A Unique Approach to Design Potent and Selective Cyclic Adenosine Monophosphate Response Element Binding Protein, Binding Protein (CBP) Inhibitors

Sarah M. Bronner,[†] Jeremy Murray,[†] F. Anthony Romero,[†] Kwong Wah Lai,[‡] Vickie Tsui,[†] Patrick Cyr,[†] Maureen H. Beresini,[†] Gladys de leon Boenig,[†] Zhongguo Chen,[‡] Edna F. Choo,[†] Kevin R. Clark,[†] Terry D. Crawford,[†] Hariharan Jayaram,[§] Susan Kaufman,[†] Ruina Li,[†] Yingjie Li,[‡] Jiangpeng Liao,[‡] Xiaorong Liang,[†] Wenfeng Liu,[‡] Justin Ly,[†] Jonathan Maher,[†] John Wai,[‡] Fei Wang,[‡] Aijun Zheng,[‡] Xiaoyu Zhu,[‡] Steven Magnuson[†]*

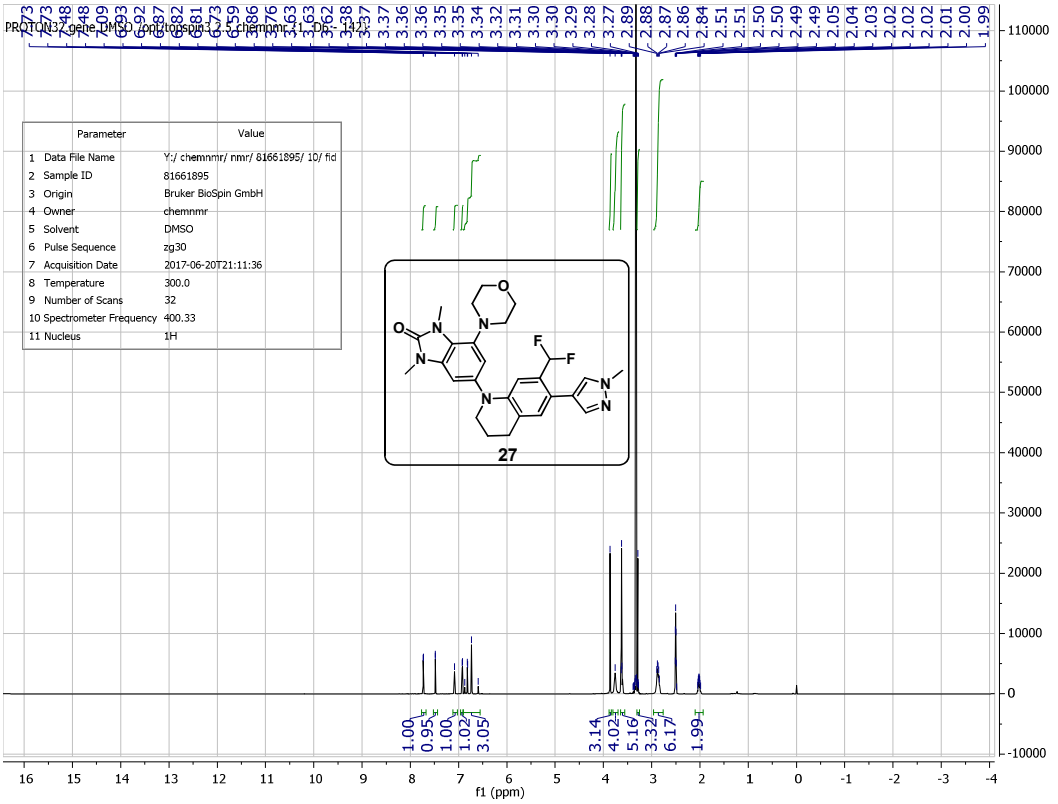
[†]Genentech, Inc. 1 DNA Way, South San Francisco, California 94080, United States

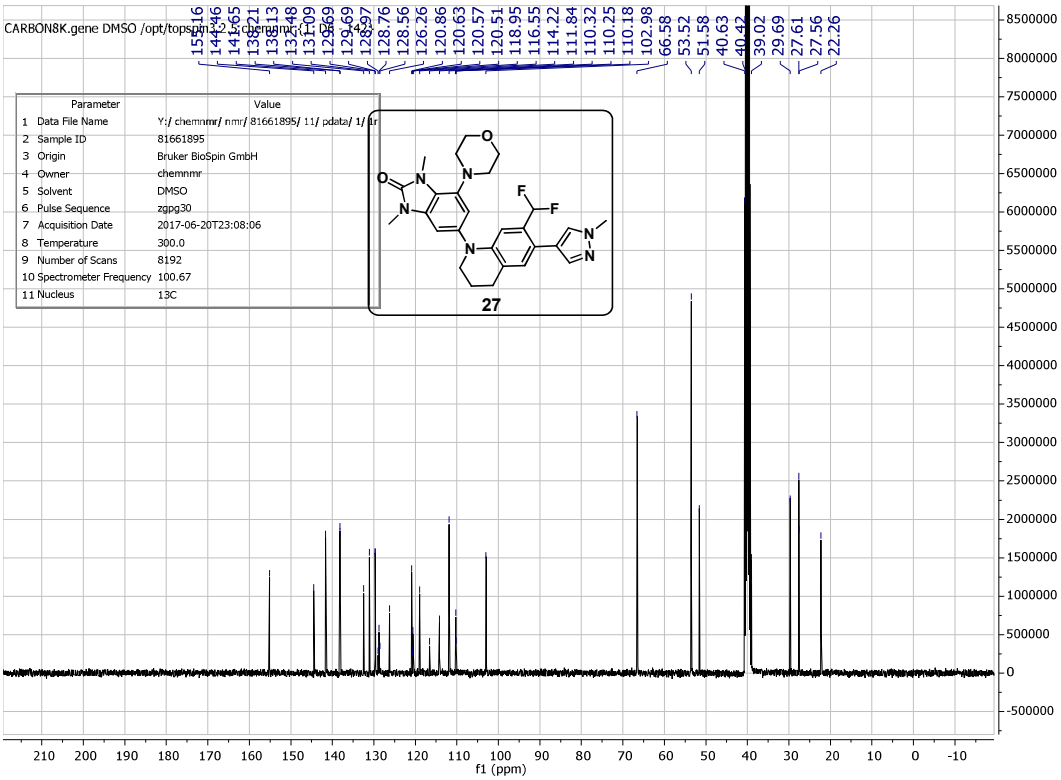
[‡]Wuxi Apptec Co., Ltd., 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, People's Republic of China

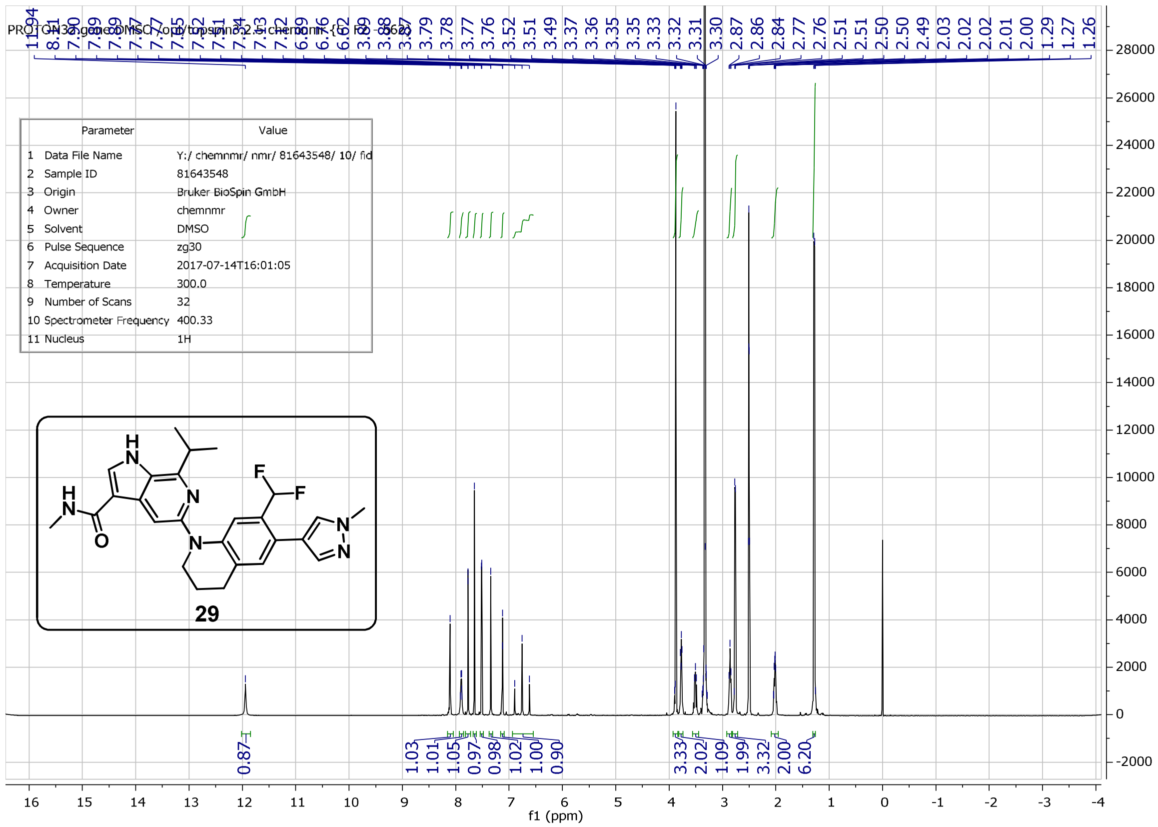
[§]Editas Medicine, Inc., 11 Hurley Street, Cambridge, Massachusetts 02141, United States

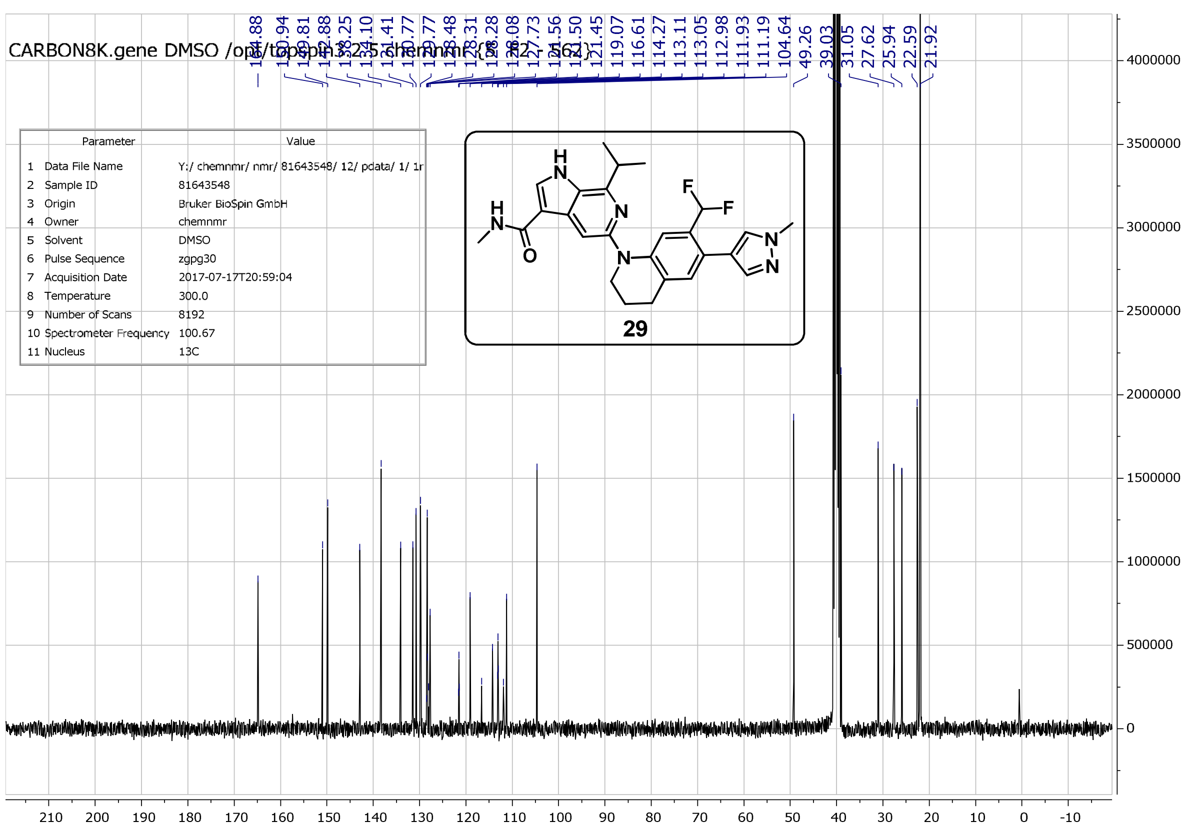
Supporting Information – Table of Contents

^{1}H NMR and ^{13}C NMR Spectra for 27 and 29






PROTON32.gene DMSO /opt/topspin3.2.5 chemnmr {C:\nmr} 0620}
Parameter | Value
1 Data File Name | Y:/ chemnmr/ nmr/ 81643548/ 10/ fid
2 Sample ID | 81643548
3 Origin | Bruker BioSpin GmbH
4 Owner | chemnmr
5 Solvent | DMSO
6 Pulse Sequence | zg30
7 Acquisition Date | 2017-07-14T16:01:05
8 Temperature | 300.0
9 Number of Scans | 32
10 Spectrometer Frequency | 400.33
11 Nucleus | 1H
29
f1 (ppm)


CARBON8K.gene DMSO /opt/topspin3.2.5 chemnmr {C:\nmr} 0620}
Parameter | Value
1 Data File Name | Y:/ chemnmr/ nmr/ 81643548/ 12/ pdata/ 1/ 1r
2 Sample ID | 81643548
3 Origin | Bruker BioSpin GmbH
4 Owner | chemnmr
5 Solvent | DMSO
6 Pulse Sequence | zgpg30
7 Acquisition Date | 2017-07-17T20:59:04
8 Temperature | 300.0
9 Number of Scans | 8192
10 Spectrometer Frequency | 100.67
11 Nucleus | 13C
29
f1 (ppm)

Bromodomain selectivity data for 27 and 29 using BROMOscan® panel from DiscoverX

Target	27	29
Gene Symbol	Kd (nM)	Kd (nM)
ATAD2A	>10000	>10000
ATAD2B	>10000	>10000
BAZ2A	>10000	>10000
BAZ2B	>10000	>10000
BRD1	>10000	>10000
BRD2(1)	>10000	>10000
BRD2(1,2)	>10000	>10000
BRD2(2)	>10000	>10000
BRD3(1)	>10000	>10000
BRD3(1,2)	>10000	>10000
BRD3(2)	8700	>10000
BRD4(1)	>10000	>10000
BRD4(1,2)	>10000	>10000
BRD4(2)	>10000	>10000
BRD4(full-length,short-	>10000	>10000
BRD7	5100	>10000
BRD8(1)	>10000	>10000
BRD8(2)	>10000	>10000
BRD9	3600	>10000
BRDT(1)	>10000	>10000
BRDT(1,2)	>10000	>10000
BRDT(2)	>10000	>10000
BRPF1	>10000	>10000
BRPF3	>10000	>10000
CECR2	>10000	>10000
CREBBP	17	31
EP300	17	23
FALZ	>10000	>10000
GCN5L2	>10000	>10000
PBRM1(2)	>10000	>10000
PBRM1(5)	>10000	>10000
PCAF	>10000	>10000
SMARCA2	>10000	>10000
SMARCA4	>10000	>10000
TAF1(2)	>10000	>10000
TAF1L(2)	>10000	>10000
TRIM24(Bromo.)	7600	>10000
TRIM24(PHD,Bromo.)	>10000	>10000
TRIM33(PHD,Bromo.)	>10000	>10000
WDR9(2)	>10000	>10000

Computational Docking Protocol. The co-crystal structure of GNE-781 (**3**) bound to the CBP bromodomain (PDB ID: 5W0E) served as a starting point for docking. The Schrodinger suite of programs available in Maestro version 9.5 (Schrodinger, Inc.) was used to remove the ligand (GNE-781) and water molecules except for the five conserved water molecules numbered 0–4.[1] The protein was prepared using default parameters in the PrepWiz module, and compound **7** was prepared using LigPrep with default parameters. Docking was carried out with Glide SP (Standard Precision), retaining default parameters for all steps except for the following modifications. Fifty poses were included for performing post-docking minimization, with strain correction terms turned on. The top ten poses were written out for visual analysis. Pose #1, with a Glide score of –7.24, presented the N-methyl of the thiazolone core towards the conserved water region, and was used for the design of **17**. Pose #2, with a Glide score of –6.88, had a similar binding mode to pose #1 but was shifted such that the vector matching the attachment of THQ was less optimal. Pose #3, with a Glide score of –6.88, presented the sulfur atom of the thiazolone core towards the conserved water region. The other eight poses that were visually inspected were de-prioritized based on the lack of vectors that made sense to attach to the THQ or the lack of interactions with the conserved Asn1168 sidechain.

Crystallography Methods. The co-crystal structures of CBP with **6, 16 & 17** were obtained by incubating 3.3 mM of each compound with protein at a concentration of 19.9 mg/mL (1.2 mM). Crystals were then grown at 4 °C using the sitting drop vapor diffusion technique by equilibrating the protein:ligand complexes against a solutions containing 3.545 M Formate, 100 mM Tris-HCl pH 8.2, 10% glycerol (**6**) or 0.2 M $MgCl_2$, 0.1 M Bis-Tris pH 6.5, 22% PEG3350 (**16** and **17**). CBP-**6** crystals were flash frozen in liquid nitrogen using a 1:1 mix of Paratone-N

and Mineral oil and data was collected at APS beamline 22BM. The CBP-**16** and CBP-**17** complex crystals were cryo-protected by the addition of 20% ethylene glycol to the well solution and data was collected at SSRF beamline 17U1.

Data Collection and Refinement Statistics

	CBP-**6** PDB **6AXQ**	CBP-**16** PDB **6AY3**	CBP-**17** PDB **6AY5**
Wavelength	0.9793	0.9793	0.9793
Resolution range	50–1.50 (1.55–1.50)	25.07–1.39 (1.44–1.39)	23.67–1.44 (1.49–1.44)
Space group	P21221	P 1 21 1	P 21 21 21
Unit cell	68.73 69.98 110.26 90.0 90.0 90.0	33.9 80.5 48.5 90 109.1 90	34.4 49.2 81.0 90 90 90
Total reflections	614,907	181476	178474
Unique reflections	614,907	49157 (4843)	25634 (2406)
Multiplicity	7.2 (7.2)	3.7 (3.6)	6.9 (6.9)
Completeness (%)	100 (100)	99.69 (98.98)	99.06 (95.10)
Mean I/sigma(I)	N/A	14.7 (2.1)	23 (2.3)
Wilson B-factor	13.3	12.15	19.78
R-merge	0.072 (0.715)	0.068 (0.57)	0.081 (0.835)
Refinement			
Reflections used in refinement	130895 (12964)	49150 (4842)	25634 (2406)
Reflections used for R-free	6589 (654)	2505 (243)	1311 (123)
R-work	0.1998 (0.3731)	0.2017 (0.3094)	0.1632 (0.2551)
R-free	0.2161 (0.3816)	0.2371 (0.3340)	0.1838 (0.3610)
Number of non-hydrogen atoms	4415	2456	1231
macromolecules	3828	2054	1025
ligands	76	73	50
solvent	511	329	156
Protein residues	450	230	115
RMS(bonds)	0.008	0.014	0.013
RMS(angles)	1.01	1.43	1.33
Ramachandran favored (%)	100	100	100

Ramachandran allowed (%)	0	0	0
Ramachandran outliers (%)	0	0	0
Rotamer outliers (%)	3.75	1.75	1.75
Clashscore	3.25	3.36	3.82
Average B-factor	17.8	18.26	25.14
macromolecules	17.18	16.87	23.67
ligands	18.17	14.71	26.06
solvent	22.35	27.74	34.53
Number of TLS groups	1	4	2

References

(1) Flynn, E. M.; Huang, O.W.; Poy, F.; Oppikofer, M.; Bellon, S.F.; Tang, Y.; Cochran A. G. *Structure* **2015**, *23*, 1801–1814.