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Discovery of potent and efficacious cyanoguanidine-containing nicotinamide phosphoribosyltransferase (Nampt) inhibitors

Xiaozhang Zheng [a,*], Timm Baumeister [a], Alexandre J. Buckmelter [a], Maureen Caligiuri [a], Karl H. Clodfelter [a], Bingsong Han [a], Yen-Ching Ho [a], Nikolai Kley [a], Jian Lin [a], Dominic J. Reynolds [a], Geeta Sharma [a], Chase C. Smith [a], Zhongguo Wang [a], Peter S. Dragovich [b], Angela Oh [b], Weiru Wang [b], Mark Zak [b], Yunli Wang [c], Po-wai Yuen [c], Kenneth W. Bair [a]

[a] Forma Therapeutics, Inc., 500 Arsenal Street, Watertown, MA 02472, USA
[b] Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[c] Pharmaron Beijing, Co. Ltd, 6 Tai He Road, BDA, Beijing 100176, PR China

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ABSTRACT

A co-crystal structure of amide-containing compound (**4**) in complex with the nicotinamide phosphoribosyltransferase (Nampt) protein and molecular modeling were utilized to design and discover a potent novel cyanoguanidine-containing inhibitor bearing a sulfone moiety (**5**, Nampt Biochemical IC_{50} = 2.5 nM, A2780 cell proliferation IC_{50} = 9.7 nM). Further SAR exploration identified several additional cyanoguanidine-containing compounds with high potency and good microsomal stability. Among these, compound **15** was selected for in vivo profiling and demonstrated good oral exposure in mice. It also exhibited excellent in vivo antitumor efficacy when dosed orally in an A2780 ovarian tumor xenograft model. The co-crystal structure of this compound in complex with the NAMPT protein was also determined.

Nicotinamide adenine dinucleotide (NAD) is an abundant and essential biomolecule used as a co-factor and/or substrate for many biological processes.[1] Mammals have multiple biosynthetic pathways to produce NAD; a de novo pathway using tryptophan and three pathways using exogenous biomolecules as the precursors—nicotinamide (NAM), nicotinic acid (NA), and nicotinamide ribose (NR).[2] NAM can be converted to NAD via a two-step pathway that uses nicotinamide phosphoribosyltransferase (Nampt) as the rate limiting enzyme (Fig. 1a). In an ATP hydrolysis coupled reaction, Nampt catalyzes the formation of NAD through the condensation of NAM with 5-phosphoribosyl-1-pyrophosphate (PRPP) to form nicotinamide mononucleotide (NMN), the immediate precursor to NAD (Fig. 1b).[3] NAD can be consumed in a manner that releases nicotinamide (NAM) by enzymes which catalyze mono- and poly-(ADP)-ribosylations, NAD dependent-protein deacetylations (Sirutins), and cyclic-ADP-ribose production. The primary mechanism of maintaining intracellular NAD levels is its synthesis from released NAM via the Nampt-dependent salvage pathway.[4] In cancers, elevated poly-ADP-ribose polymerase (PARP) activity has been well documented, which leads to an elevated rate of cellular NAD consumption. As a co-factor, NAD and its reduced form NADH are used in multiple biological redox reactions such as mitochondrial oxidative phosphorylation, glycolysis, and the citric acid cycle. These processes are used to produce ATP and assorted essential biosynthetic precursors upon whose levels cancer cells are exquisitely dependent. Additionally, NAD and NADH have essential functions in maintaining the reductive environment that protects cells from reactive oxygen species which are elevated in cancers.[5] Cancer cells that consume more NAD and are more dependent upon NAD driven processes should be extremely sensitive to inhibition of the NAD salvage pathway. Therefore, Nampt is an inviting target for potential development of novel cancer therapies.

Several classes of Nampt inhibitors have been reported in the scientific literature[6,7] and the most advanced compounds GMX-1778[8] (**1**), its prodrug GMX-1777[9] (not shown), and APO-866[10] (**2**) progressed to clinical trials during the past decade (Fig. 2). We recently reported the structure-based identification of urea- and amide-containing Nampt inhibitors, exemplified by compounds **3** and **4** (Fig. 2).[11,12] In the current work, we describe the continuation of our Nampt-related research leading to the discovery of novel and highly potent cyanoguanidine-containing Nampt inhibitors.

Our previous exploration of urea- and amide-containing Nampt inhibitors indicated that changes within the linker region of the compounds were tolerated as long as the positioning of the left hand side (LHS) aromatic ring and the right hand side (RHS) moiety remained unaffected (Fig. 3). In particular, crystallographic water

* Corresponding author.
E-mail address: xzheng@formatherapeutics.com (X. Zheng).

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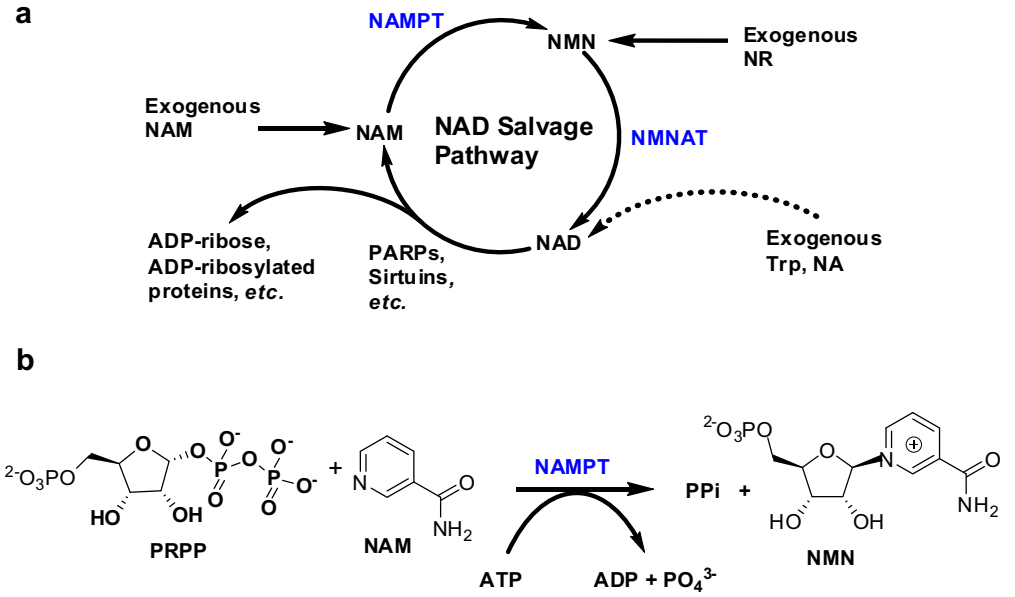

Figure 1. (a) Schematic representation of NAD biosynthetic pathways found in mammalian cells. Nampt, the rate limiting step in the biosynthesis of NAD from NAM, catalyzes the conversion of NAM to NMN. NMN is further converted to NAD by the enzyme nicotinamide mononucleotide adenylyltransferase (NMNAT). NAD can also be produced through alternative pathways using Trp (tryptophan), NA (nicotinic acid), and NR (nicotinamide ribose) as the precursors. (b) The phosphoribosyltransferase reaction catalyzed by Nampt.

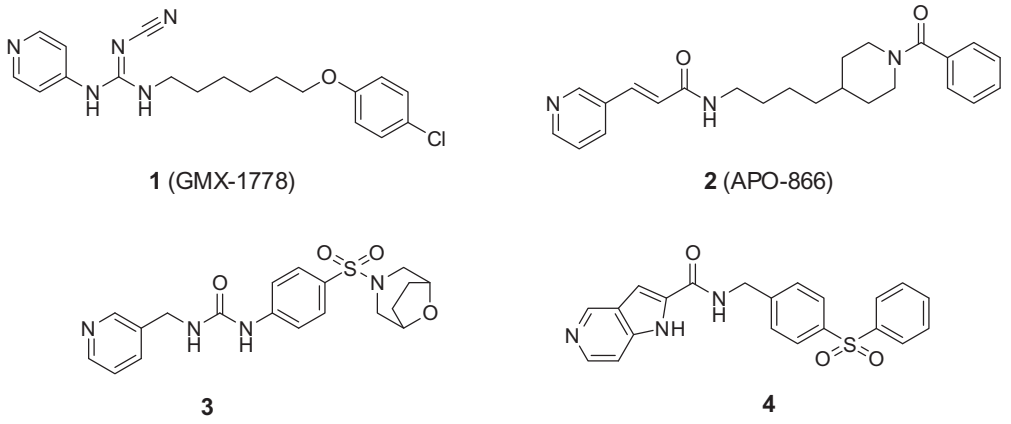

Figure 2. Examples of Nampt inhibitors.

molecules in the linker region shifted to accommodate variation in the linker hydrogen bonding network between Nampt residues Ser275 and Asp219.[11,12] To further examine the effects of different linker groups, we used molecular modeling to explore whether the cyanoguanidine linker in compound **1** could replace the amide moiety present in compound **4**, for which a co-crystal structure in complex with NAMPT was obtained (Fig. 3). The docked pose of the resulting hybrid molecule **5** overlapped well with both the pyridine and cyanoguanidine portion of docked **1** as well as with the RHS of compound **4** (Fig. 4). Compound **5** was therefore synthesized, and it demonstrated very good biochemical and cellular *anti*-Nampt potency (Table 1).[13] The molecule thus provided an excellent starting point for further inhibitor optimization.

As shown in Table 1, a compound containing a sulfonamide RHS moiety was also a potent Nampt inhibitor in biochemical and cell-based assays. Interestingly, moving the pyridine nitrogen atom from the 4-position to 3-position (compounds **7** and **8**) resulted in equal biochemical potency but also produced a several-fold loss of cellular activity (compare to **5** and **6**). The reduced cell potencies of **7** and **8** relative to **5** and **6** may be due to improper positioning of the LHS pyridine atom which prevents facile formation of the corresponding inhibitor-PRPP ribose adducts in the Nampt active site.[15] The sulfone containing compounds (**5** and **7**) displayed better mouse liver microsomal stability than the sulfonamide-containing inhibitors (**6** and **8**), and this trend was also observed with other Nampt inhibitors we previously studied.[11,12]

With this information, our research then focused on 4-pyridine-cyanoguanidine sulfones by exploring various substituents on the RHS of the molecules. Our previous work with related urea- and amide-containing compounds indicated that alterations to the biarylsulfone portion of the inhibitor could greatly improve the potency and biopharmaceutical properties.[11,12] Similar modifications were expected to be tolerated in the cyanoguanidine-containing Nampt inhibitor series since the biarylsulfone terminus was anticipated to be highly solvent-exposed. As shown in Table 2, inserting a methylene group between the sulfone and phenyl ring led to compound **9** which maintained good biochemical activity