Bioorganic & Medicinal Chemistry Letters 24 (2014) 954–962

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fragment-based design of 3-aminopyridine-derived amides as potent inhibitors of human nicotinamide phosphoribosyltransferase (NAMPT)

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ARTICLE INFO

Article history:
Received 17 October 2013
Revised 12 December 2013
Accepted 16 December 2013
Available online 21 December 2013

Keywords:
Nicotinamide phosphoribosyltransferase
NAMPT
Fragment-based design
Structure-based design
X-ray crystal structure
Surface plasmon resonance

ABSTRACT

The fragment-based identification of two novel and potent biochemical inhibitors of the nicotinamide phosphoribosyltransferase (NAMPT) enzyme is described. These compounds (**51** and **63**) incorporate an amide moiety derived from 3-aminopyridine, and are thus structurally distinct from other known anti-NAMPT agents. Each exhibits potent inhibition of NAMPT biochemical activity (IC_{50} = 19 and 15 nM, respectively) as well as robust antiproliferative properties in A2780 cell culture experiments (IC_{50} = 121 and 99 nM, respectively). However, additional biological studies indicate that only inhibitor **51** exerts its A2780 cell culture effects via a NAMPT-mediated mechanism. The crystal structures of both **51** and **63** in complex with NAMPT are also independently described.

Nicotinamide phosphoribosyltransferase (NAMPT, also known in the literature as pre-B cell colony-enhancing factor (PBEF) as well as visfatin; EC 2.4.2.12) plays a critical role in cellular metabolism.[1] The enzyme catalyzes the rate-limiting step in the conversion of nicotinamide (NAM) to the important enzyme co-factor nicotinamide adenine dinucleotide (NAD).[2] This process enables the efficient intracellular recycling of NAM, which is produced by the catalytic action of NAD-consuming enzymes such as the PARPs and Sirtuins, back into NAD (Fig. 1).[3] Proper maintenance of NAD levels is known to be critical to sustaining energetics required for many cellular functions.[4] Inhibition of NAMPT has therefore emerged as a novel strategy for impairing the proliferation of tumors whose high growth rates may make them more susceptible to NAMPT disruption relative to non-cancerous cells.[5]

Multiple examples of NAMPT inhibitors are known in the scientific and patent literature, and the most advanced of these agents [GMX-1778 (**1**)[6] and APO-866 (**2**)[7]; Fig. 2] have progressed to human clinical trials.[8,9] Our own prior discovery efforts identified potent urea and amide-derived NAMPT inhibitors which also contained terminal biaryl sulfone moieties (compounds **3**–**6**; Fig. 2).[10] In an effort to further diversify these molecules, we also conducted a surface plasmon resonance (SPR)-based screen to identify small, structurally novel NAMPT-binding moieties ('fragments') which could be combined with our existing compounds.[11] In this report, we describe the structure-based elaboration of two SPR screening hits into potent anti-NAMPT agents that are structurally distinct from other known NAMPT inhibitors.

Compound **7** was identified from our SPR-based screening methods as a moderately potent NAMPT binder with high ligand efficiency (Table 1).[11,12] The compound also demonstrated the ability to inhibit NAMPT in biochemical assessments, although its potency was somewhat attenuated relative to its binding properties (Table 1).[13] A co-crystal structure of the molecule in complex with NAMPT was subsequently determined and revealed that the molecule occupied the nicotinamide-binding region of the protein's active site (Fig. 3).[14] Not unexpectedly, the pyridine portion of **7** formed face-to-face pi-stacking interactions with the side

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http://dx.doi.org/10.1016/j.bmcl.2013.12.062

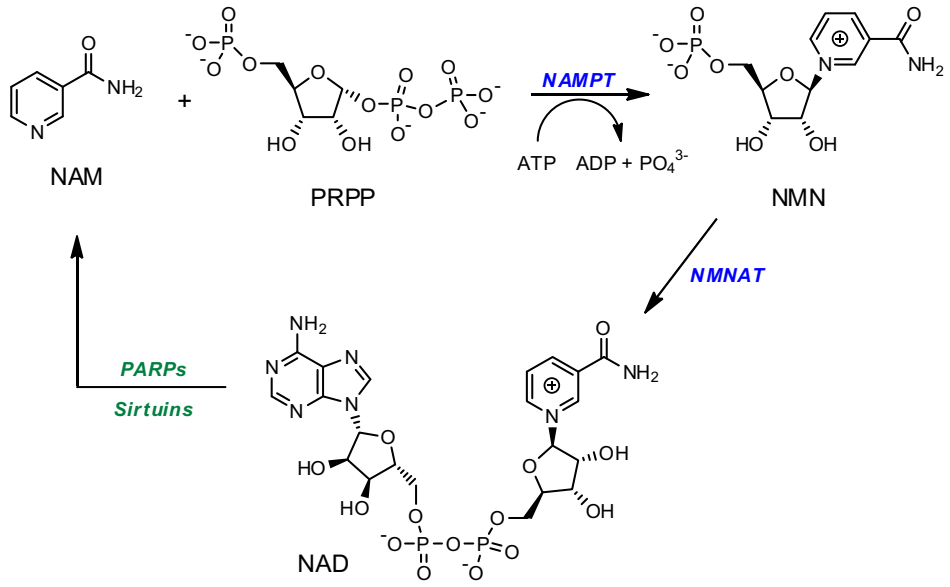

Figure 1. NAD recycling and NAMPT biochemical mechanism.

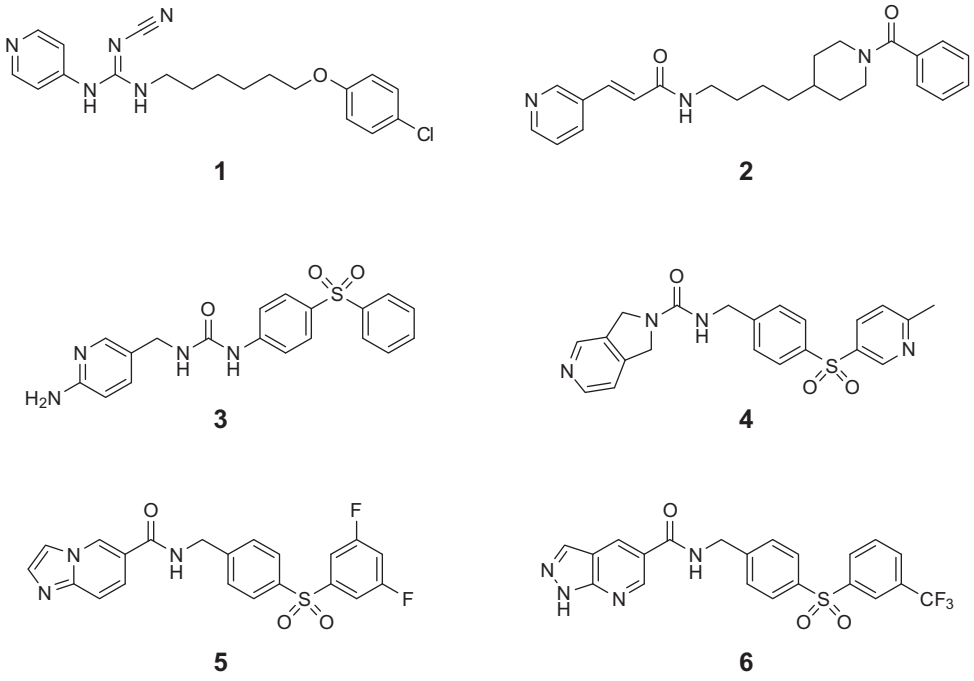

Figure 2. Examples of known NAMPT inhibitors.

chains of Phe-193 and Tyr-18′ in a manner that was similar to that observed for many other NAMPT inhibitors.[10] A hydrogen bond was also observed between the amide NH of **7** and the NAMPT Asp-219 residue. However, in contrast to the binding of previously-studied amide-containing NAMPT inhibitors such as **5**,[10c] the amide moiety of **7** was positioned much deeper in the nicotinamide-binding region of the protein (Fig. 3). This location oriented the amide carbonyl of **7** directly toward the Arg-311 side chain instead of toward a water-filled region of the active site that was typically occupied by the carbonyl group present in other known NAMPT inhibitors (e.g., **4**–**6**, Fig. 2).[10c–e] Importantly, the observed binding of **7** positioned the compound's pyridine nitrogen in a location that was similar to that observed for the N-atoms present in many other heterocycles which bound to the same NAMPT region (e.g., compare the location of the imidazopyridine moiety present in **5** with that of the pyridine contained in **7**; Fig. 3). Such positioning was consistent with **7** functioning as a NAMPT substrate and undergoing enzyme-catalyzed condensation with PRPP in the NAMPT active site, although this possibility was not examined experimentally (c.f., Fig. 1).[15]

Our first attempts to improve the inhibitory activity of **7** involved replacing the *N*-methyl-pyrazole moiety with other isosteric methyl-containing heterocycles. However, as shown in Table 1, none of these modifications afforded significant improvements in SPR-binding affinity to NAMPT or biochemical inhibitory activity (compare **7** with compounds **8**–**16**).[16] Attempts to extend various methyl-containing heterocycles into the NAMPT tunnel region occupied by the benzyl portion of **5** (c.f., Fig. 3) were also not