (1636)
Multiplicity	12.9 (12.8)
Completeness (%)	100 (100)
Mean I/sigma(I)	26.53 (11.28)
Wilson B-factor	11.75
R-merge	0.081 (0.244)
R-meas	0.085 (0.254)
CC1/2	0.999 (0.99)
CC*	1 (0.998)
Reflections used in refinement	16971 (1636)
Reflections used for R-free	873 (84)
R-work	0.163 (0.158)
R-free	0.190 (0.173)
CC(work)	0.964 (0.953)
CC(free)	0.968 (0.934)
Number of non-hydrogen atoms	1216
macromolecules	1037
ligands	22
Protein residues	125
RMS(bonds)	0.007
RMS(angles)	0.91
Ramachandran favored (%)	98
Ramachandran allowed (%)	1.6
Ramachandran outliers (%)	0
Rotamer outliers (%)	0.85
Clashscore	1.91
Average B-factor	15.09
macromolecules	13.34
ligands	23.10
solvent	25.49