Discovery of a B-Cell Lymphoma 6 Protein-Protein Interaction Inhibitor by a Biophysics-driven Fragment-based Approach

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Abstract

B-cell lymphoma 6 (BCL6) is a transcriptional factor that expresses in lymphocytes and regulates the differentiation and proliferation of lymphocytes. Therefore, BCL6 is a therapeutic target for autoimmune diseases and cancer treatment. This report presents the discovery of BCL6–corepressor interaction inhibitors by using a biophysics-driven fragment based approach. Using the surface plasmon resonance (SPR)-based fragment screening, we successfully identified fragment **1**(SPR K_D = 1200 μM, ligand efficiency (LE) = 0.28), a competitive binder to the natural ligand BCoR peptide. Moreover, we elaborated **1** into the more potent compound **7** (SPR K_D = 0.078 μM, LE = 0.37, cell-free protein–protein interaction (PPI) IC50 = 0.48 μM (ELISA), cellular PPI IC50 = 8.6 μM (M2H)) by a structure-based design and structural integration with a second high-throughput screening hit.

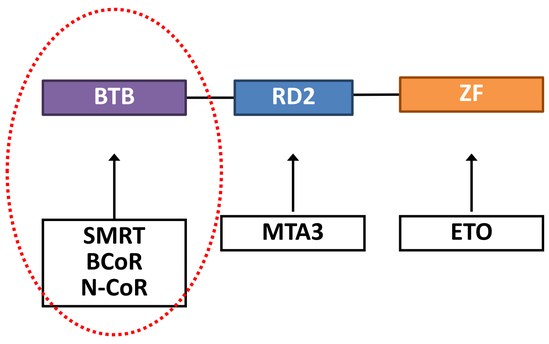
Objective and Background

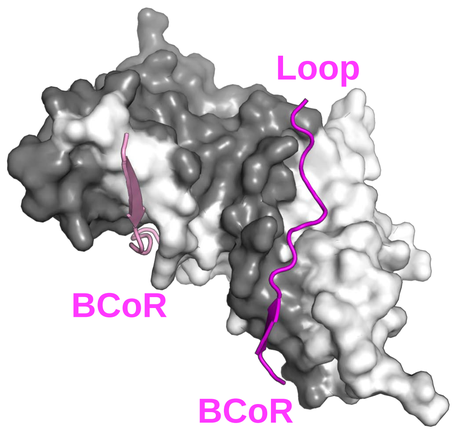
Objective

➢ **To discover potent BCL6 small molecule inhibitors by biophysical approach**

Background

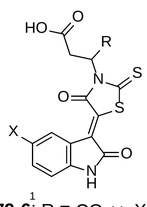
- BCL6: B-cell lymphoma 6 protein
- Autoimmune diseases (Inflammation)
- BTB-Zinc finger family
- Function: A transcriptional repressor



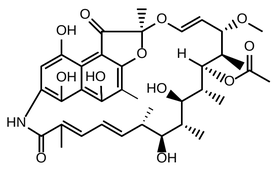


BCL6
PDB: 3BIM[5]

- Interaction with corepressor
- BTB domain: Homodimer
- A shallow groove at the interface



79-6[1]: R = CO_2H, X = Br
K_D = 129 μM
FX1: R = H, X = Cl
K_D = 7 μM



Rifamycin SV[2]
K_D = ~ 1 mM

Ac -LWYTDIRMSWRVP-OH

F1324[3]
K_D = 0.57 nM

TAT-GRGIEHISR-Fu

RI-BPI[4]
GI_{50} = ~10 μM

Known BCL6 inhibitors. No high potent small molecule inhibitors have been reported.

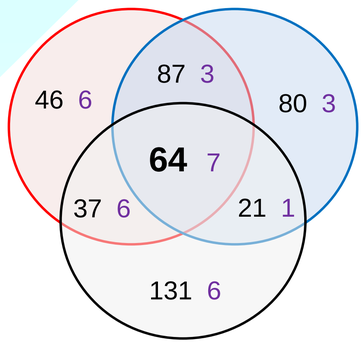
Workflow of Fragment Screening

Primary screening

Fragment library (1494 compounds)
<350 Da (ave. 180 Da)

SPR
BIACORE 4000
Single point assay at 1 mM
mutantBCL6^{BTB}, wild typeBCL6^{BTB}, wild type BCL6, Neturavidin

➢**64 compounds (hit rate: 4.3%)**



Captured wild type BCL6^{BTB}
Captured mutant BCL6^{BTB}
Coupled wild type BCL6^{BTB}
Neturavidin

Hit confirmation

Dose response (@ 0.25, 0.5, 1, 2 mM)

➢**64 compounds**

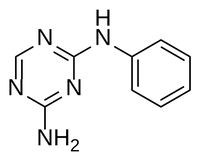
STD-NMR
Bruker 600 MHz with cryoprobe

➢**7 compounds (0.47%)**

Competition experiment by SPR

Crystal Structure analysis

➢**1 compound (0.067%)**

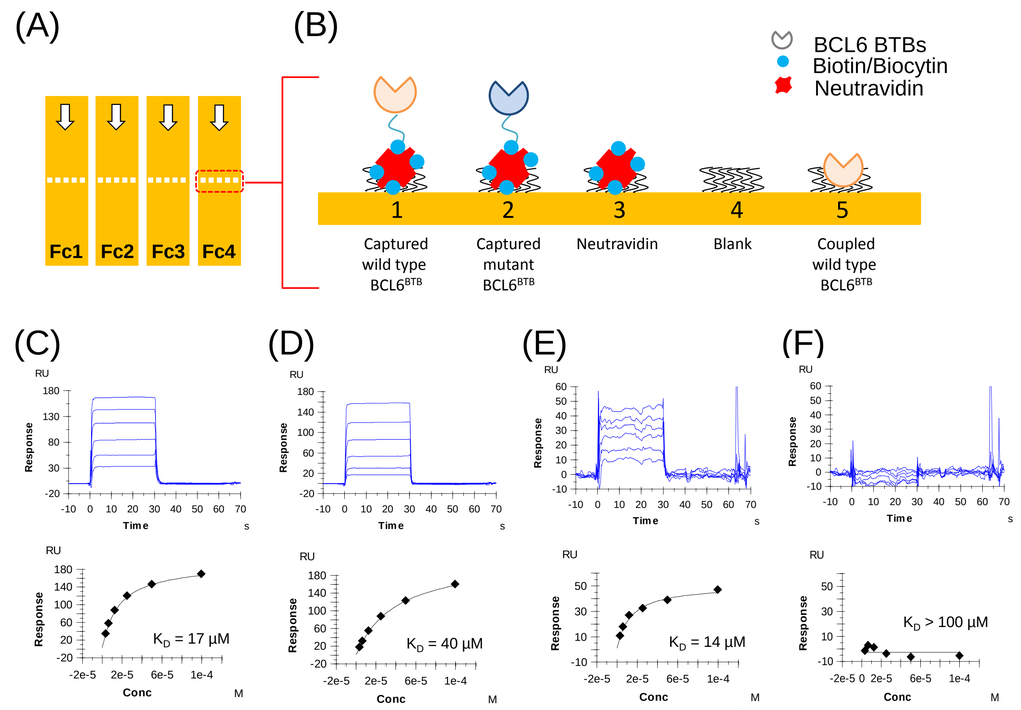


Fragment 1

SPR K_D = 1200 μM, LE = 0.28

SPR-based screening of 1494 fragments tested against (A) captured wild type BCL6^{BTB}, (B) captured mutant BCL6^{BTB}, (C) coupled wild type BCL6^{BTB}, and (D) NeutrAvidin. The color of each plot indicates fast binding (blue-circle), slow binding (red-circle), and 100 μM BCoR peptide as a positive control (gray-square).

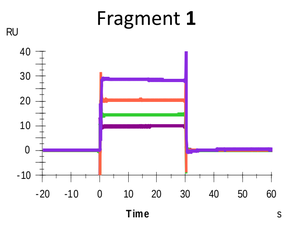
Fragment Screening by SPR

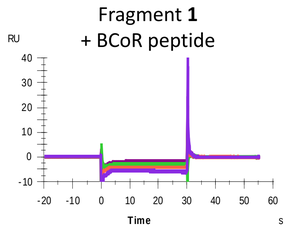


Layout of the sensor chip. (A) Four independent flow cells (Fc), each with five detection spots. (B) Protein immobilized in each flow cell. Binding responses of captured wild type BCL6^{BTB}, captured mutant BCL6^{BTB}, coupled wild type BCL6^{BTB}, and NeutrAvidin are detected on spots 1−3, spots 2−3, spots 5−4, and spots 3−4, respectively. Sensorgrams of BCoR peptide binding to (C) captured wild type BCL6^{BTB}, (D) captured mutant BCL6^{BTB}, (E) coupled wild type BCL6^{BTB}, and (F) NeutrAvidin. Lower graphs indicate the fit plots of the response measured at equilibrium plotted against BCoR peptide concentration. Top concentration is 100 μM; dilution step is 2-fold.

Validation by Biophysics

A

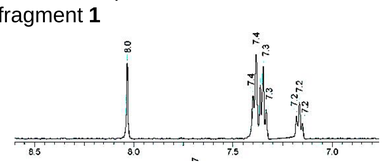


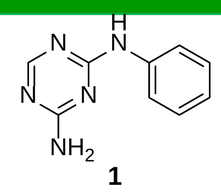


B

STD spectrum of fragment **1** (difference)

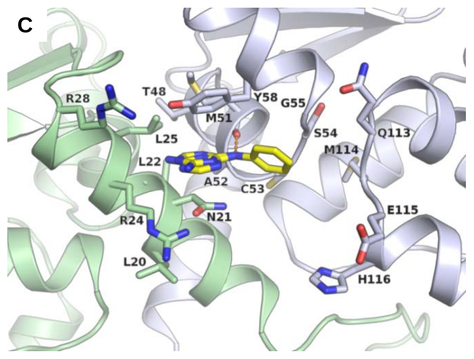
Reference spectrum ^{1}H NMR of fragment **1**





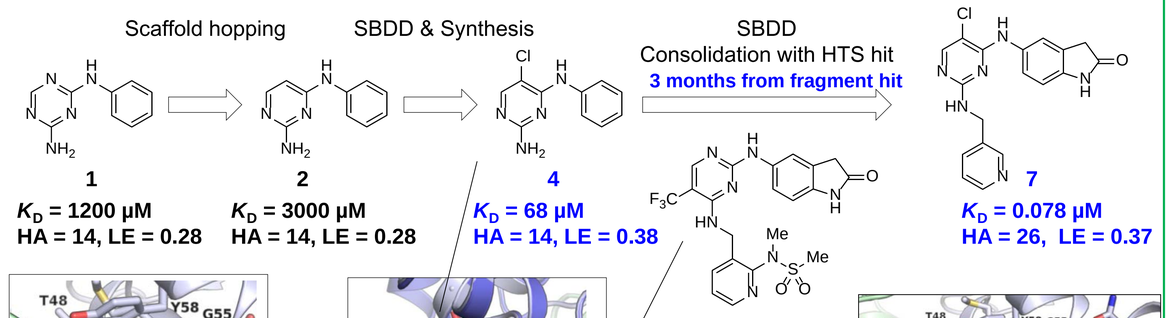
1

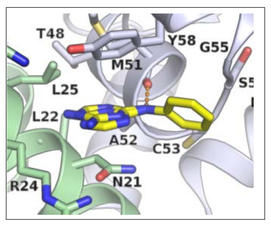
C

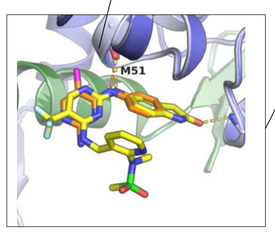


Characterization of fragment **1**. (A) SPR competition experiments. Sensor grams of fragment **1** to mutant BCL6^{BTB} in the absence or presence of 100 μM BCoR peptide. Top concentration is 1 mM; dilution step is 2-fold. (B) STD-NMR experiments. The STD spectrum of fragment **1** (difference). ^{1}H spectrum of fragment **1** for off-resonance as reference , and (C) Co-crystal structure of fragment **1** in complex with BCL6^{BTB}.

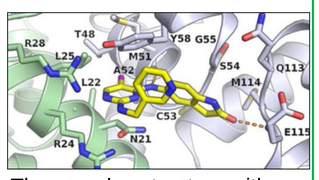
Optimization of Fragment compound 1





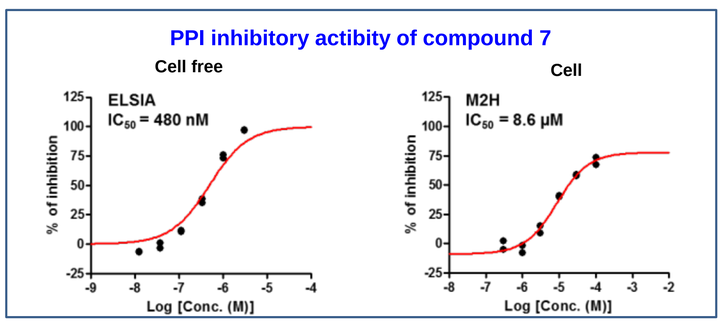


The complex structures with compounds **4** (orange, PDB: 5X4N) and **5** (yellow, PDB: 5X4O)



The complex structure with compounds **7** (yellow) (PDB: 5X4Q)

PPI inhibitory actibity of compound 7



Conclusion

- We identified fragment **1** by biophysics-driven fragment screening using SPR.
- We successfully developed a potent BCL6 PPI inhibitor compound **7** from fragment **1**.
- The combination of biophysics-driven FBDD/SBDD and FADD is a promising strategy for hit identification and lead generation against challenging targets such as PPIs.

Kamada, Y.; Sakai, N.; Sogabe, S.; Ida, K.; Oki, H.; Sakamoto, K.; Lane, W.; Snell, G.; Iida, M.; Imaeda, Y.; Sakamoto, J.; Matsui, *J. Med. Chem.* **2017**, 60, 4358−4368

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