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Structure based design of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors from a phenotypic screen

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ABSTRACT

Nicotinamide phosphoribosyltransferase is a key metabolic enzyme that is a potential target for oncology. Utilizing publicly available crystal structures of NAMPT and *in silico* docking of our internal compound library, a NAMPT inhibitor, **1**, obtained from a phenotypic screening effort was replaced with a more synthetically tractable scaffold. This compound then provided an excellent foundation for further optimization using crystallography driven structure based drug design. From this approach, two key motifs were identified, the (*S,S*) cyclopropyl carboxamide and the (*S*)-1-*N*-phenylethylamide that endowed compounds with excellent cell based potency. As exemplified by compound **27e** such compounds could be useful tools to explore NAMPT biology *in vivo*.

Regulation of nicotinamide adenine dinucleotide (NAD+) levels within cells is critical given the importance of NAD+ in both maintaining cellular energy homeostasis and its role as an enzymatic cofactor.[1] There are multiple mechanisms within eukaryotic cells to sustain cellular NAD+ levels but the rate determining source is the conversion of nicotinamide (NAM) to nicotinamide mononucleotide (NMN) by the enzyme nicotinamide phosphoribosyltransferase (NAMPT). The NMN produced by NAMPT is then further processed to NAD+ by the enzyme Nicotinamide mononucleotide adenylyltransferase (NMNAT, Fig. 1A).[2] Several reports have demonstrated that cancerous cells express higher levels of NAMPT and have a higher NAD+ demand than normal cells.[3] This evidence therefore suggests that blocking NAD+ production through the inhibition of NAMPT enzymatic activity could be selectively lethal to cancerous cells and consequently provide a therapeutic benefit.[4]

Recently, we disclosed the pyrrolo-pyrimidine NAMPT inhibitor **1** (Fig. 1B), which was discovered through a phenotypic screening campaign.[5] While **1** is very potent in an A2780 Cell-Titer Glo™ (CTG) assay (IC_{50}: 6 nM) the construction of the pyrrolo-pyrimidine core requires a significant investment of synthetic effort.[6] Given the structures of known NAMPT inhibitors,[7] we hypothesized that this complex core is not necessary for NAMPT activity and we could identify an alternative to **1** that was more synthetically tractable and therefore more amenable to iterative optimization. In this vein, we used the crystal structure of NAMPT/FK866 (PDB code: 2GVJ)[8] as the template for an *in silico* screen for alternative chemical matter. The pyridine of FK866 is in a pi-stacking position between Tyr18′ and Phe193 which mimics the position of the natural ligand NMN to competitively inhibit enzyme function. Docking **1** into this pocket suggested that the pyridine nitrogen could form the same hydrogen-bond with the phenol of Tyr18 as observed for the analogous nitrogen in the FK866 crystal structure. To potentially enhance this interaction, a 2-aminopyridine was selected as a new head group because of the enhanced basicity of this pyridine nitrogen relative to an unsubstituted pyridine. The 2-aminopyridine was then used as the substructure search query to filter our internal compound collection, followed by docking into the FK866 binding pocket. A dozen top-scoring docking hits were selected and submitted for biological activity testing in the A2780 CTG assay. From this effort, the most promising ligand identified was 2-aminopyridine **2,** which we found to be equipotent to **1** in both the A2780 and CORL23 CTG assays.[9] Furthermore, the cellular activity of **2** was rescued by concurrent treatment with 100

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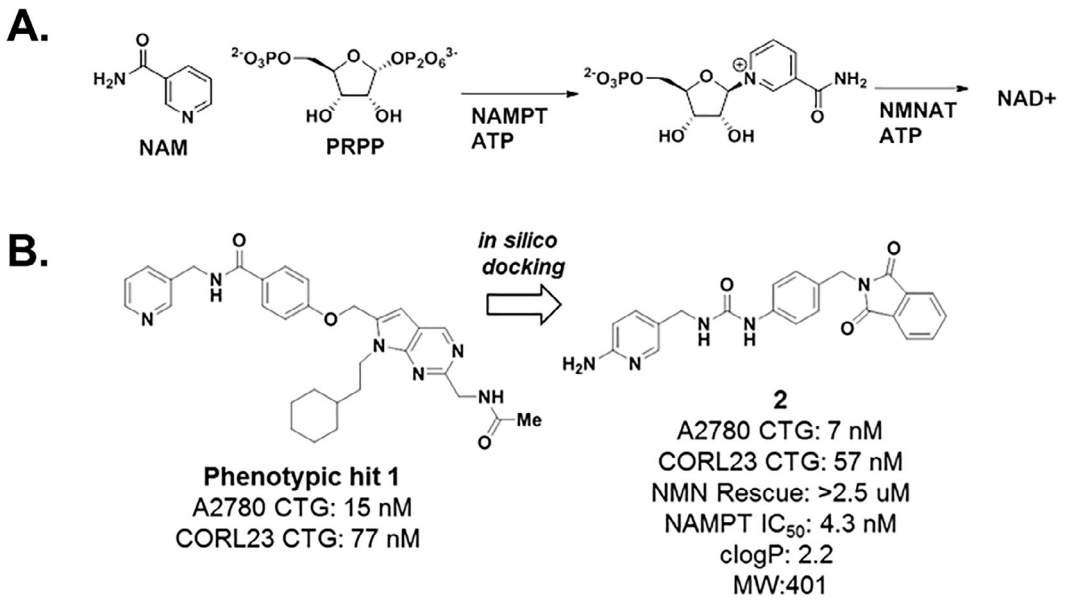


Fig. 1. Identification of cyclopropyl carboxamide NAMPT inhibitors from phenotypic hit **1**. A. NAMPT converts NAM to NMN on the path to NAD^+. B. Structure of phenotypic hit **1** and *in silico* derived **2**.

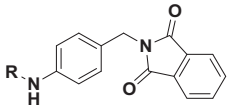
μM of the NAMPT product NMN. Compound **2** was also found to inhibit NAMPT in a biochemical assay (Fig. 1B). This data strongly supports the conclusion that **2** is decreasing the viability of A2780 and CORL23 cells through direct inhibition of NAMPT. In addition to the potent activity of **2**, this small molecule has promising physiochemical properties (clogP: 2.2, MW: 401) and can be readily synthesized in a convergent fashion. Thus, **2** appeared to be a promising foundation to build a lead optimization campaign.

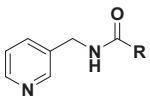
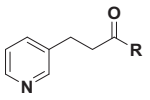
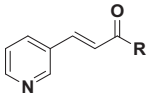
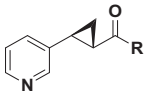
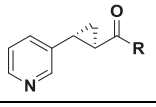
Examining the structure of **2**, we initially sought to explore the structure activity relationship (SAR) of the urea motif. We hypothesized that replacing the urea functionality could potentially improve the aqueous solubility and membrane permeability of the scaffold by decreasing the number of NH bonds. Furthermore, given the α,β unsaturated amide in FK866[10] and the bicyclic NAM mimetics that are known in the literature,[11,12] we postulated that the benzylic urea nitrogen was not engaged in a direct interaction with the target protein and could be replaced. As shown in Table 1 we first removed the aniline on the 2 position of the pyridine to simplify analog synthesis and found that pyridine **3** was approximately ten fold less potent in the A2780 CTG assay.[13]

Next, deleting the benzylic nitrogen gave amide **4**, which was a potent NAMPT inhibitor (IC_{50}: 25 nM) but was inactive in the CORL23 CTG assay. This type of disconnect between biochemical and cell based assays has also been reported for other NAMPT inhibitors.[11] We suspected that the increased flexibility of the ethyl bridge in **4** led to its greatly attenuated activity and we therefore rigidified the system by introducing unsaturation to give amide **5**. However, we were surprised to find that α,β unsaturated amide **5** was only moderately active with an IC_{50} of 2000 nM in the CORL23 CTG assay. To further explore conformational constraints at this position, we utilized the Corey-Chaykovsky[14] reaction to synthesize cyclopropyl carboxamide **6** and separated the two *trans* cyclopropane enantiomers. Upon testing, we found the (*S,S*) enantiomer afforded significantly improved activity over the parent urea **3**. We also found that the cellular activity of the cyclopropane motif is highly stereoselective as the corresponding (*R,R*) enantiomer **7** was inactive in both the A2780 and CORL23 CTG assays. Confirmation that NAMPT is the target of **6** was achieved through demonstration of the biochemical inhibition of NAMPT (Table 1). Though 3-pyridine cyclopropyl carboxamide NAMPT inhibitors have been previously reported in the literature,[15] this effort was limited to a phenylsulfone core. With this in mind, we were intrigued by the potential of the phthalimide moiety to provide new directions for the optimization of **6**.

The absolute configuration of the cyclopropane was determined through co-crystallization with NAMPT, as shown in Fig. 2. In addition to determining the absolute stereochemistry, the crystal structure also suggests the basis for the enhanced cellular activity of the

Table 1
SAR of the amide linker.



Cmpd	R	A2780 CTG (nM)	CORL23 CTG (nM)	NAMPT IC_{50} (nM)
3		100	NT[a]	3.1
4		>10,000	>10,000	25
5		NT	2000	10
6		27	35	<1
7		>2500	>10,000	>2500

[a] Not tested.