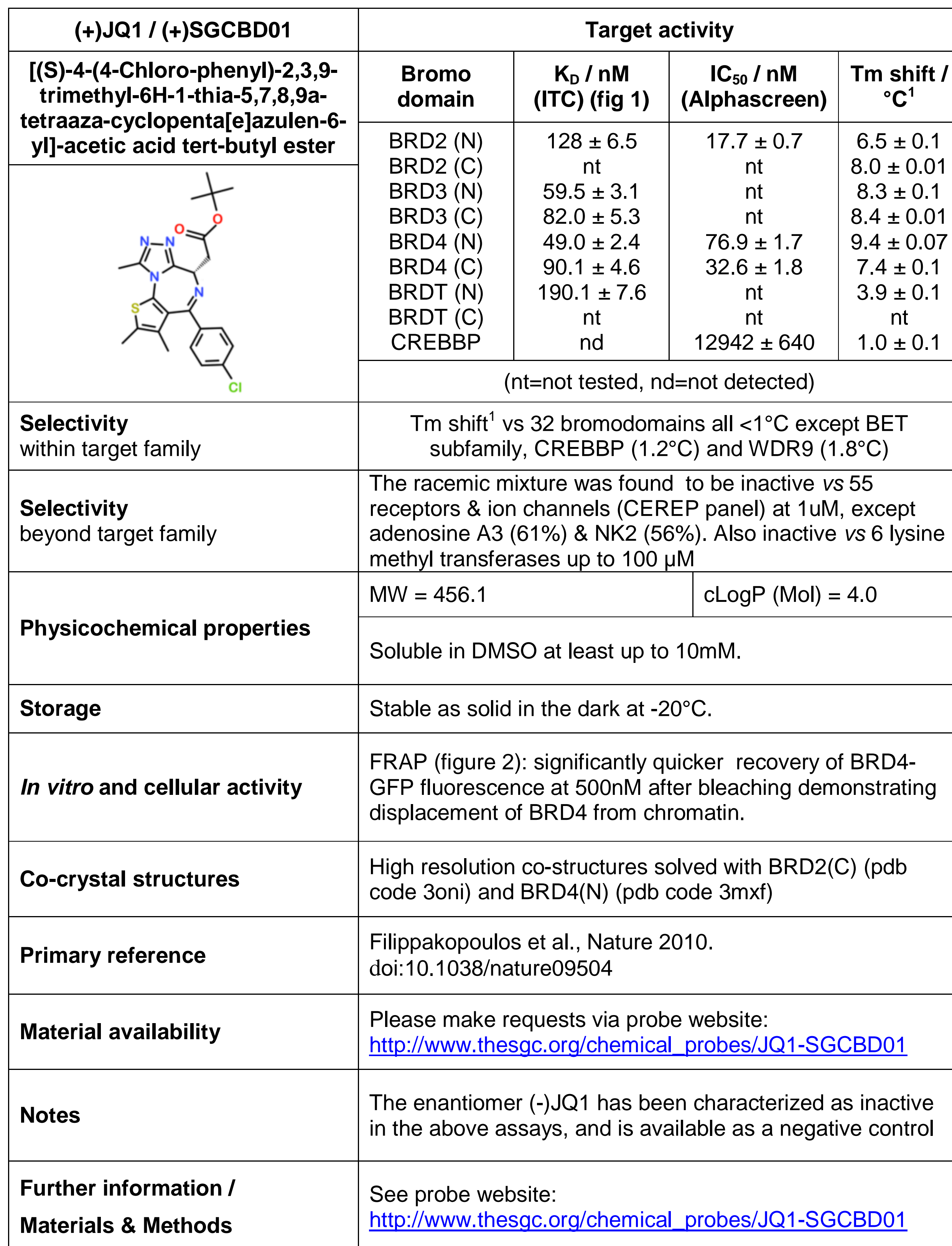
JQ1 / SGCBD01: A BET-family Selective Bromodomain Chemical Probe

(+)JQ1 / (+)SGCBD01	Target activity			
[(S)-4-(4-Chloro-phenyl)-2,3,9-trimethyl-6H-1-thia-5,7,8,9a-tetraaza-cyclopenta[e]azulen-6-yl]-acetic acid tert-butyl ester	**Bromo domain**	**K_D / nM (ITC) (fig 1)**	**IC_{50} / nM (Alphascreen)**	**Tm shift / °C[1]**
	BRD2 (N)	128 ± 6.5	17.7 ± 0.7	6.5 ± 0.1
	BRD2 (C)	nt	nt	8.0 ± 0.01
	BRD3 (N)	59.5 ± 3.1	nt	8.3 ± 0.1
	BRD3 (C)	82.0 ± 5.3	nt	8.4 ± 0.01
	BRD4 (N)	49.0 ± 2.4	76.9 ± 1.7	9.4 ± 0.07
	BRD4 (C)	90.1 ± 4.6	32.6 ± 1.8	7.4 ± 0.1
	BRDT (N)	190.1 ± 7.6	nt	3.9 ± 0.1
	BRDT (C)	nt	nt	nt
	CREBBP	nd	12942 ± 640	1.0 ± 0.1
	(nt=not tested, nd=not detected)			

Selectivity within target family	Tm shift[1] vs 32 bromodomains all <1°C except BET subfamily, CREBBP (1.2°C) and WDR9 (1.8°C)
Selectivity beyond target family	The racemic mixture was found to be inactive *vs* 55 receptors & ion channels (CEREP panel) at 1uM, except adenosine A3 (61%) & NK2 (56%). Also inactive *vs* 6 lysine methyl transferases up to 100 μM
Physicochemical properties	MW = 456.1; cLogP (Mol) = 4.0 Soluble in DMSO at least up to 10mM.
Storage	Stable as solid in the dark at -20°C.
***In vitro* and cellular activity**	FRAP (figure 2): significantly quicker recovery of BRD4-GFP fluorescence at 500nM after bleaching demonstrating displacement of BRD4 from chromatin.
Co-crystal structures	High resolution co-structures solved with BRD2(C) (pdb code 3oni) and BRD4(N) (pdb code 3mxf)
Primary reference	Filippakopoulos et al., Nature 2010. doi:10.1038/nature09504
Material availability	Please make requests via probe website: http://www.thesgc.org/chemical_probes/JQ1-SGCBD01
Notes	The enantiomer (-)JQ1 has been characterized as inactive in the above assays, and is available as a negative control
Further information / Materials & Methods	See probe website: http://www.thesgc.org/chemical_probes/JQ1-SGCBD01

[1] **Tm shift protocol described in** Niesen, F. H., Berglund, H. & Vedadi, M. The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability. *Nat Protoc* **2**, 2212-2221, (2007).

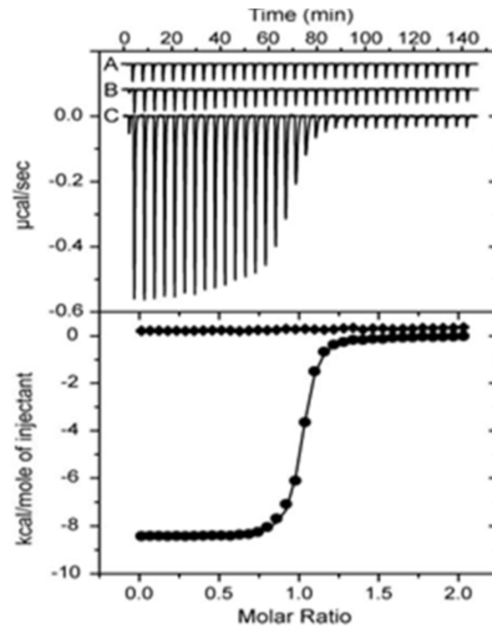


Figure 1: Isothermal titration calorimetry (ITC). The upper panel shows raw injection heats for blank titration of BRD4 into buffer (A), into inactive (-)JQ1/(-)SGCBD01 (B) and active (+)JQ1/(+)SGCBD01 (C). Normalized binding isotherms are shown in the lower panel for (-)JQ1/(-)SGCBD01 (squares) and (+)JQ1 /(+)SGCBD01 (spheres).

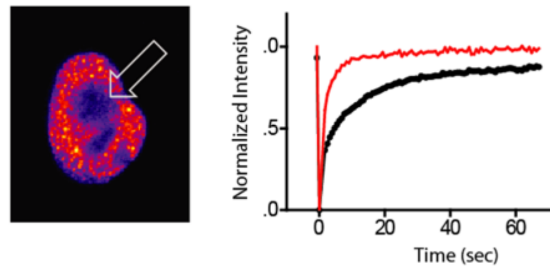


Figure 2: Fluorescence recovery after photobleaching (FRAP): The panel on the left shows a GFP-BRD4 fluorescent nucleus. The arrow indicates the zone of bleaching. GFP-BRD4 showed significantly quicker recovery in the bleached zone when treated with 500nM (+)JQ1/(+)SGCBD01.