

## Supporting Information

Discovery of QCA570 as an Exceptionally Potent and Efficacious Proteolysis Targeting Chimera (PROTAC) Degradator of the Bromodomain and Extra-Terminal (BET) Proteins Capable of Inducing Complete and Durable Tumor Regression

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## **Contents of Supporting Information:**

**Figure S1.** Western blotting analysis of BRD2, BRD3, and BRD4 proteins in RS4;11 cells treated with BET inhibitor **22**, compound **26** and BET degraders **27, 28, 29, 30,** and **31**.

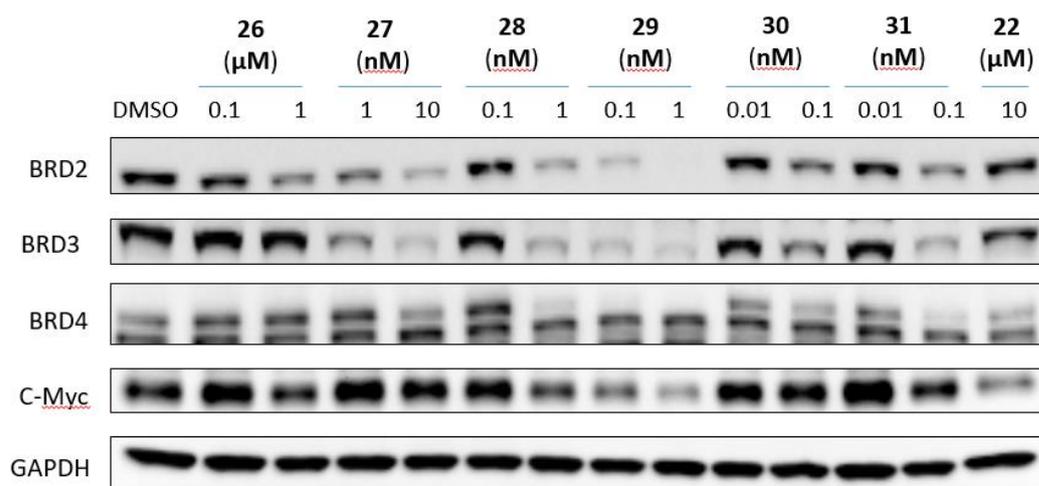
**Figure S2.** Western blotting analysis of GSPT1 proteins in RS4;11 cells treated with BET degrader **35**.

**Figure S3.**  $^1\text{H}$  NMR spectrum of compound **35**.

**Figure S4.**  $^{13}\text{C}$  NMR spectrum of compound **35**.

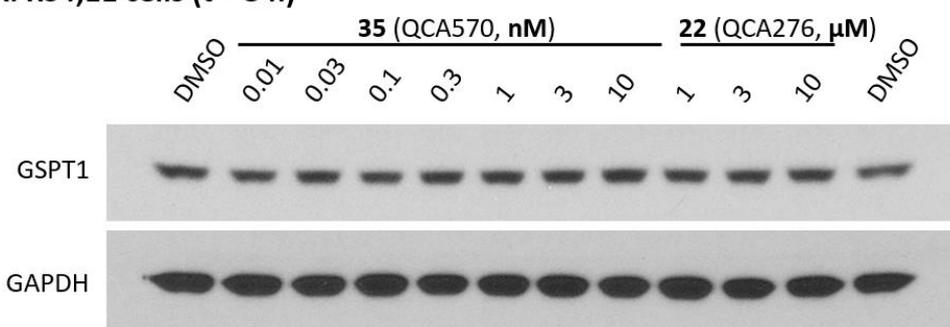
**Figure S5.** UPLC-MS chromatogram of compound **35**.

**Figure S1.** Related to Figure 3. Western blotting analysis of BRD2, BRD3, and BRD4 proteins in RS4;11 cells treated with BET inhibitor **22**, compound **26** and BET degraders **27**, **28**, **29**, **30**, and **31**. RS4;11 cells were treated for 3 h with each individual compound at indicated concentrations. BET-BRD protein degradation was determined by western blot probed with specific antibodies. Decreased levels of c-Myc were used to determine the biological activity of each compound. GAPDH was used as loading control.



**Figure S2.** Western blotting analysis of GSPT1 proteins in RS4;11 cells treated with BET degrader **35**. RS4;11 and MV-4-11 cells were treated with BET degrader **35** (QCA570) and the corresponding BET inhibitor **22** (QCA276) at the indicated concentrations for 3 hours. Then cell lysates were analyzed by western blotting for protein level of GSPT1, probed with a specific GSPT1 polyclonal antibody (ab126090, Abcam). GAPDH was used as loading control.

**A. RS4;11 cells (t = 3 h)**



**B. MV-4-11 cells (t = 3 h)**

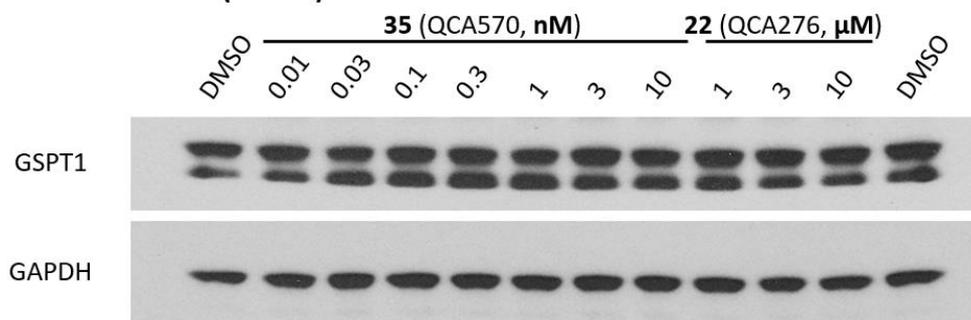
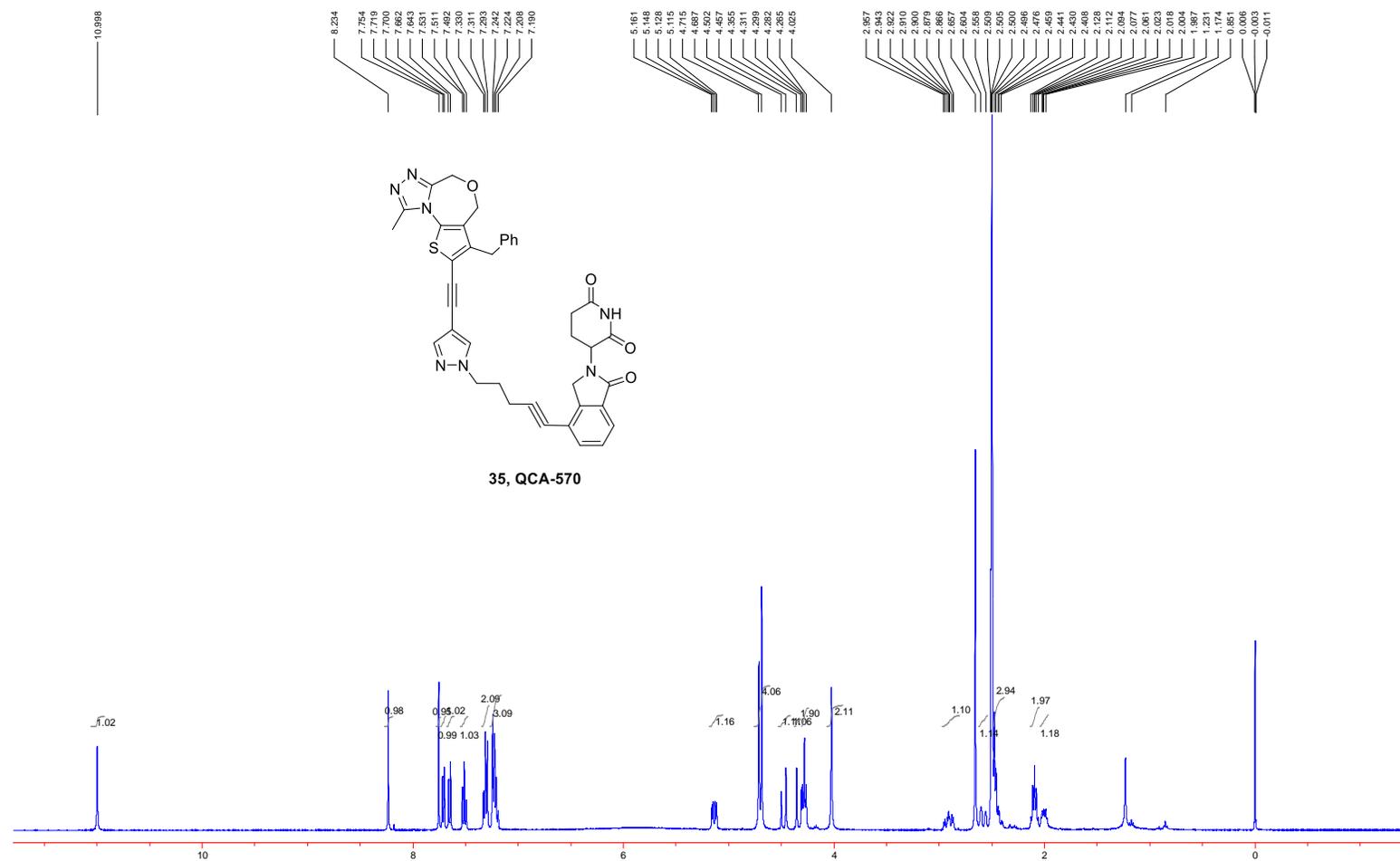
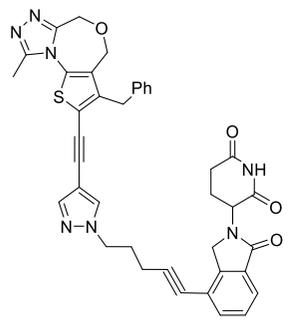
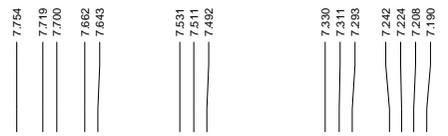
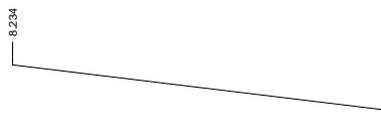


Figure S3.  $^1\text{H}$  NMR of 35.





35, QCA-570

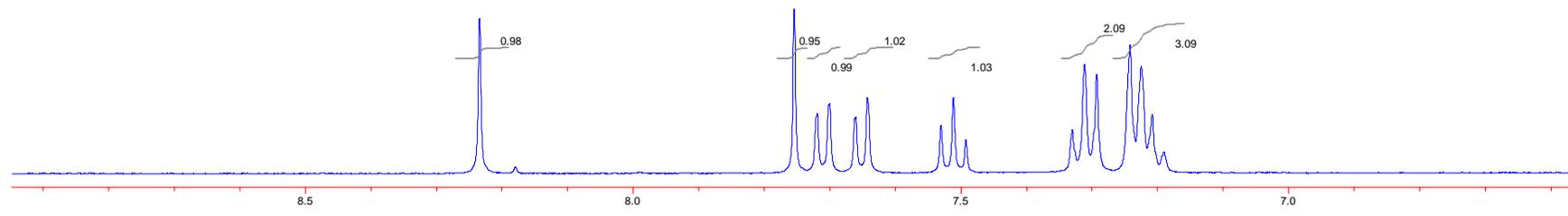
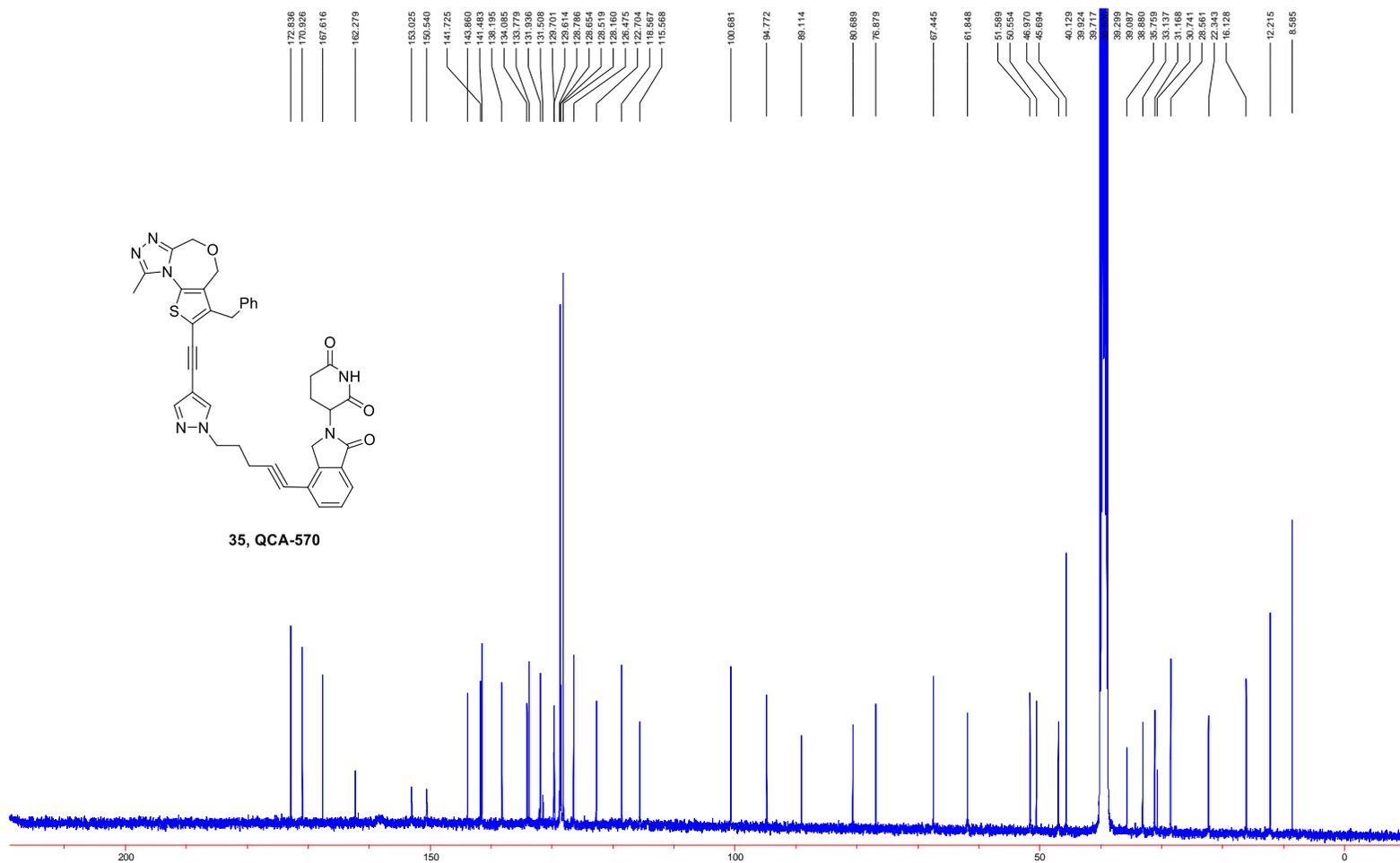
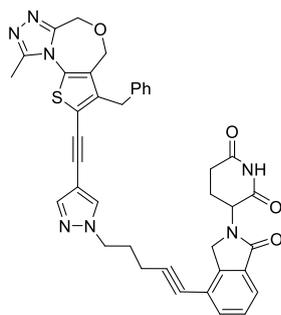
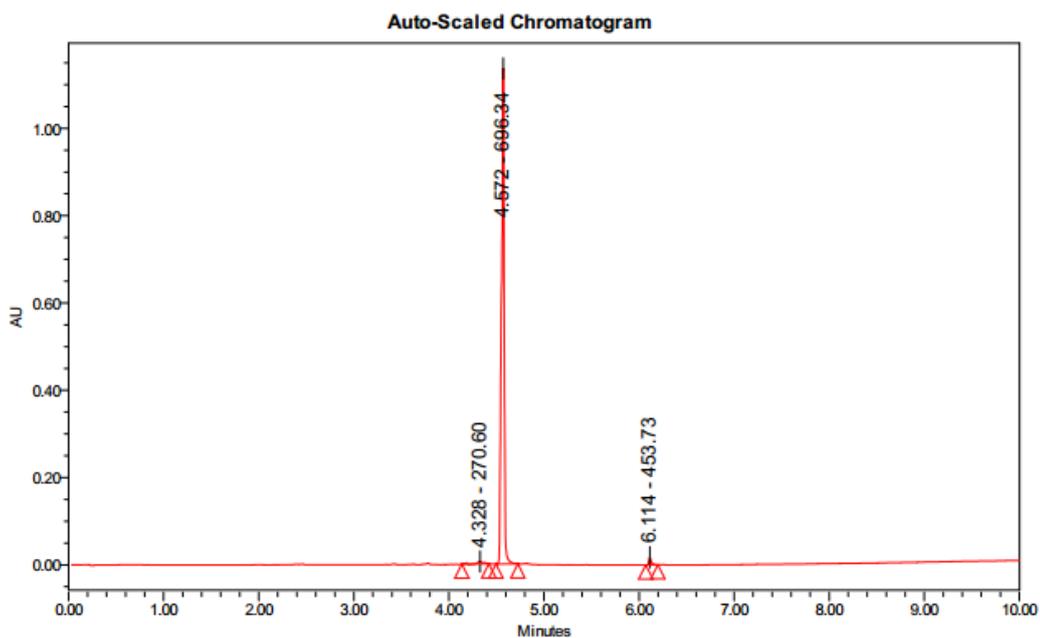


Figure S4.  $^{13}\text{C}$  NMR spectrum of compound **35**.



**Figure S5.** UPLC-MS results for compound **35**.

SAMPLE INFORMATION			
Sample Name:	QCD-200-1	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	3/20/2018 2:38:51 PM EDT
Vial:	1:D,6	Acq. Method Set:	10to100% Bin 10
Injection #:	1	Date Processed:	3/20/2018 5:19:15 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Caffeine PDA
Run Time:	10.0 Minutes	Channel Name:	254.0nm@2
Sample Set Name:	Shilin	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm



**35, QCA-570**

**Peak Results**

	RT	Area	Height	% Area
1	4.328	30497	5606	1.33
2	4.572	2226514	1135770	97.26
3	6.114	32245	17953	1.41

**Peak Results**

	Base Peak (m/z)
1	270.60
2	696.34
3	453.73