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Identification of 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine-derived ureas as potent inhibitors of human nicotinamide phosphoribosyltransferase (NAMPT)

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ABSTRACT

Potent nicotinamide phosphoribosyltransferase (NAMPT) inhibitors containing 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridine-derived ureas were identified using structure-based design techniques. The new compounds displayed improved aqueous solubilities, determined using a high-throughput solubility assessment, relative to previously disclosed urea and amide-containing NAMPT inhibitors. An optimized 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridine-derived compound exhibited potent anti-NAMPT activity (**18**; BC NAMPT IC_{50} = 11 nM; PC-3 antiproliferative IC_{50} = 36 nM), satisfactory mouse PK properties, and was efficacious in a PC-3 mouse xenograft model. The crystal structure of another optimized compound (**29**; NAMPT IC_{50} = 10 nM; A2780 antiproliferative IC_{50} = 7 nM) in complex with the NAMPT protein was also determined.

Nicotinamide phosphoribosyltransferase (NAMPT; also known in the literature as pre-B cell colony-enhancing factor/PBEF and visfatin) catalyzes the condensation of nicotinamide (NAM) and phosphoribosyl pyrophosphate (PRPP) to produce nicotinamide mononucleotide (NMN; Fig. 1).[1] This transformation is dependent on the phosphorylation of a histidine residue in the NAMPT active site by ATP, and is the rate-limiting event in the two-step conversion of NAM to the important enzyme co-factor nicotinamide adenine dinucleotide (NAD; Fig. 1).[2] The NAM–NAD conversion represents the only known mechanism for mammalian cells to efficiently produce NAD from NAM that is liberated by NAD-consuming enzymes such as PARPs and Sirtuins.[3] Accordingly, NAMPT plays a key role in maintaining appropriate intracellular NAD levels, and blocking NAMPT activity may thus impair the growth and/or function of cells which are highly reliant on NAD-dependent processes for survival. Given the elevated metabolisms and high proliferation rates associated with many tumors and/or cancer cell lines, NAMPT inhibition is currently viewed as a novel strategy for the development of new anticancer agents.[4,5]

We previously described the identification of urea-containing NAMPT inhibitors exemplified by general structure **1** (Fig. 2) and compound **5** (Table 1).[6–8,32] We also disclosed the preparation of NAMPT inhibitors bearing amides derived from various bicyclic heteroaromatic moieties (e.g., structures **2** and **3**, Figure 2 and compounds **6** and **7**, Table 1).[9] Many examples of both the urea-containing inhibitors and the heteroaromatic compounds exhibited desirable biological properties including potent NAMPT inhibition activity, acceptable oral bioavailability, and efficacy in mice bearing human tumor xenografts. However, compounds from both of these inhibitor classes often displayed poor aqueous solubilities which complicated their further development. We therefore hypothesized that the introduction of a urea derived from a cyclic, aliphatic amine into the inhibitor design (structure **4**, Fig. 2) might favorably alter intra/inter hydrogen bonding characteristics relative to ureas such as **1** and thereby improve aqueous solubility properties. This design modification also involved the saturation of an aromatic ring present in structures **2** and **3**, and such alterations were also anticipated to beneficially impact

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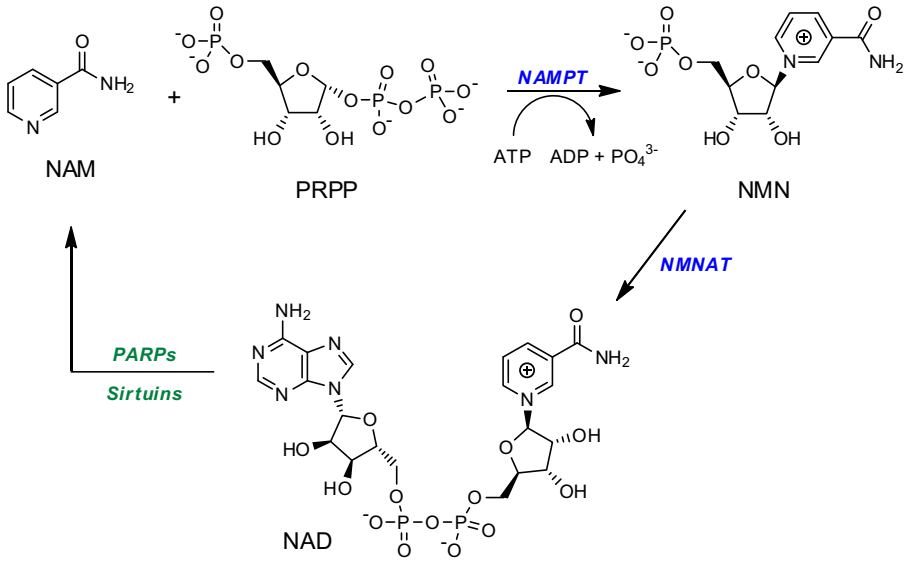

Figure 1. NAD recycling and NAMPT biochemical mechanism.

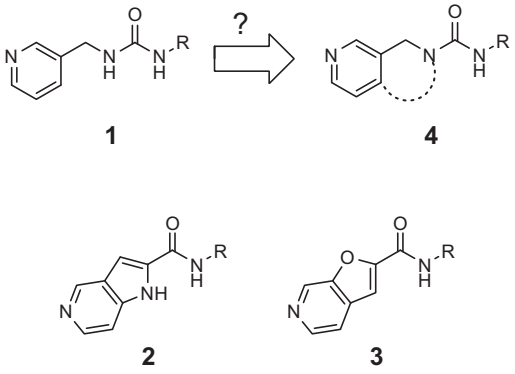

Figure 2. Design of ureas derived from cyclic, aliphatic amines as NAMPT inhibitors.

solubility parameters.[10] In this work, we describe the identification of NAMPT inhibitors bearing such aliphatic urea moieties as well as the subsequent transformation of the initial lead molecules into more optimized compounds.[11]

As shown in Table 1, an inhibitor containing a urea moiety derived from 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridine exhibited potent anti-NAMPT properties in both biochemical and cell culture assessments (compound **8**).[12] The compound's antiproliferation effects were ameliorated by the addition of 0.33 mM of NMN (the product of NAMPT catalysis; c.f., Fig. 1) to the cell assay, strongly implicating NAMPT inhibition as the causative mechanism. Importantly, the molecule also displayed improved aqueous solubility relative to our previously characterized NAMPT inhibitors bearing unsubstituted ureas or heteroaromatic amides (compare to compounds **5**, **6**, and **7**; Table 1).[13] Introduction of an amino substituent adjacent to the pyridine nitrogen atom present in **8** significantly worsened NAMPT inhibition and solubility properties (compound **9**). Relocation or removal of the pyridine nitrogen atom contained within **8** afforded molecules (**10** and **11**) which retained potent NAMPT biochemical inhibition properties but which were devoid of anti-NAMPT cell activity. This phenomenon likely arises from the inability of these compounds to form PRPP-derived phosphoribosylated adducts in the NAMPT active site.[14] Incorporation of larger aliphatic rings into the urea inhibitor design (compounds **12** and **13**, Table 1) was not tolerated, likely due to tight steric constraints in the NAMPT active site. Similarly, replacing the urea moiety present in **8** with the corresponding sulfonyl urea functional group led to complete loss of biological activity (compound **14**, Table 1). The above SAR studies identified the 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridine-derived urea as optimal for obtaining potent biochemical and cell-based NAMPT inhibition properties, and this functionality was therefore incorporated into all molecules described in the remainder of this work.

We next explored variation of the biaryl sulfone moiety present in compound **8**. As shown in Table 2, numerous substituted aromatic and heteroaromatic groups were tolerated at the inhibitor terminus (compounds **15**–**21**). These results paralleled those from our previous SAR studies of related NAMPT inhibitors and were consistent with this portion of the molecules residing in a solvent-exposed region in the NAMPT binding site.[9] We also explored the inclusion of various aliphatic sulfones and sulfonamides into the inhibitor design. As shown in Table 2, saturation of the terminal phenyl ring present in **8** afforded a compound (**22**) with encouraging NAMPT inhibition properties but unacceptably low stability in human liver microsomes. The poor in vitro stability of **22** could be improved by blocking metabolism on the cyclohexane ring (**23**) or by reducing compound lipophilicity through the incorporation of various polar functional groups in this region of the molecule (**24**–**31**). However, many of these modifications were also detrimental to anti-NAMPT potency in the biochemical and/or cell-based assessments. Compounds containing terminal sulfonamide moieties (**32**–**40**) were generally weaker NAMPT inhibitors than the related sulfones, but several exceptions to this trend were identified (**38**–**40**). With the exception of compound **22**, in vitro human liver microsomal stabilities for all compounds depicted in Table 2 were in the stable to moderate range. Measured aqueous solubilities were also generally favorable with many compounds displaying values >100 μM in the high-throughput assessment.[13]

In anticipation of conducting in vivo experiments with the described NAMPT inhibitors, we profiled selected examples in a variety of additional in vitro DMPK assessments. We focused these efforts on molecules which exhibited potent anti-NAMPT activity (A2780 $IC_{50} \leqslant 0.020$ μM), good in vitro stability toward human liver microsomes (HLM CL $\leqslant$ 10 mL/min/kg), and appropriate aqueous solubility (>50 μM in the high throughput assessment). As shown in Table 3, the majority of these compounds were not