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Identification of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors with no evidence of CYP3A4 time-dependent inhibition and improved aqueous solubility

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ABSTRACT

Herein we report the optimization efforts to ameliorate the potent CYP3A4 time-dependent inhibition (TDI) and low aqueous solubility exhibited by a previously identified lead compound from our NAMPT inhibitor program (**1**, GNE-617). Metabolite identification studies pinpointed the imidazopyridine moiety present in **1** as the likely source of the TDI signal, and replacement with other bicyclic systems was found to reduce or eliminate the TDI finding. A strategy of reducing the number of aromatic rings and/or lowering $c\mathrm{Log}D_{7.4}$ was then employed to significantly improve aqueous solubility. These efforts culminated in the discovery of **42**, a compound with no evidence of TDI, improved aqueous solubility, and robust efficacy in tumor xenograft studies.

Nicotinamide adenine dinucleotide (NAD) is an important biomolecule implicated in a large number of cellular processes ranging from cell metabolism and survival to calcium homeostasis.[1] NAD carries out its biological functions by at least two distinct modes of action. In one case NAD is an electron-transfer agent, where it acts as a cofactor of enzymes involved in metabolism. In the other case NAD is a substrate for enzymes such as the PARPs and sirtuins, which cleave NAD by transferring its ADP-ribose unit to acceptor molecules while liberating nicotinamide (NAM).[1b] The constant consumption of NAD by enzymes such as the PARPs and sirtuins, combined with its essential roles in multiple cellular functions, requires cells to regenerate NAD in order to survive.[2] Indeed, multiple pathways for NAD biosynthesis have been characterized,[3] including a pathway which recycles NAM back to NAD (Fig. 1). Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the first and rate-determining step of this sequence, namely the condensation of NAM and phosphoribosyl pyrophosphate (PRPP) to produce the corresponding phosphoribose adduct, nicotinamide mononucleotide (NMN).[4] A subsequent reaction then converts NMN to NAD under the catalysis of a second enzyme (nicotinate/nicotinamide mononucleotide adenyltransferase, NMNAT). Due to their high demand for NAD, rapidly proliferating cells such as those found in cancers may be particularly sensitive to mechanisms that reduce intracellular NAD concentrations.[5] Blockade of the NAM-NAD recycling pathway via NAMPT inhibition may, therefore, be an appropriate therapeutic strategy for oncology indications.

Due to the compelling biological rationale, several groups have targeted small molecule NAMPT inhibitors.[6] Indeed, previous efforts by our group led to the identification of compound **1** (GNE-617, Table 1), a highly potent inhibitor of NAMPT activity containing a bicyclic imidazopyridine group in the left-hand portion of the molecule.[7] Despite multiple favorable attributes including excellent in vivo preclinical pharmacokinetics and

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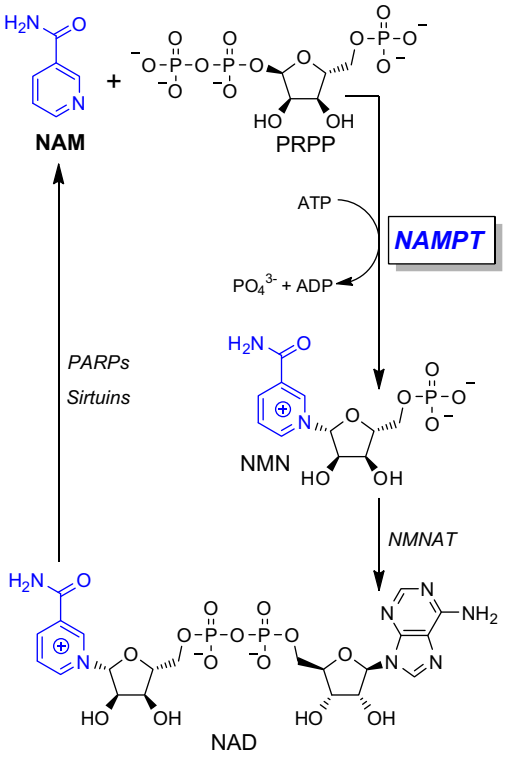


Figure 1. The NAM-NAD recycling pathway.

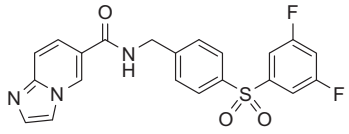
outstanding efficacy in tumor xenograft studies, **1** possessed two undesirable features we wished to address with additional optimization. Paramount among these was the potent time-dependent inhibition (TDI) of CYP3A4 caused by **1**. In addition to the potential for drug–drug interactions due to irreversible CYP3A4 inactivation, we were concerned this TDI signal was indicative of a reactive and potentially toxic metabolite. A secondary concern associated with **1** was its low aqueous solubility, a potential issue for adequate and consistent oral exposure with a solid dosage form. Notably, compound **1** possessed aqueous solubility below the lower limit of detection in both kinetic and thermodynamic solubility assay formats.

To gain insight into the potential origin of the TDI signal, we performed in vitro metabolite identification studies with compound **1**. Multiple metabolites were observed after human hepatocyte incubation, with the major products possessing molecular masses and fragmentation patterns consistent with compounds **3** and **4**. A potential mechanism for the formation of **3** and **4** from **1** is also delineated in Figure 2. A trace metabolite with a mass and fragmentation pattern consistent with **2** was observed, which could produce **3** via water opening of the epoxide. Ejection of a glyoxal moiety could then give rise to product **4**. The small abundance of **2** relative to **3** and **4** may indicate that the epoxide is highly reactive and, once formed, may be rapidly consumed. The potentially high reactivity of **2** led us to hypothesize that it could be the species responsible for the strong TDI observed with compound **1**.

With the imidazopyridine portion of **1** suspected as the source of TDI activity, we conducted TDI screening on several of our previously reported[7] bicyclic replacements (Table 2). Imidazopyrimidine **5**, also containing a bridgehead N atom in the bicycle, likewise exhibited very potent TDI, and led us to focus on bicyclic groups with all carbon bridgeheads. Azaindazole **6** offered an improvement, however, weak but measurable TDI was still observed. Giving further credence to this weak signal was the fact that multiple other analogs containing the bicyclic azaindazole system present in **6** also exhibited weak to moderate TDI signals (data not shown). Satisfyingly, transposing the positions of the 5- and 6-membered rings of the bicyclic system with respect to the remainder of the inhibitor led to attenuation of the TDI signal. Indeed, no TDI activity was detected with azabenzofuran **7**, azaindole **8**, or azabenzothiophene **9**.

As shown in Table 3, the three compounds with no evidence of TDI (**7**, **8**, **9**) all possessed similar biochemical and cell-based NAMPT potency to **1**, although aqueous solubility remained low. Thus, the focus of further SAR exploration became improving solubility within the azabenzofuran, azaindole, and azabenzothiophene sub-series while maintaining potency. Towards this goal, our general strategy was to reduce the number of aromatic rings present in the inhibitors and/or to lower $c\mathrm{Log}D_{7.4}$. Others have previously defined the sum of $c\mathrm{Log}D_{7.4}$ and number of aromatic rings as the 'solubility forecast index (SFI)' and have demonstrated that reducing this parameter correlates positively to improved solubility.[11] As will be discussed later in this disclosure, X-ray crystallography indicated that the extreme right-hand side of the inhibitors protruded into a relatively open and solvent-exposed region of the

Table 1
Selected data for previously reported NAMPT inhibitor **1** (GNE-617)



1 (GNE-617)

Entry	NAMPT IC_{50}[a] (μM)	A2780 IC_{50}[b] (μM)	CYP3A4 TDI (%AUC shift)[c]		Aqueous solubility (μM, pH 7.4)		$c\mathrm{Log}D_{7.4}$[h]
			M[d]	T[e]	Kinetic[f]	Thermo[g]	
1	0.005	0.002	57	48	<1	<2	3.1

All assay results are reported as the arithmetic mean of at least two separate runs. See Ref. 8 for experimental details.
[a] NAMPT biochemical inhibition.
[b] Antiproliferation activity determined in cell culture experiments using A2780 cell line. This inhibition can be reversed by addition of 0.33 μM of NMN, strongly implicating NAMPT inhibition as the causative MOA.
[c] Time-dependent inhibition of cytochrome P450 3A4. >15% AUC shift considered possible TDI risk. Experiments carried out as described in Ref. 9.
[d] Substrate = midazolam.
[e] Substrate = testosterone.
[f] 10 mM DMSO stock solution diluted to 200 μM with pH 7.4 aqueous buffer. Final DMSO concentration: 2% (v/v). Solubility measured after shaking for 24 h.
[g] Thermodynamic solubility. Solubility of crystalline powder after shaking for 24 h in aqueous pH 7.4 buffer.
[h] See Ref. 10 for the method used to calculate $c\mathrm{Log}D_{7.4}$.