The Journal of Experimental Medicine

SUPPLEMENTAL MATERIAL

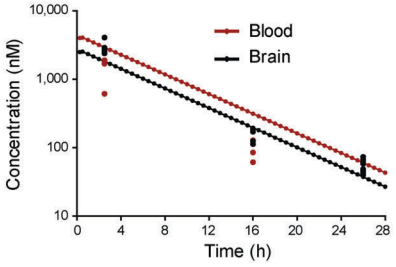
Sullivan et al., http://www.jem.org/cgi/content/full/jem.20151271/DC1

A Table1: Functional Comparison of I-BET Compounds

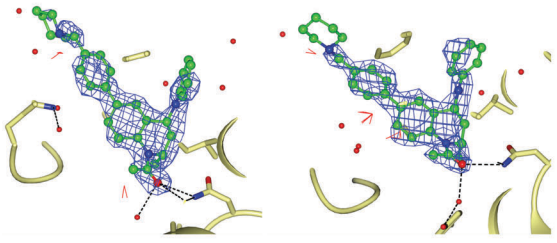
Assay	Target/Cell	GSK-1024858 (IC_{50} nM)	GSK-525762 (IC_{50} nM)	GSK-1210151 (IC_{50} nM)
Fluorescence Resonance Energy Transfer (FRET)	BRD4/BD1	27	61	30
	BRD4/BD2	6	31	339
	BRD3/BD1	56	71	30
	BRD3/BD2	21	31	83
	BRD2/BD1	100	117	55
	BRD2/BD2	17	50	209
	BRDT/BD1	87	57	12
	BRDT/BD2	23	135	1549
Surface Plasmon Resonance Affinity (BIACORE)	BRD4	23 (Kd)	230 (Kd)	158 (Kd)
Inhibition of IL-6 Production from Lipopolysaccharide (LPS) Stimulated Cells	Human PBMC	3	316	200
	Human Blood	200	398	794
	Rat Blood	50	316	200

B Table 2: Pharmacokinetic parameters of I-BET858 compounds

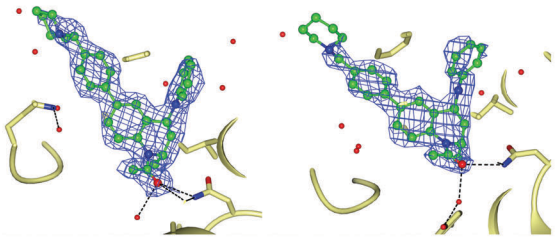
PK Parameter	Mouse IP n =3	Rat IV n=3	Rat PO n=3
Dose (mg/kg)	30 (3)	1 (1h infusion)	5
CLb (ml/min)kg)	-	26	-
Vss (L/kg)	-	13.4	-
Cmax (ng/ml)	2001 (166)	116	133
Tmax (h)	0.5-4.0	-	2.0
$T_{1/2}$	-,(4.2)	6.8	9.8
%Fpo	-	-	47
Brain:Blood	0.63 (4h)	0.85 (steady state)	-
F_{ub} (%)	2.6	3.6	-



C



OMIT (Fo-Fc)map of GSK1024858A
in the active site contoured at ±3sigma (blue/red)



OMIT (2Fo-Fc)map of GSK1024858A
in the active site contoured at +1sigma (blue)

D Table 3: Crystallography data collection & refinement statistics

(collection on a single crystal)	BRD4-BD1 ligand complex
Data collection	
Space group	$P2_12_12_1$
Cell dimensions *a*, *b*, *c* (Å) α, β, γ (°)	 42.149, 59.497, 108.025 90.000, 90.000, 90.000
Resolution (Å)	36.01-2.01 (2.13-2.01)
R_{merge}	0.082 (0.352)
$I/\sigma I$	8.1 (2.7)
Completeness (%)	97.0 (98.2)
Redundancy	2.7 (2.8)
Refinement Resolution (Å)	36.01-2.01
No. reflections	49497 (8136)
No. uniq reflections	18016 (2893)
R_{work}/R_{free}	0.214/0.271
No. atoms Protein Ligand/ion Water	2518 2131 68/12 307
B-factors Protein Ligand/ion Water	 29.16 25.99/43.47 39.93
R.m.s deviations Bond lengths (Å) Bond angles (º)	 0.007 1.174

*Highest resolution shell is shown in parenthesis

Figure S1. Identification and characterization of a novel brain-permeable inhibitor of BET proteins (I-BET858). (A) Acetyl lysine ligand displacement, direct Biacore binding and functional BET inhibitory activity in human blood by I-BET858 and related I-BET151 and I-BET762 compounds was measured using standard protocols (Nicodeme et al., 2010; Dawson et al., 2011). (B) Pharmacokinetic parameters of in vivo I-BET858 treatment were determined for mouse and rat brain tissue and blood as referenced in Methods section. Graph shows mouse brain (black) and blood (red) I-BET858 concentrations in nM at 2, 16, and 26 h after a single i.p. injection of 30 mg/kg (n = 4–10 mice/time point). (C) OMIT maps of different electron density at the acetylated-lysine site are shown. (D) X-ray crystallography of the BRD4–BD1 complex with I-BET858 was performed as described previously (Nicodeme et al., 2010).

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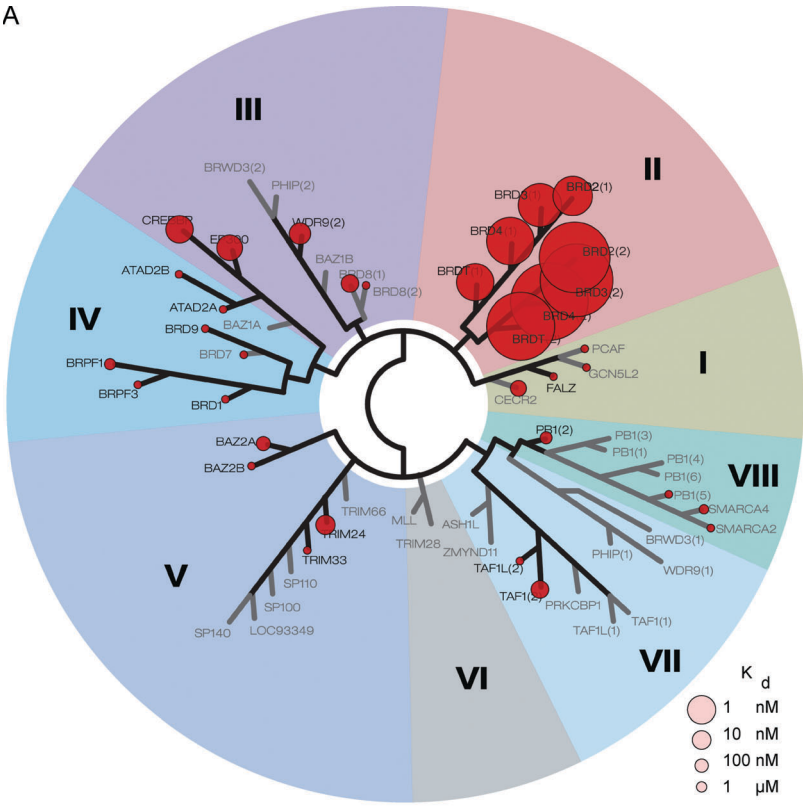

B Table 4: Bromodomain Selectivity of I-BET858

Target Gene Symbol	I-BET858 Kd nM	Target Gene Symbol	I-BET858 Kd nM
BRD2(1)	23	BRPF1	20000
BRD2(2)	0.47	BRPF3	>30000
BRD3(1)	10	CECR2	9500
BRD3(2)	0.39	CREBBP	270
BRD4(1)	6.4	EP300	410
BRD4(2)	0.25	FALZ	>30000
BRDT(1)	35	GCN5L2	>30000
BRDT(2)	0.59	PBRM1(2)	17000
ATAD2A	>30000	PBRM1(5)	>30000
ATAD2B	>30000	PCAF	>30000
BAZ2A	12000	SMARCA2	>30000
BAZ2B	>30000	SMARCA4	24000
BRD1	>30000	TAF1(2)	5000
BRD7	>30000	TAF1L(2)	>30000
BRD8(1)	4900	TRIM24 (PHD Bromo.)	2700
BRD8(2)	>30000	TRIM33 (PHD Bromo.)	>30000
BRD9	>30000	WDR9(2)	1200

Figure S2. Affinity analysis of I-BET858 for bromodomain-containing proteins. (A) I-BET858 binding affinities across 34 bromodomain-containing proteins, including the BET subfamily with Kd > 1 μM are indicated by pink circles. (B) I-BET858 affinities across 34 bromodomain-containing proteins are shown using BROMO*scan* Bromodomain Profiling (Theodoulou et al., 2015). I-BET858 shows highest affinity for the BET subfamily with >1,000 fold selectivity.

Table S1 (available as an Excel file) contains the effects of I-BET858 treatment on gene expression in primary neurons in vitro (2 and 12 h). Table S2 (available as an Excel file) lists the effects of I-BET858 on BDNF-inducible gene expression in primary neurons in vitro (2 and 12 h). Table S3 (available as an Excel file) displays the effects of acute and chronic I-BET858 on striatal gene expression in vivo. Table S4 (available as an Excel file) shows the gene lengths analysis of I-BET858 suppressed genes in vitro and in vivo.

SUPPLEMENTAL REFERENCES

Dawson, M.A., R.K. Prinjha, A. Dittmann, G. Giotopoulos, M. Bantscheff, W.I. Chan, S.C. Robson, C.W. Chung, C. Hopf, M.M. Savitski, et al. 2011. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature*. 478:529–533. http://dx.doi.org/10.1038/nature10509

Nicodeme, E., K.L. Jeffrey, U. Schaefer, S. Beinke, S. Dewell, C.W. Chung, R. Chandwani, I. Marazzi, P. Wilson, H. Coste, et al. 2010. Suppression of inflammation by a synthetic histone mimic. *Nature*. 468:1119–1123. http://dx.doi.org/10.1038/nature09589

Theodoulou, N.H., P. Bamborough, A.J. Bannister, I. Becher, R.A. Bit, K.H. Che, C.W. Chung, A. Dittmann, G. Drewes, D.H. Drewry, et al. 2015. Discovery of I-BRD9, a selective cell active chemical probe for bromodomain containing protein 9 inhibition. *J. Med. Chem*. http://dx.doi.org/10.1021/acs.jmedchem.5b00256.