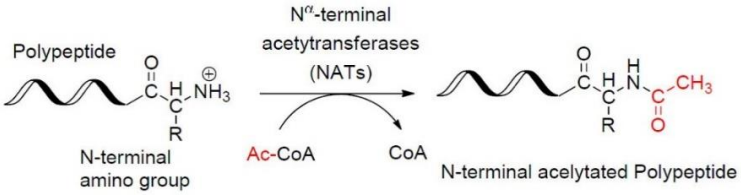
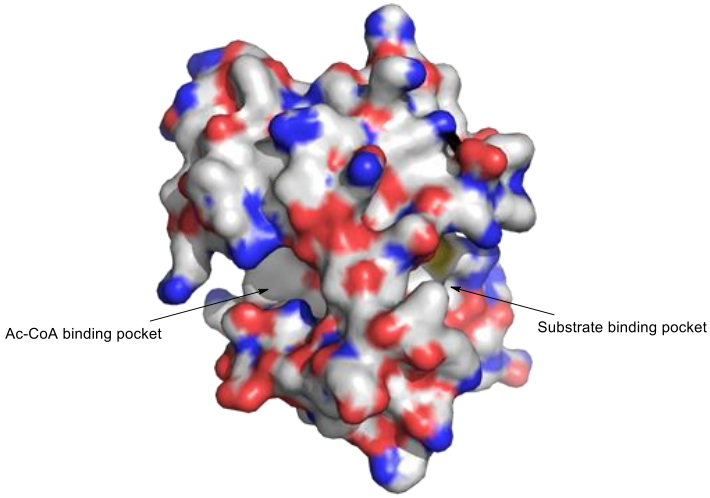
Identification of Transferase NAA50 Inhibitors by DEL Selection*

(Collaboration Project with Pfizer, ACS Med. Chem. Lett. 2020, 11, 1175−1184)

Protein NAA50 and its Function

The N-terminal-acetylation of a protein can affect its nuclear import and export and can also act as a degradation signal to control the protein's cellular stability. The Nα-terminal acetyltransferase (Naa50) enzyme is a member of the Nα-terminal acetyltransferase NAT protein family. It coexists with Naa10 and Naa15 in the NatE complex and is responsible for the enzymatic function of the complex. Naa50 is also found to be essential for normal sister chromatid cohesion and chromosome condensation. Therefore, an inhibitor of the Naa50 enzyme might have therapeutic applications in oncology indications. The enzymatic catalysis and protein structure are shown below.

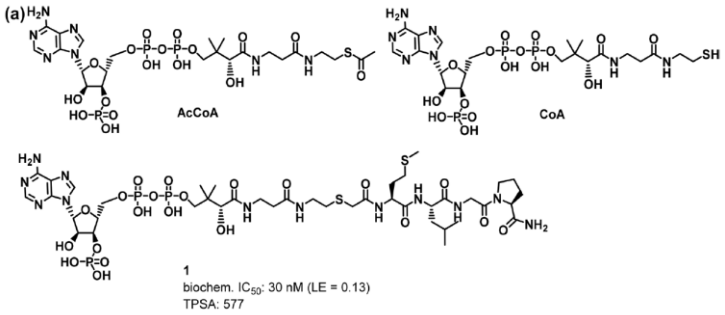




NAA50 Known Inhibitor and Objective of DEL Selection

Compound **1** is designed by studies of the NAA50 biochemical mechanism which indicated formation of a ternary complex between the AcCoA cofactor, an appropriate protein substrate (tetra-peptide MLGP), and the enzyme. Although compound **1** is a potent Naa50 inhibitor, the molecule is not particularly efficient due to its large molecular weight (ligand efficiency (LE)10 = 0.13). In addition, its high molecular weight (MW = 1223) and high polarity (tPSA = 577 and cLogP = −4.1) likely prevents facile permeability across cell membranes and may thus compromise the use of the molecule as a robust in vitro tool compound.

The DEL selection is to identify potent and selective NAA50 inhibitors with improved physiochemical properties relative to compound 1 (i.e., reduced molecular weight and tPSA; increased logD).



DEL Selection Plan

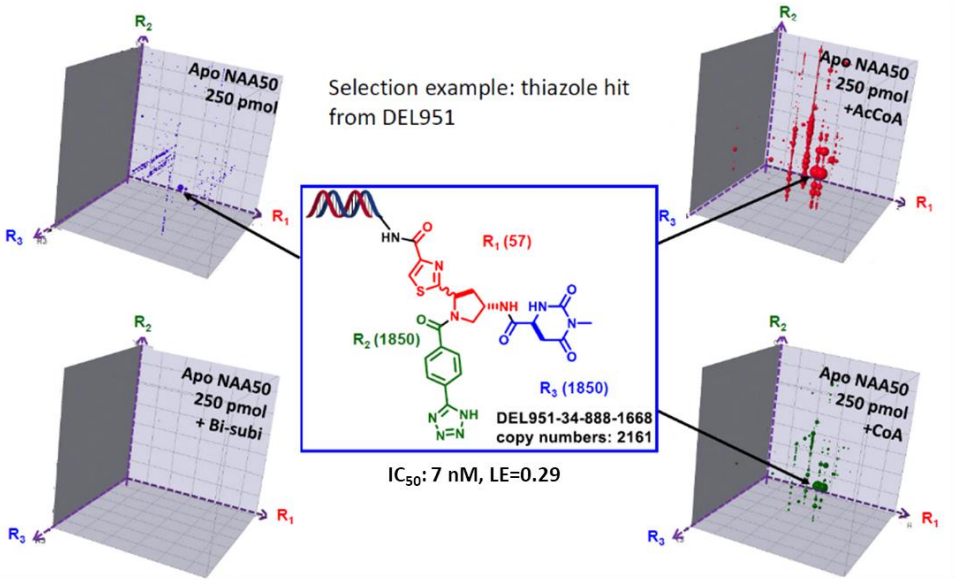
In a typical DEL selection plan, we set up 3 samples (1. protein alone, Apo NAA50 in this case; 2. protein and inhibitor in saturated concentration; 3. Blank control). However, during the studies of NAA50, we realized that NAA50 protein involves conformational change in the catalytical process. By comparison of selection results of Sample **1** and **2** aforementioned, we are not able to effectively identify inhibitors. Consequently, we included two additional samples by addition of AcCoA and CoA, seeking for compounds binding to the transition state. The final Selection Plan are summarized below.

No	Target	Library	Supplement	Purpose
1	His-NAA50 (250 pmol)	HitGen DEL	-	Identify all the possible binders
2	His-NAA50 (250 pmol)	HitGen DEL	AcCoA	Identify all the binders in the presence of AcCoA
3	His-NAA50 (250 pmol)	HitGen DEL	CoA	Identify all the binders in the presence of CoA
4	His-NAA50 (250 pmol)	HitGen DEL	Compound **1**	Combine with Sample 1,2,3 to identify inhibitors
5	No Protein	HitGen DEL	-	Identify beads binders

Representative DEL Selection Results

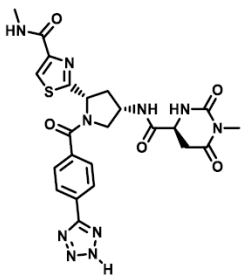
DEL selection is typically presented in a cubic layout, where the axis represents the corresponding building block and bubble size represents the sequence count of each compound. If the compounds have more engagement to the protein (high binding affinity or slow off-rate), the sequence counts will be presented as bigger bubbles. As we can see from the first four samples (blank sample 5 is now shown), Apo NAA50, Apo NAA50 + AcCoA, Apo NAA50 + CoA and Apo NAA50 + Compound 1 (also called Bi-subi) resulted different enrichment pattern, proving that NAA50 has conformational changes during the catalysis. Selection of compounds binding to all the protein stages and competing with Compound 1 obviously will yield NAA50 inhibitors. Such a selected compound structure is shown in the figure below, where the compound is built in the DEL as a racemic mixture.

The selected compound DEL951-34-888-1668 is resynthesized for validation.

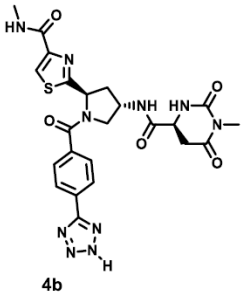


DEL Compound Validation

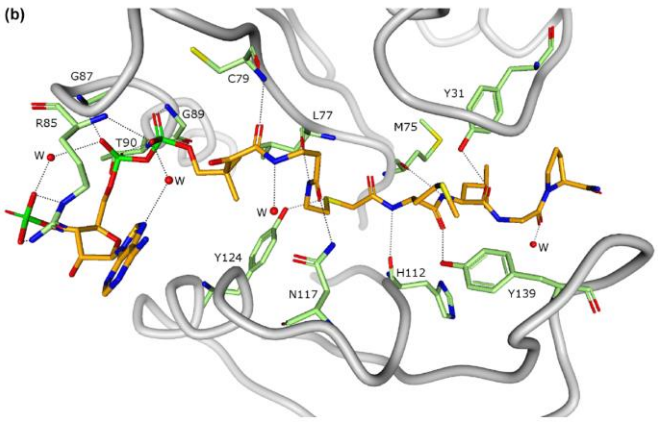
The two isomers of the selected compound DEL951-34-888-1668 were synthesized and tested by SPR in the presence of CoA and AcCoA and biochemical assay. The chiral isomer **4a** has been found as a very potent inhibitor with improved MW, Ligand Efficiency, and tPSA. The interaction of compound **4a** and NAA50 has been further confirmed by co-crystal structure by Pfizer (pdb code: 6WFN).



4a
SPR (Naa50+CoA) Kd: 32 nM
SPR (Naa50+AcCoA) Kd: 27 nM
biochem. IC_{50}: 7 nM
LE: 0.29
TPSA: 224



4b
SPR (Naa50+CoA) Kd: 471 nM
SPR (Naa50+AcCoA) Kd: 470 nM
biochem. IC_{50}: 1600 nM
LE: 0.2
TPSA: 224



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