Supporting Information

Discovery of Protein–Protein Interaction BCL6 Inhibitor by Biophysics-Driven Fragment-Based Approach

Yusuke Kamada*[,†], Nozomu Sakai*[,†], Satoshi Sogabe[†], Koh Ida[†], Hideyuki Oki[†], Kotaro Sakamoto[†,§], Weston Lane[‡], Gyorgy Snell[‡], Motoo Iida[†], Yoshihiro Imaeda[†], Junichi Sakamoto[†]and Junji Matsui[†]

[†]Takeda Pharmaceutical Company Limited, 26-1 Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

[‡]Takeda California Inc., 10410 Science Center Dr., San Diego, California 92121, United States

*E-mail: yusuke.kamada1@takeda.com. Tel:+81-0466-32-2782;

*E-mail: nozomu.sakai@takeda.com. Tel:+81-0466-32-1273

TABLE OF CONTENTS

(A) (B)

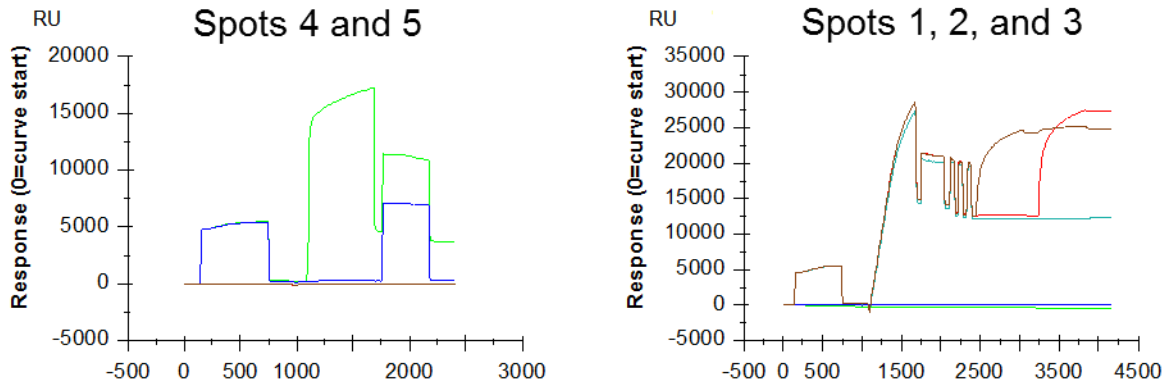

Figure S1. SPR sensorgrams for parallel immobilization of $BCL6^{BTB}$s. (A) wt $BCL6^{BTB}$ was immobilized on spot 5 (green) via the standard amine coupling procedure. (B) Avi-tagged wt $BCL6^{BTB}$ (brown) and avi-tagged mt $BCL6^{BTB}$ (red) were immobilized on spots 1 and 2, respectively via the steptavidin–biotin binding.

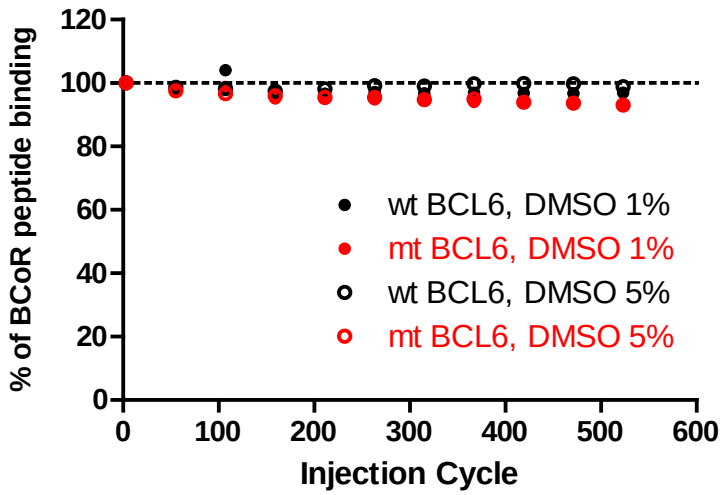

Figure S2. Evaluation of stability and DMSO effect. For every 52nd cycle, the BCoR peptide was injected at 50 μM over each captured $BCL6^{BTB}$.

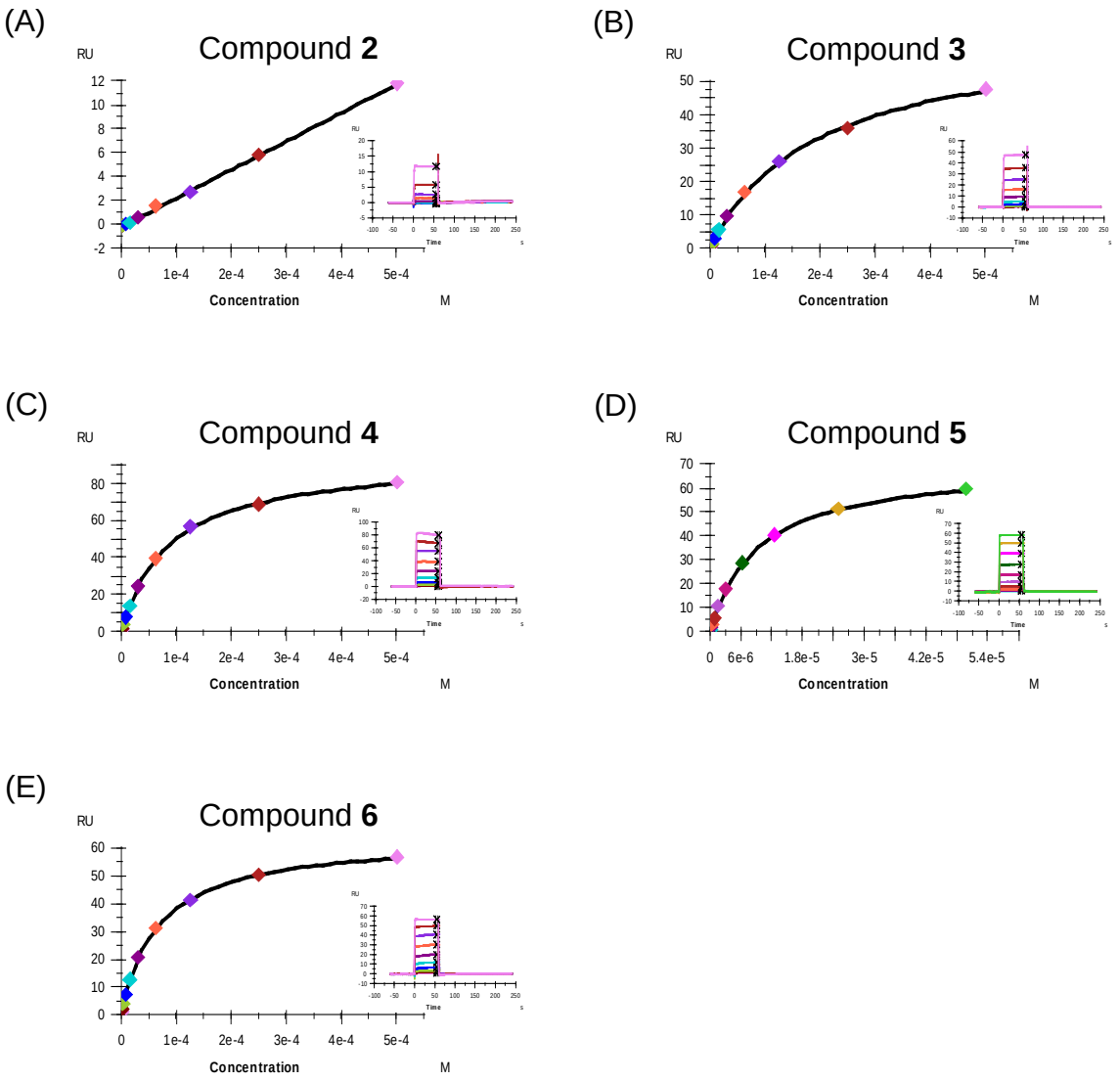

Figure S3. SPR sensorgrams (insert) and equilibrium plots for compounds **2**–**6**. Top concentration is 50 µM; dilution step is 2-fold.

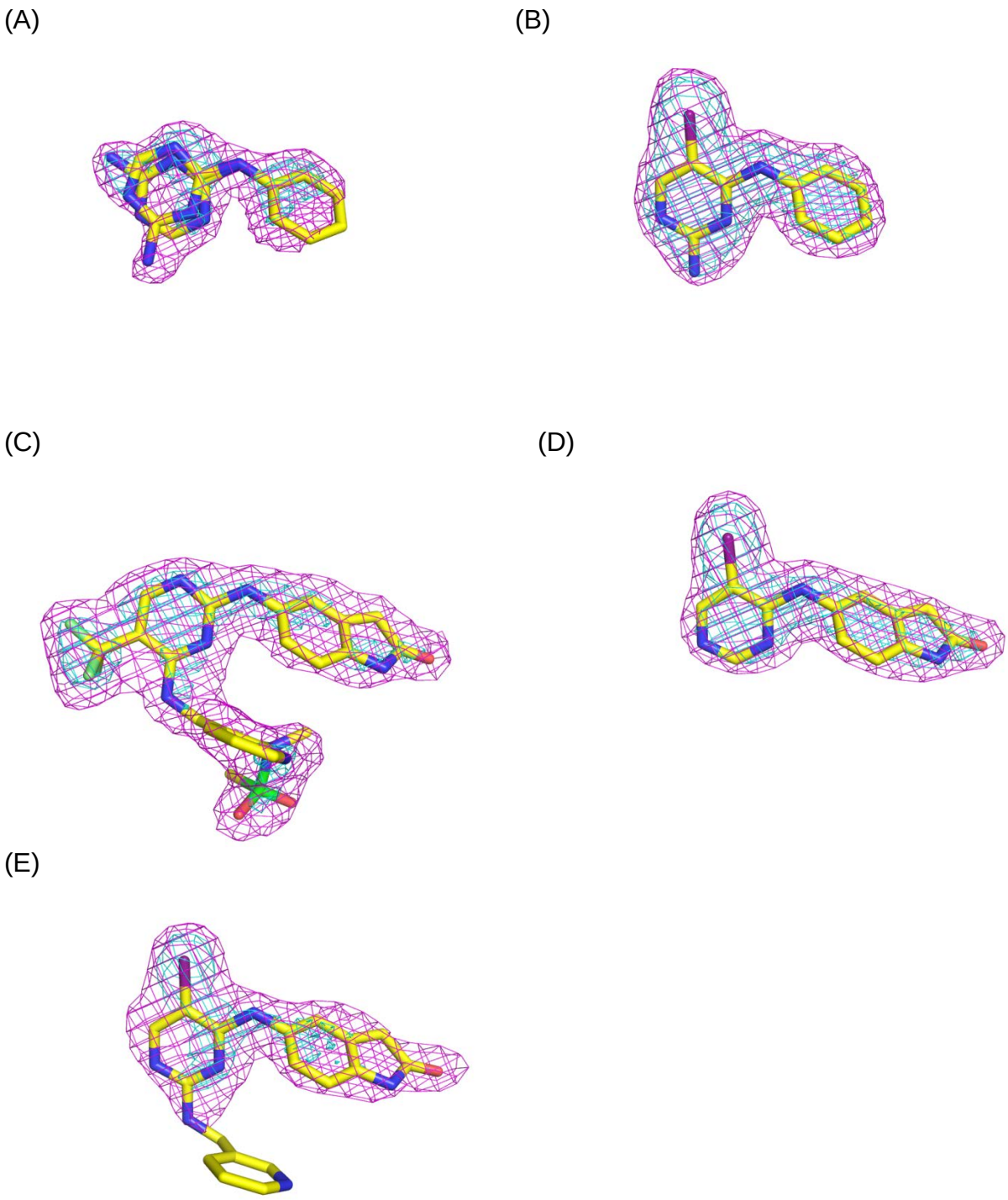

Figure S4. Fobs–Fcalc electron density omit maps contoured at 3σ (magenta) and 6σ (cyan) for the vicinity of the compounds. (A) compound **1**, (B) compound **4**, (C) compound **5**, (D) compound **6**, and (E) compound **7**.

Table S1. Data collection and refinement statistics

Crystal	$BCL6^{BTB}$/**1**	$BCL6^{BTB}$/**4**	$BCL6^{BTB}$/**5**	$BCL6^{BTB}$/**6**	$BCL6^{BTB}$/**7**
Data collection					
Space group	$P6_122$	$P6_122$	$P6_122$	$P6_122$	$P6_122$
Unit cell dimensions					
a, b, c (Å)	66.4, 66.4, 152.2	66.6, 66.6, 154.6	66.4, 66.4, 152.8	66.7, 66.7, 155.3	66.9, 66.9, 153.8
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	50–1.65 (1.68–1.65)	50–1.94 (1.97–1.94)	50–2.05 (2.09–2.05)	50–2.06 (2.10–2.06)	50–2.00 (2.03–2.00)
Observed reflections	307212	205900	87052	149914	160248
Unique reflections	24802	15813	13221	13032	14486
Redundancy	12.4 (5.4)	13.0 (8.7)	6.6 (5.3)	11.5 (8.7)	11.1 (9.4)
Completeness (%)	99.8 (96.6)	100.0 (100.0)	99.8 (100.0)	97.5 (86.0)	100.0 (100.0)
I/σ	36.2 (1.2)	37.2 (1.5)	23.9 (1.9)	28.5 (1.3)	29.0 (1.3)
R_{sym} [a]	0.057 (>1.000)	0.063 (>1.000)	0.059 (0.704)	0.069 (>1.000)	0.067 (>1.000)
Refinement					
Resolution (Å)	40–1.65 (1.69–1.65)	40–1.94 (1.99–1.94)	40–2.05 (2.10–2.05)	40–2.06 (2.12–2.06)	40–2.00 (2.05–2.00)
Reflections	23303	14958	12503	12329	13687
R_{work} [b]	0.179 (0.299)	0.188 (0.283)	0.195 (0.254)	0.193 (0.272)	0.198 (0.293)
R_{free} [b]	0.206 (0.300)	0.209 (0.303)	0.233 (0.272)	0.219 (0.374)	0.230 (0.286)
Number of atoms					
Protein	1064	1042	1042	1042	1042
Ligand/Ion	21	19	35	18	31
Water	98	40	31	25	31
Average B factor ($Å^2$) [c]	30.6	51.0	50.6	58.6	53.4
Rms deviation from ideal geometry					
Bond lengths (Å)	0.010	0.010	0.010	0.010	0.010
Bond angles (°)	1.520	1.419	1.620	1.514	1.534
Ramachandran plot (%) [d]					
Preferred regions	97.6	97.7	97.7	96.6	98.4
Allowed regions	2.4	2.3	2.3	3.9	1.6
Outliers	0.0	0.0	0.0	0.0	0.0
PDB code	**5X4M**	**5X4N**	**5X4O**	**5X4P**	**5X4Q**

[a] $R_{sym} = \Sigma_h\Sigma_i|I(h)_i - \langle I(h)\rangle|/\Sigma_h\Sigma_i\langle I(h)\rangle$, where $\langle I(h)\rangle$ is the mean intensity of symmetry-related reflections. [b] $R_{work} = \Sigma||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|$. R_{free} was calculated for randomly chosen 5% of reflections excluded from refinement. [c] B-factor includes contributions from TLS parameters. [d] Calculated with Coot. Values in parentheses are for the highest resolution shell.