

**AZD5438-PROTAC: A Selective CDK2 Degradator that Protects Against Cisplatin-
and Noise-Induced Hearing Loss**

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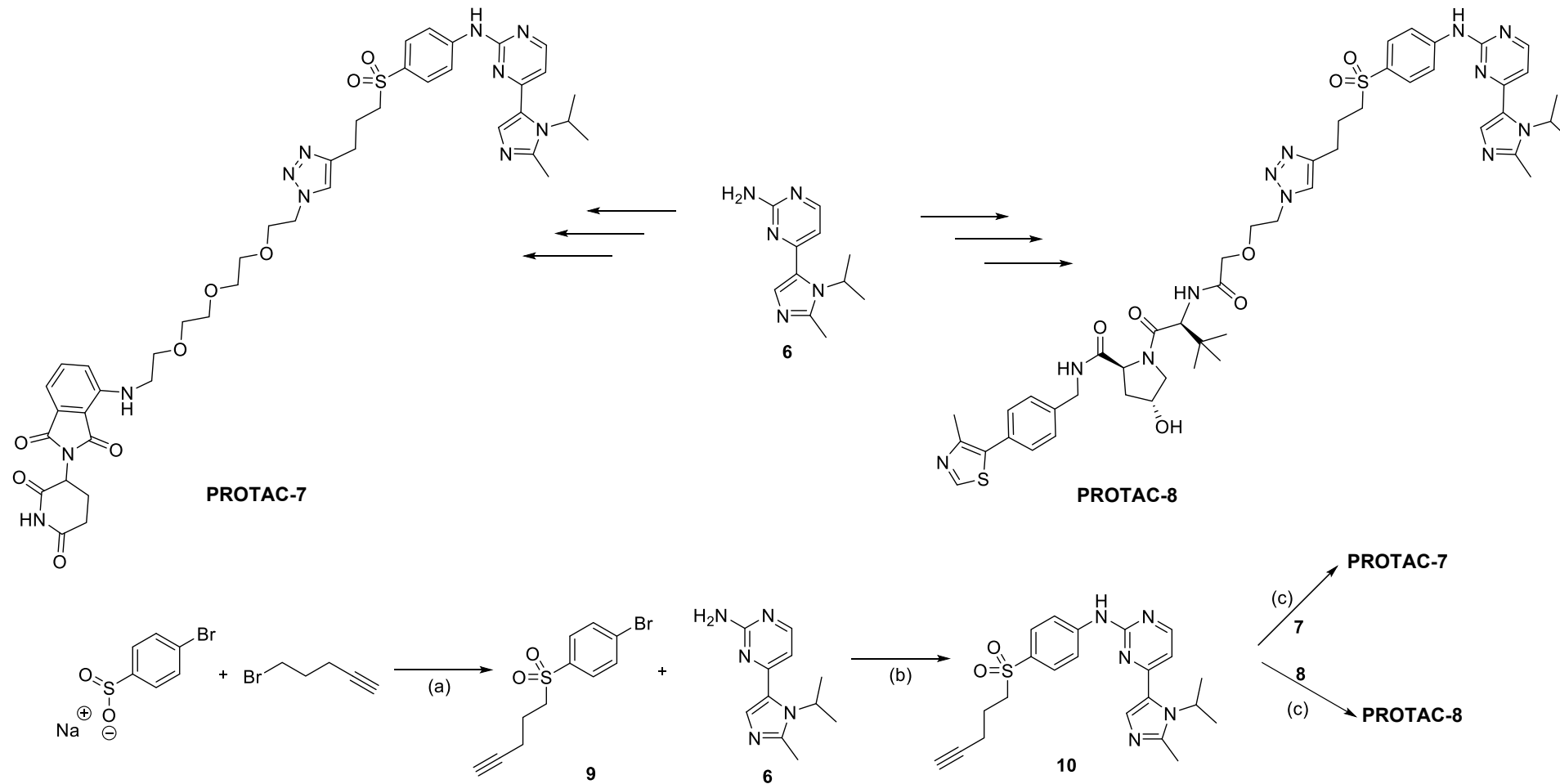
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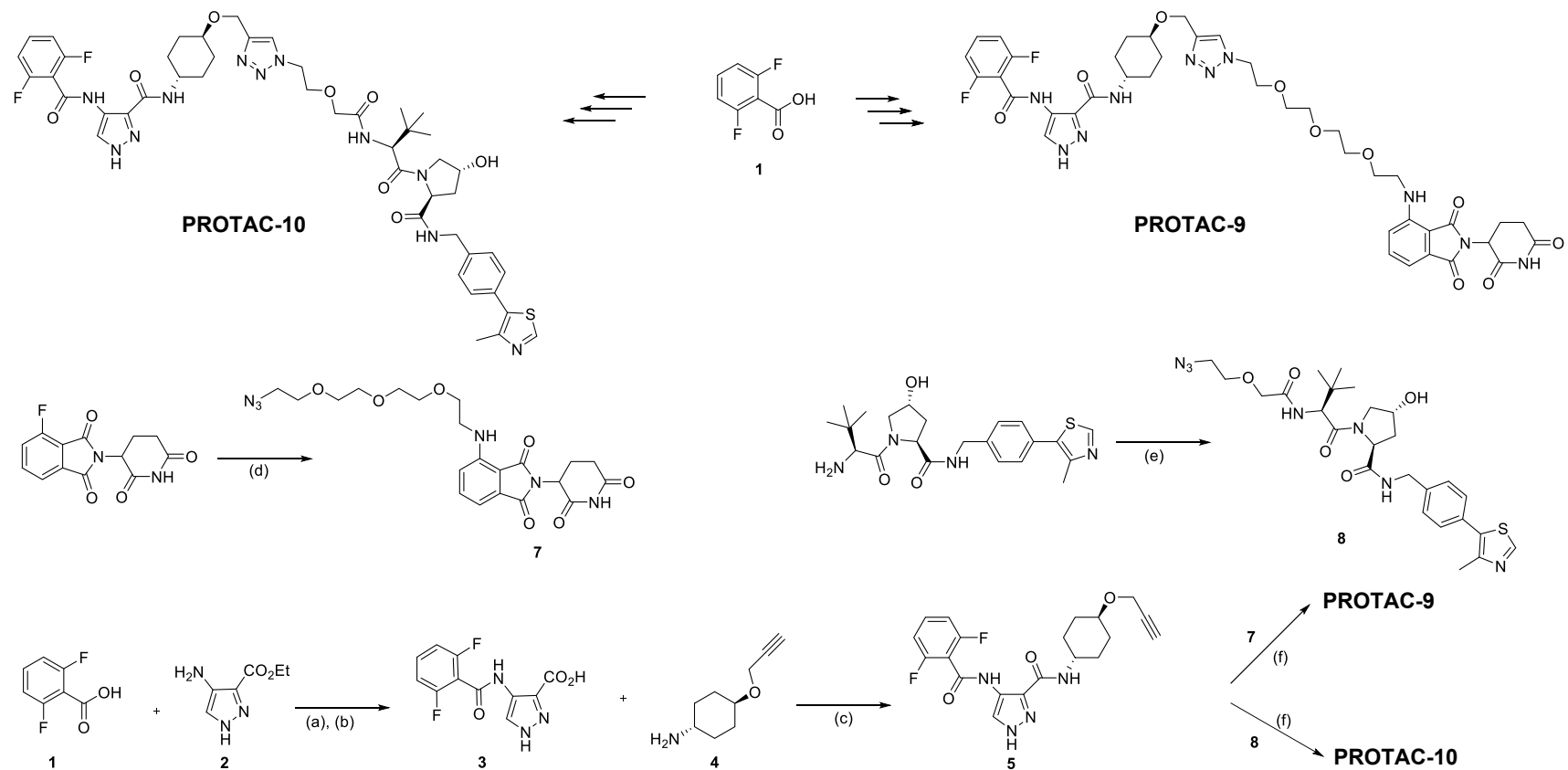
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Scheme 1



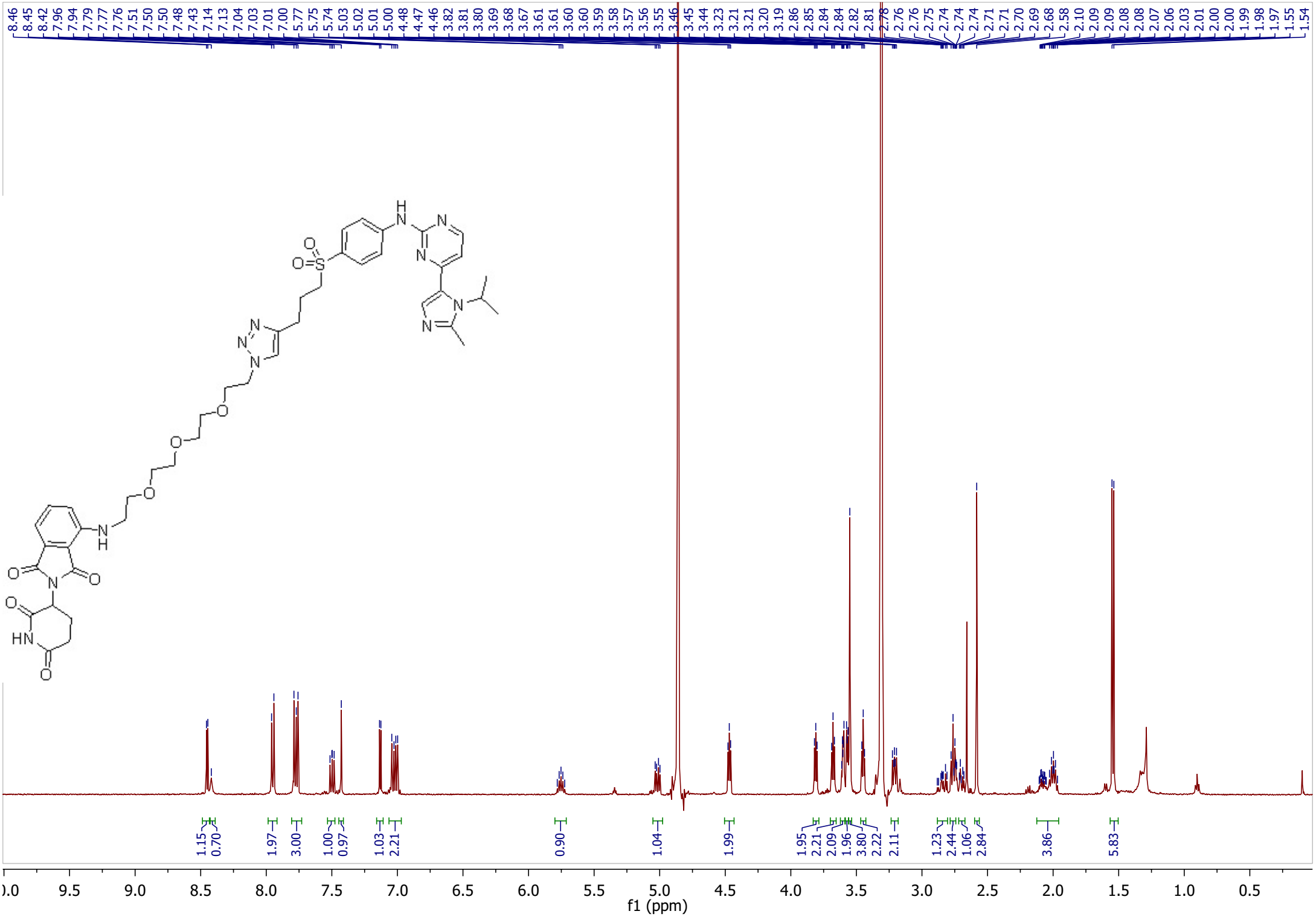
Scheme 1. Reagents and conditions: (a) DMF, rt, 36h. (b) NaOtBu (3.0 equiv.), BINAP (0.1 equiv.), Pd₂(dba)₃ (0.05 equiv.), 1,4-Dioxane, 80 °C, 2h. (c) CuSO₄ (1.1 equiv.), Sodium ascorbate (2.2 equiv), DMSO:H₂O (1:1), rt, 20h.

Scheme 2

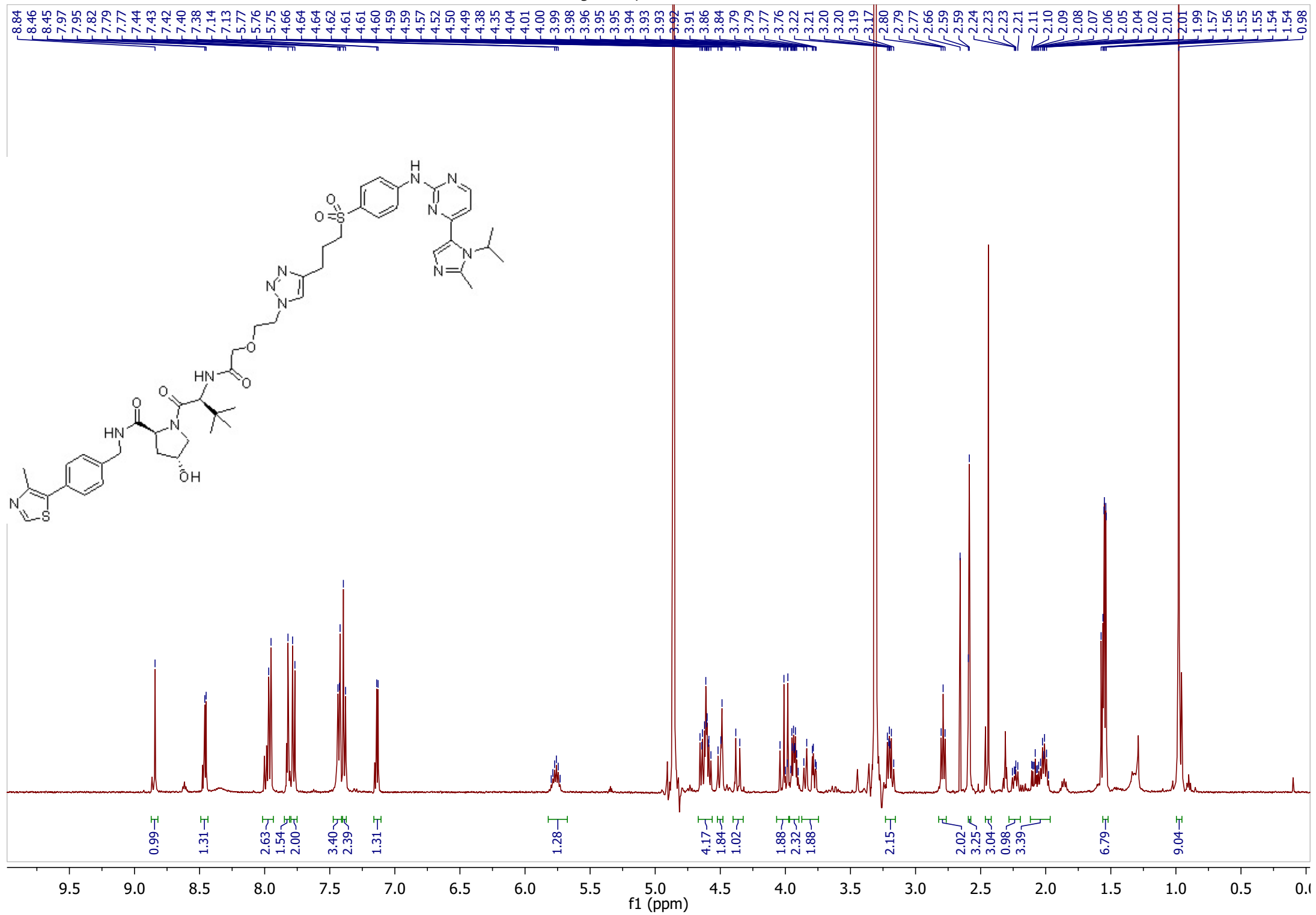


Scheme 2. Reagents and conditions: (a) EDC (1.2 equiv.), HOBt (1.2 equiv.), DMF, rt, 20h (b) NaOH/EtOH (2M), rt, 20h. (c) (1*r*,4*r*)-4-(prop-2-yn-1-yloxy)cyclohexan-1-amine (1.0 equiv.), EDC (1.2 equiv.), HOBt (1.2 equiv.), DMF, rt, 18h. (d) 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (1.0 equiv.), DIPEA (2.0 equiv.), DMF, 90 °C, 18h. (e) 2-(2-azidoethoxy)acetic acid (1.0 equiv.), DIPEA (6.0 equiv.), HATU (4.0 equiv.), DMF, 0°C-rt, 20 min (f) CuSO₄ (1.1 equiv.), Sodium ascorbate (2.2 equiv.), DMSO:H₂O (1:1), rt, 20h.

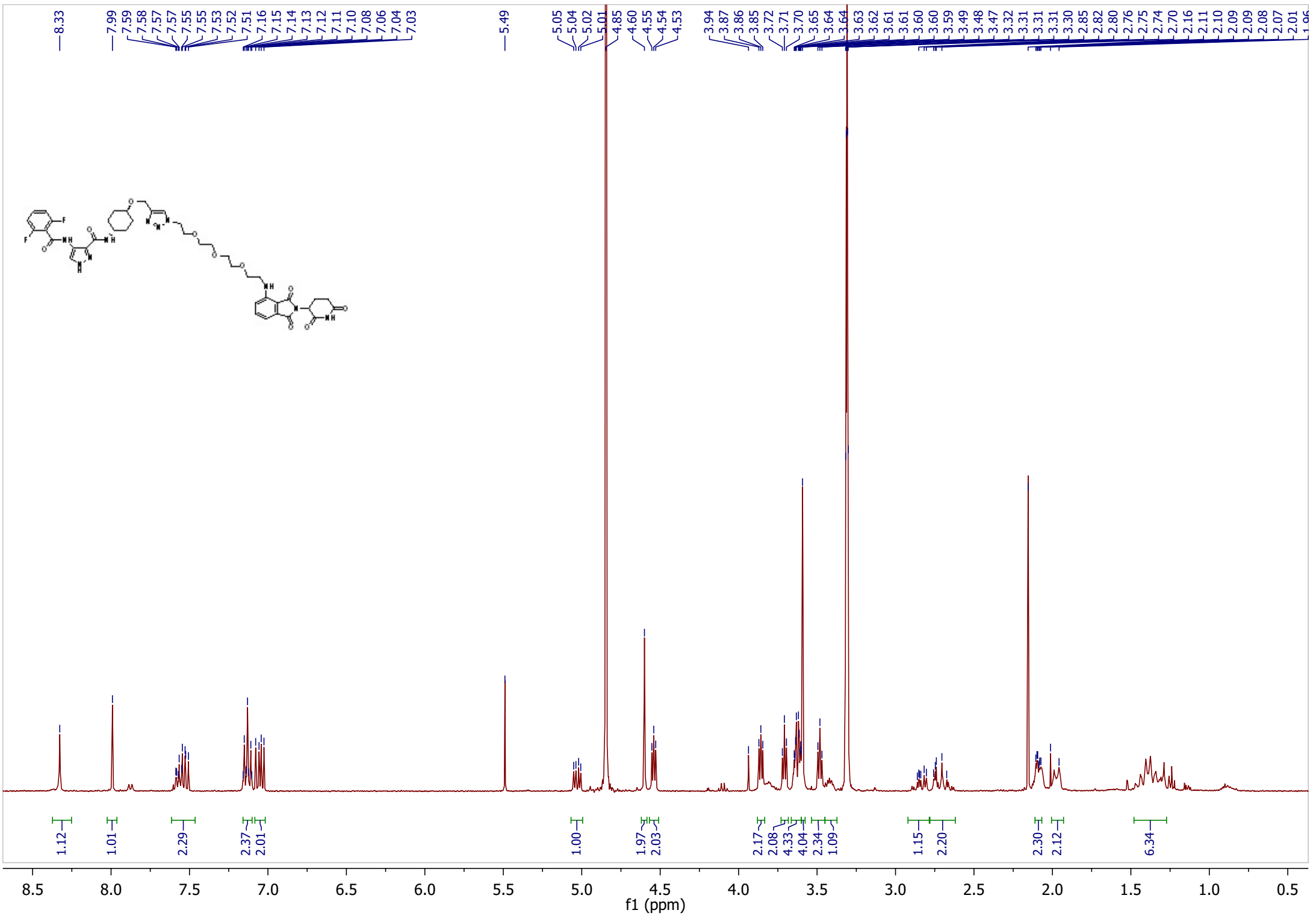
SI Figure 1-A, 1H NMR-PROTAC-7



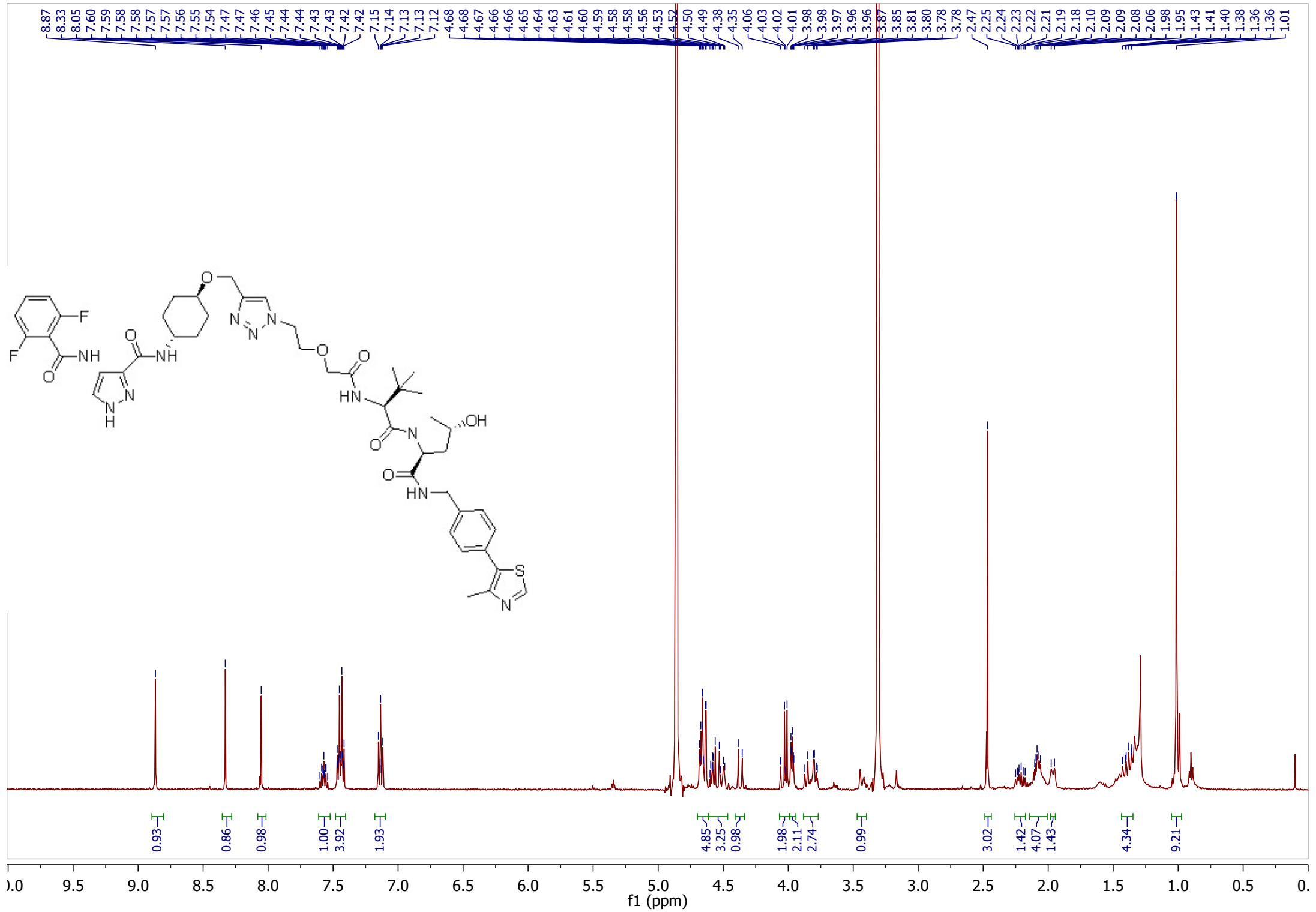
SI Figure 1-B, 1H NMR-PROTAC-8



SI Figure 1-C, ¹H NMR-PROTAC-9

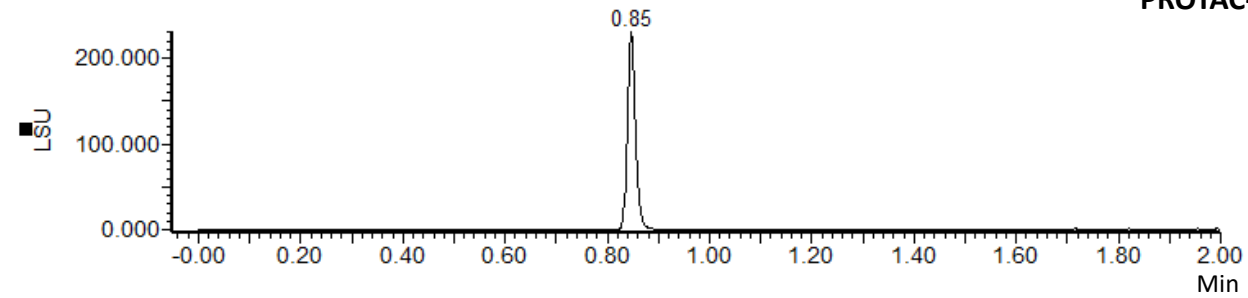


SI Figure 1-D, 1H NMR-PROTAC-10

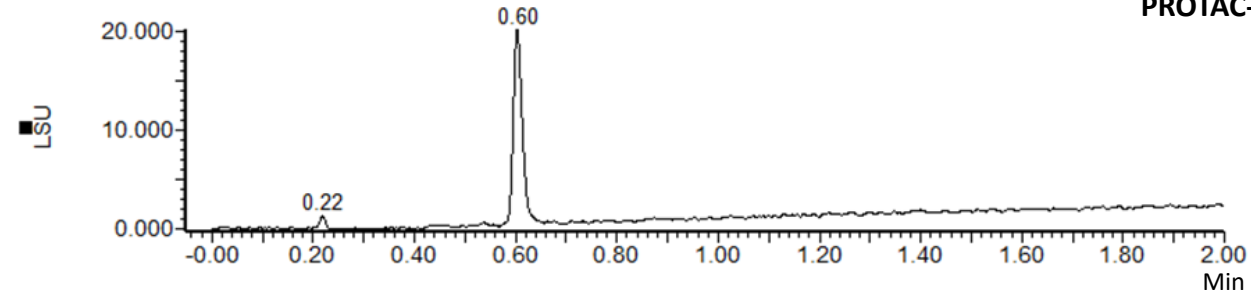


HPLC-Traces

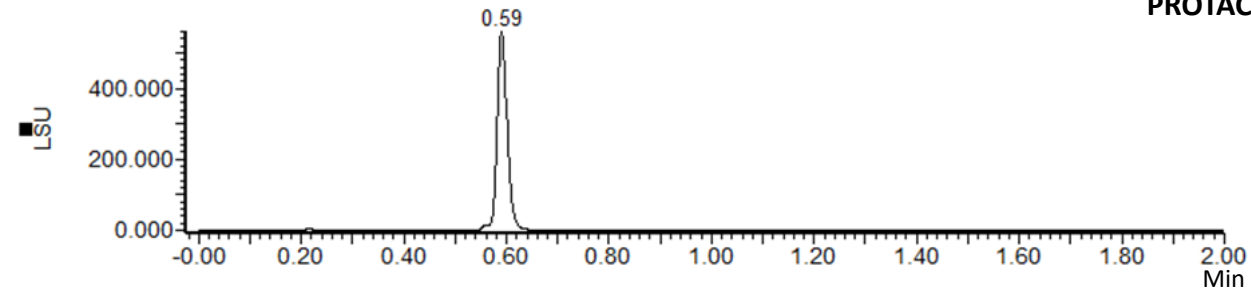
PROTAC-9



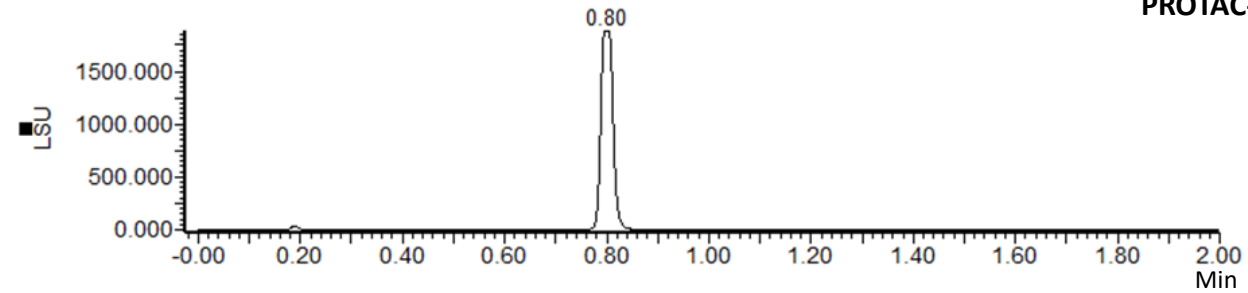
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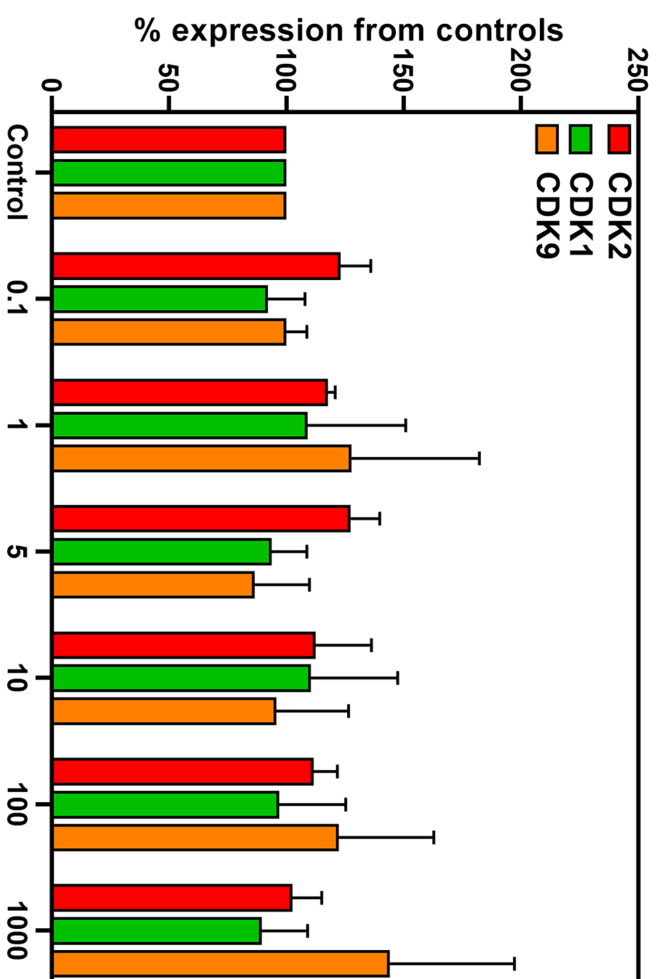
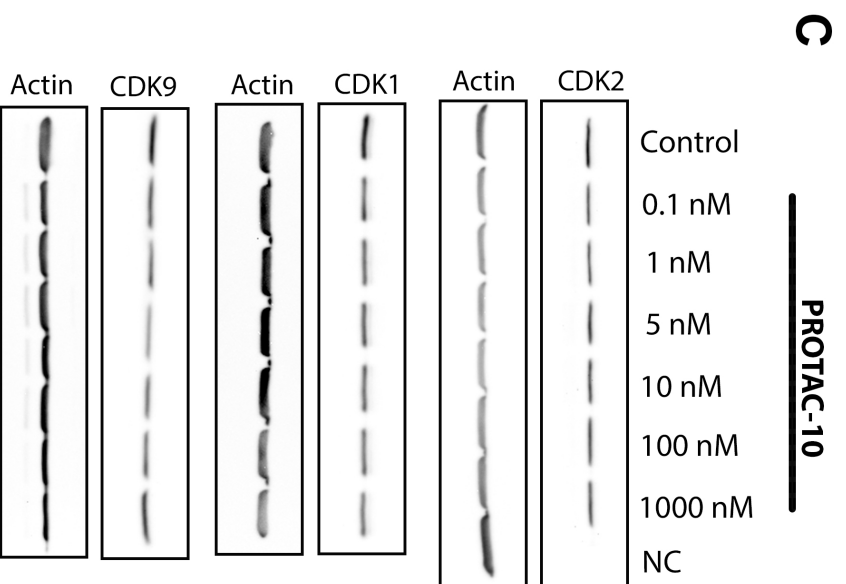
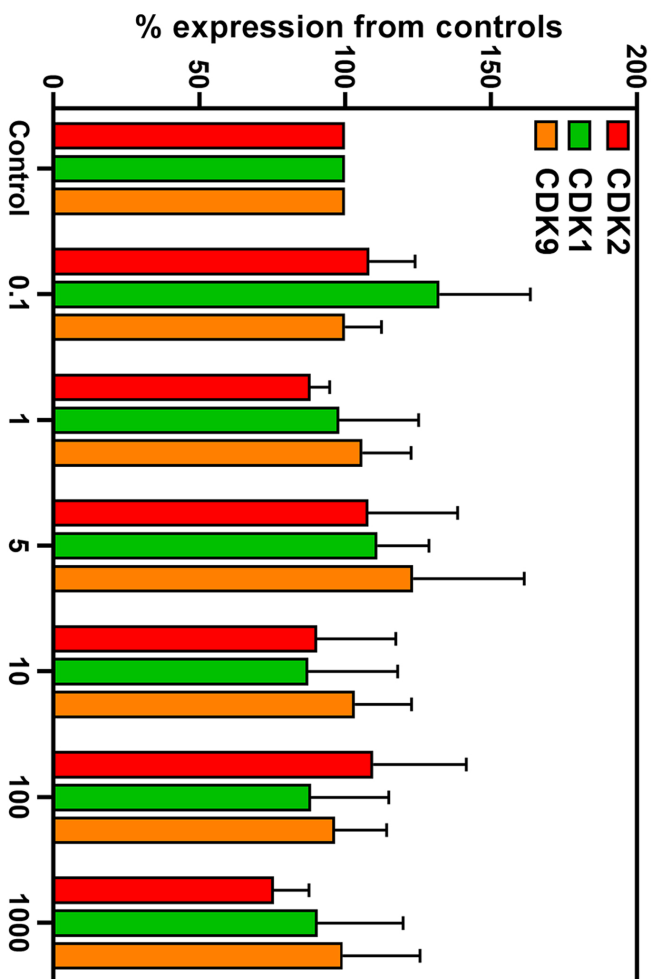
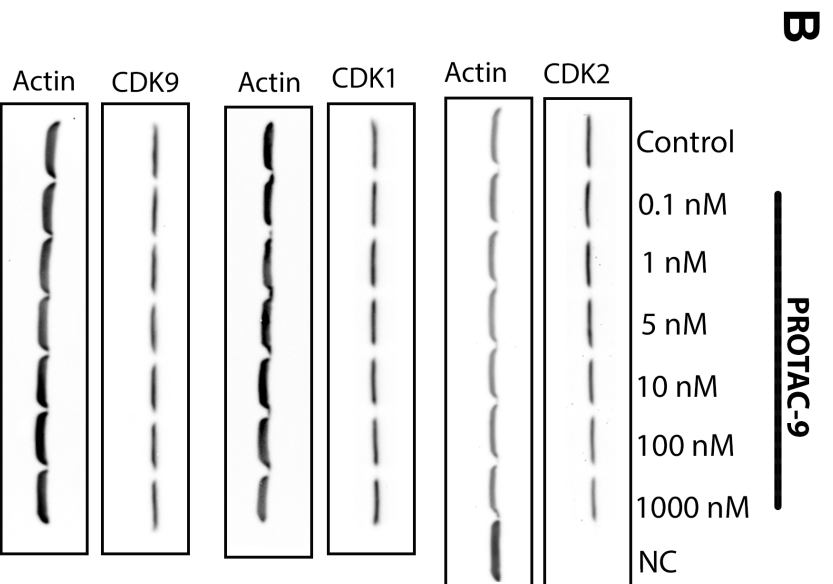
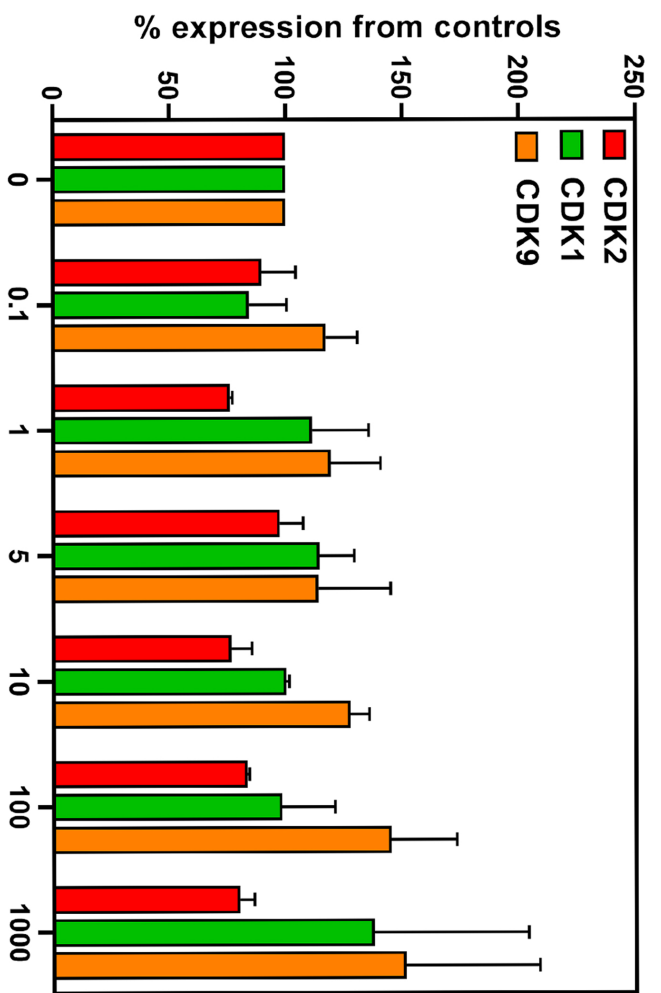
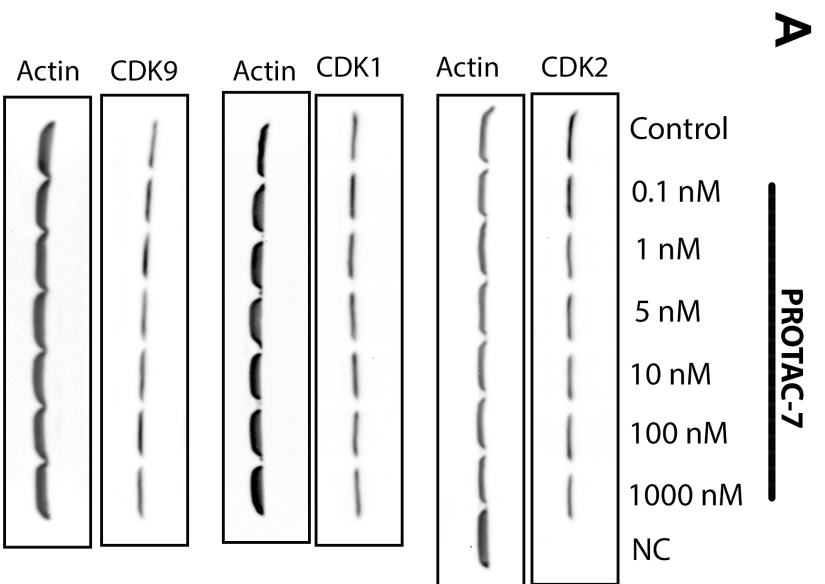


PROTAC-8

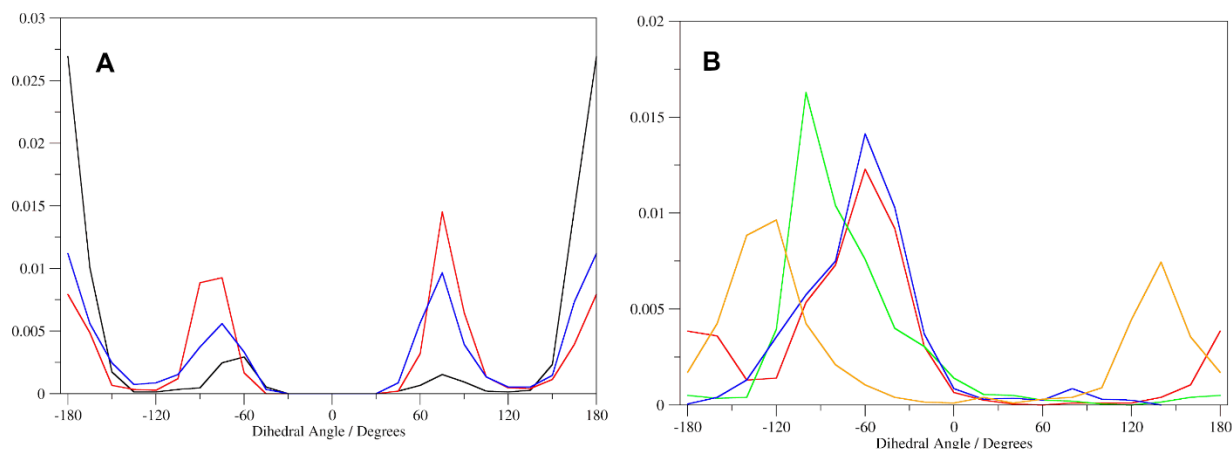


PROTAC-10

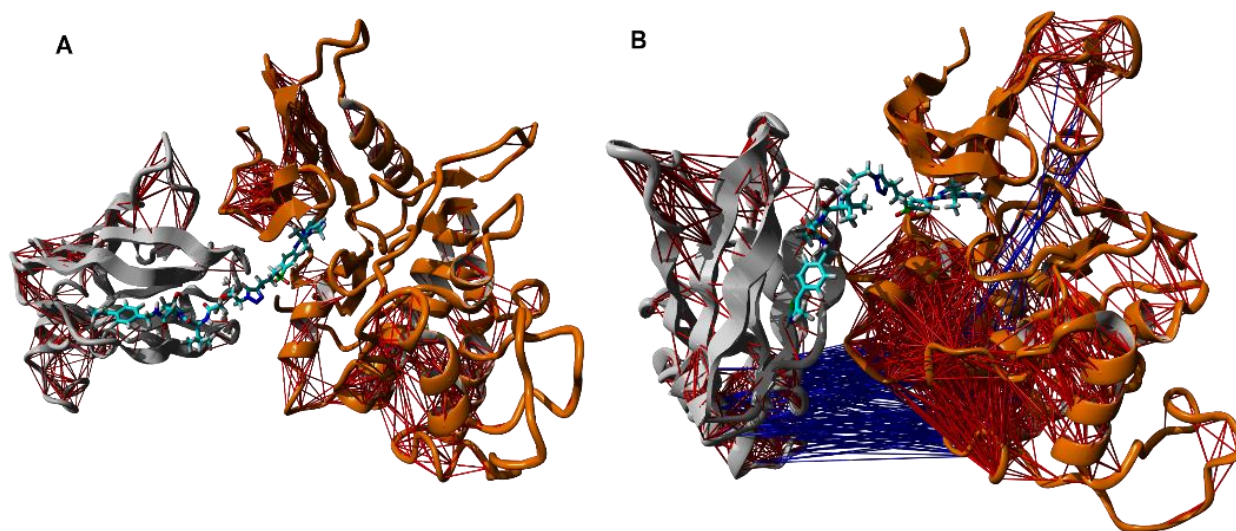




SI Figure 2. Characterization of PROTACs in HEI-OC1 cells. HEI-OC1 cells were incubated for 24 hours with different concentrations of PROTAC-7 (**A**), PROTAC-9 (**B**) and PROTAC -10 (**C**). Cells were harvested and processed for immunoblot detection of CDK2, CDK1 and CDK9. Left: Representative immunoblots. Right: Quantification analysis of the specific bands for CDK2, CDK1 and CDK9 from two independent experiments. Results were expressed as mean +/- SEM. Actin was used as a loading control. NC: Kidney lysate from CDK2 knockout mouse used as a negative control for CDK2 immunodetection.



SI Figure 3. Distribution of α_1 , α_2 , α_3 and α_4 dihedral angles during MD simulations. Definition of the dihedral angles are given in the legend of Figure 3 in the main text. **A)** Distributions of α_1 (black), α_2 (red), and α_3 (blue) dihedral angles for MD simulation 1; **B),** α_4 dihedral angle in MD simulations 1, 2, 3, and 5, respectively black, red, blue and green is in gauche(-) conformations, whereas in MD simulation 5 (orange) α_4 explored both gauche(+) and gauche(-) conformations. MD simulations 1 and 2 resulted in similar α_4 dihedral angle distribution therefore the two curves are indistinguishable.



SI Figure 4. Dynamic cross-correlation matrix (DCCM) analysis of trajectories. pVHL (grey) connected through PROTAC-8 to CDK2 (orange), red and blue lines are shown between correlated and anti-correlated Ca atom pairs, respectively. **A)** In MD simulations 1-4 the two protein move independently from each other and only intradomain movement can be observed; **B)** in MD simulation 5 both intradomain correlated and interprotein anti-correlated movements are observed.