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Medicinal Chemistry of Nicotinamide Phosphoribosyltransferase (NAMPT) Inhibitors

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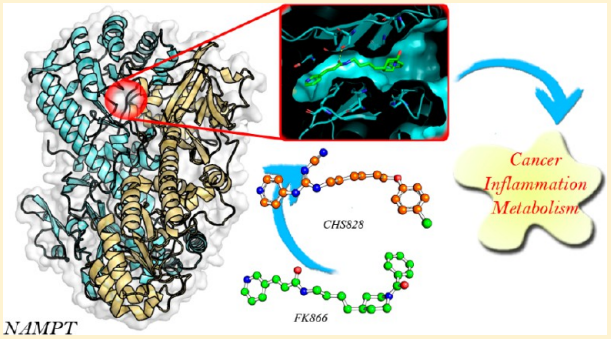
ABSTRACT: Nicotinamide phoshophoribosyltransferase (NAMPT) plays a key role in the replenishment of the NAD pool in cells. This in turn makes this enzyme an important player in bioenergetics and in the regulation of NAD-using enzymes, such as PARPs and sirtuins. Furthermore, there is now ample evidence that NAMPT is secreted and has a role as a cytokine. An important role of either the intracellular or extracellular form of NAMPT has been shown in cancer, inflammation, and metabolic diseases. The first NAMPT inhibitors (FK866 and CHS828) have already entered clinical trials, and a surge in interest in the synthesis of novel molecules has occurred. The present review summarizes the recent progress in this field.



INTRODUCTION

Otto Heinrich Warburg, over 80 years ago, hypothesized that basic metabolism was profoundly altered in cancer cells, but only recently it is becoming apparent that this could be exploited as a therapeutic strategy in cancer.[1] Among the specific targets being explored, the biosynthetic pathways leading to NAD are receiving considerable attention.[2] Indeed, in cancer cells, NAD synthesis and/or replenishment is constantly required because of the high proliferation rate, the high energy requirements, and the high activity of NAD-depleting enzymes (PARPs, sirtuins, etc.). It is interesting to note that already in the 1960s Gholson proposed that a constant cellular NAD turnover existed. Later, Rechsteiner suggested that the half-life of NAD in cultured cells is approximately 1 h.[3]

BIOSYNTHETIC PATHWAYS LEADING TO NAD IN MAMMALS

As NAD is rapidly consumed in cells, replenishing pathways must exist. While NAD is conserved through evolution as an electron acceptor/donor, it has been shown that there are differences between prokaryotes and eukaryotes on how this replenishment takes place. It is therefore not surprising that the enzymes involved in its synthesis in microorganisms have been proposed as druggable targets for the development of novel antibiotics.[4]

NAD can be synthesized from various precursors containing the pyridine moiety (nicotinic acid (NA), nicotinamide (Nam), and nicotinamide riboside (NR)) and from tryptophan. The pathways leading to NAD biosynthesis have been carefully reviewed elsewhere.[5] While lower eukaryotes and prokaryotes use nicotinic acid (a form of vitamin B3) as a major NAD precursor, mammals predominantly use nicotinamide (another form of vitamin B3) rather than nicotinic acid for NAD biosynthesis. In lower organisms, such as bacteria and yeast, Nam is converted to NA by nicotinamidase, but this activity appears to be lacking in mammals.

The de novo biosynthesis of NAD starts with the essential amino acid L-tryptophan, which is taken up from the diet through the rate-limiting enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO), both requiring molecular oxygen, and which have also been proposed to be druggable targets in humans.[6]

It is likely, nonetheless, that in humans the main source of cellular NAD is from salvage pathways, which require the uptake of precursors other than tryptophan (i.e., NA, Nam, and NR) from the diet or their reuse intracellularly after the activity of NAD-utilizing enzymes. In mammals, Nam is thought to be the main niacin-derived NAD precursor, although the enzymatic pathway that uses NA is conserved. The schematic representation of these pathways is presented in Figure 1.

Given that most NAD-utilizing reactions liberate nicotinamide, it is not surprising that mammals have elected the most direct and economical route as their major NAD source. Briefly, this pathway involves the synthesis of NMN from nicotinamide and 5-phosphoribosyl pyrophosphate (PRPP) by the enzyme NAMPT and the subsequent conversion of NMN and ATP to

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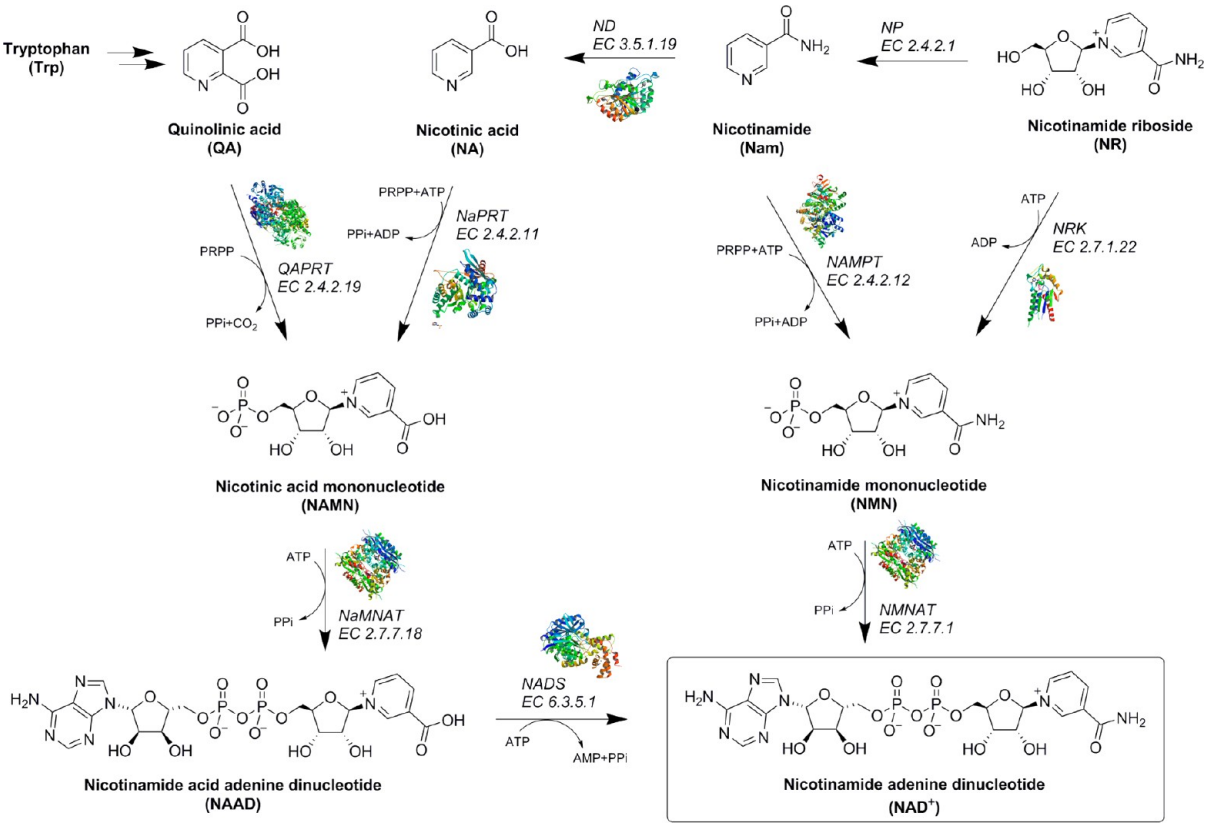

Figure 1. Schematic representation of the salvage NAD pathways. Multiple pathways allow NAD biosynthesis from the different sources (QA, NA, Nam, and NR), and these are reviewed elsewhere.[5b,c]

NAD by nicotinamide mononucleotide adenylyltransferase (NMNAT).

The present review focuses on the therapeutic potential and medicinal chemistry of NAMPT inhibitors.

NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT)

The enzymatic activity of NAMPT was originally reported by Preiss and Handler in 1957.[7] The main form of human NAMPT consists of 491 amino acids with a molecular weight of approximately 55 kDa. The X-ray crystal structure of NAMPT has also been determined and has established that NAMPT belongs to a dimeric class of type II phosphoribosyltransferases.[8] Gel filtration and co-immunoprecipitation assays have confirmed that NAMPT is indeed present as a dimer in cells. Furthermore, mutagenesis experiments have shown that NAMPT mutants that do not dimerize properly display a decreased enzymatic activity.[8,9] A number of splice variants and truncated protein forms have been detected (reviewed in ref 10), but the physiological role of these is at present unknown and requires further investigation. Likewise, in humans a number of single nucleotide polymorphisms (SNPs) have also been found, and some of these have been associated with increased risk of disease (e.g., acute respiratory distress syndrome, diabetes) or with other risk factors (high-density lipoprotein, triglycerides) (reviewed in ref 10). However, a nonsystematic approach to the link between SNPs of the NAMPT gene and disease as well as an incomplete understanding of the functional consequences of these SNPs has reduced the impact of such findings. Last, the activity of human NAMPT is profoundly affected by histidine autophosphorylation at residue His247, with the phosphorylated form displaying at least 1000-fold higher activity. In brief, activation of NAMPT by His247 phosphorylation causes stabilization of the enzyme−phosphoribosyl pyrophosphate complex, permitting efficient capture of Nam and likely representing a phosphoenzyme intermediate of the reaction.[11] To our knowledge, no other regulatory post-translational modifications have been described, but bioinformatics suggest that a number of residues conserved across species could be phosphorylated, ubiquitinated, or acetylated. Given the pleiotropic roles described below, it is likely that at least some of these post-translational modifications play a role in NAMPT function and role.

In the cell, NAMPT is abundant in the cytosol and present in the nucleus,[12] while there is controversy on whether it might also be present in the mitochondria.[12,13] To great surprise, the protein NAMPT is not exclusively intracellular, as an extracellular secreted form has been described. This protein is usually referred in the literature as visfatin, as it was initially described as being secreted by the adipose tissue, or as PBEF, as it functions also as an enhancing factor for pre-B-cell maturation.[14] In the present review, to avoid confusion, we will use the term eNAMPT and the term iNAMPT when referring to the extracellular or intracellular form, respectively. Whether the extracellular form presents specific differences in terms of truncations or post-translational modifications is at present unclear and requires urgent further clarification. Similarly, whether its extracellular effects are all linked to an enzymatic function or not is still a matter of debate. A possibility raised by the literature is that a yet unknown receptor exists that is able to bind to eNAMPT and transduce

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Table 1. Evidence That NAMPT Is Involved in Cancer[a]

condition	level of investigation	main finding	ref
Breast cancer	Tissutal protein	Associated with poor disease-free and overall survival	78
Oostmenopausal breast cancer (PBC)	Serum level	Elevated (associated with risk of PBC)	79
Gastric cancer	Tissutal protein and mRNA	Increased	80
	Serum level	Increased (positive correlation with stage progression)	81
	Tissutal protein	Increased (correlation with VEGF-A expression, negatively correlated with survival)	82
Lymphomas	Tissutal protein	Increased (highly expressed in Hodgkin's lymphoma)	83
Prostate cancer	Tissutal protein	Increased	84
Ovarian cancer	Tissutal protein	Increased	85
Colorectal cancer	Blood level	Increased (correlated with stage progression)	86
Malignant astrocytomas: anaplastic astrocytoma (AA, grade III) and glioblastoma (GBM, grade IV)	Serum level tissutal protein and mRNA	Increased (correlated with tumor grade, coexpression with p53 in GBM tissue was associated with poor survival)	87
Esophageal cancer	Serum levels, tissutal mRNA	Increased (independent factor of mortality)	88
Melanoma	Tissutal protein and mRNA	Increased (independent of BRAF mutations and Clark's levels)	54

[a]PubMed was used to retrieve the evidence using "NAMPT or PBEF or visfatin" AND "cancer" as a search string. Evidence collected through microarrays or tissues arrays, albeit present, was excluded. Evidence on the role of NAMPT in other diseases, including inflammation or metabolism, is also abundant in the literature.

at least some of its effects. For example, extracellular application of eNAMPT is able to trigger ERK and MAPK phosphorylation.[15] Last, it has been shown that a regulated positive secretory process exists,[9] although the exact mechanism of release is at present under investigation. As the protein does not present a secretory signal peptide or a caspase I cleavage site, the most accredited hypothesis, yet to be confirmed in most cell types, is that eNAMPT is secreted through a nonclassical secretory pathway, which is not blocked by inhibitors of the classical endoplasmatic reticulum (ER)−Golgi secretory pathway, such as monensin and brefeldin A.[9,16] A growing number of cell types have been shown to release eNAMPT, including adipocytes, hepatocytes, cardiomyocytes, and activated immune cells, e.g., LPS-activated monocytes.[16,17]

Physiological Roles of NAMPT. Although NAMPT's well-established role is in bioenergetics, maintaining intracellular NAD levels, over the past 10 years there has been a growing body of research suggesting that NAMPT functions are pleiotropic. However, given the limitations of the experimental approaches used (e.g., pharmacological, molecular), it is not yet possible to assign specific functions to either iNAMPT or eNAMPT or understand whether both forms work in concert.

First, it has been shown by a number of laboratories and by a variety of experimental approaches that NAMPT activity is a key regulator of NAD consuming enzymes. Among the key enzymes that use NAD as substrate are poly (ADP-ribose) polymerases (PARPs), mono (ADP-ribose) transferases (ARTs), and sirtuins. For example, considerable attention recently has been given to the ability of NAMPT to modulate sirtuin activity, as these enzymes appear to be regulated by cellular NAD levels. For instance, in human vascular smooth muscle cells, reduced NAMPT expression results in premature senescence, whereas a significant delay in senescence is observed upon overexpression of NAMPT.[18] Furthermore, two other studies have shown that NAMPT participates in the circadian clock.[19] The core clock components of the circadian machinery regulate the recruitment of SIRT1 to the NAMPT promoter to increase NAMPT expression. This is followed by NAD biosynthesis, which in turn will activate sirtuins as well as other NAD-dependent enzymes. In a negative feedback loop, SIRT1 will repress the clock components and thereby NAMPT expression.[19] It is suggested that through this mechanism, NAMPT and SIRT1[19,20] may play a crucial role in the circadian regulation of metabolism.

Second, a possibility that has not been fully explored on further functions of NAMPT is that its direct product NMN (see Figure 1) may have a role in signaling, which has indeed been suggested by some.[21] Likewise, as nicotinamide is an inhibitor of a number of NAD-utilizing enzymes, it could be envisaged that NAMPT could act as a scavenger of nicotinamide itself.

Third, growing evidence suggests that eNAMPT is a cytokine that binds to a yet unknown extracellular receptor. As such, it has been shown, for example, to potentiate the functions of stem cell factor (SCF) and interleukin-7 (IL-7) in enhancing pre-B colony formation from normal human or mouse bone marrow.[14b] Moreover, the possible role of eNAMPT as an immunomodulating mediator has been investigated. eNAMPT exerts direct proinflammatory effects on macrophages by increasing MMP expression and activity,[22] and the treatment with eNAMPT induces the production of cytokines in human PBMCs, dependent on MAPK signaling, and induces chemotaxis in monocytes.[23] Furthermore, an essential role of eNAMPT in myelopoiesis has been postulated. eNAMPT induces granulocytic differentiation of CD34+ hematopoietic progenitor cells through sirtuin activation and up-regulation of G-CSF and the G-CSF receptor.[24] Similarly, it had also been postulated that eNAMPT exerted insulin-mimetic actions by binding directly to the insulin receptor, but this report was eventually retracted.[25] Nonetheless, there is ample evidence that a cross-talk exists between eNAMPT signaling and insulin signaling pathways. It is at present unclear whether eNAMPT requires its enzymatic activity for these functions and whether enough substrate (PRPP or nicotinamide)[9,26] would be present in the extracellular space to yield either NMN or, eventually, NAD (although there is no evidence that NMNAT, the enzyme required to yield the final product, is present extracellularly).

Evidence of an Involvement of NAMPT in Disease. The first strong evidence that the bioenergetics of cancer cells were different from that of healthy nonaffected cells dates from

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Table 2. Experimental Evidence on the Therapeutic Potential of FK866 and Cancer[a]

cancer type	main finding	experimental model	ref
Multiple myeloma	Cytotoxic (autophagy), antitumor activity	Cell lines/xenograft	41, 42b
Breast cancer	Cytotoxic in combination with olaparib, increase antitumoral potential of olaparib in xenograft (reduction tumor volume)	Cell lines/xenograft	44b
Leukemia	Cytotoxic in myeloid leukemic cells (correlation with p53 function and acetylation)	Cell lines	89
	Cytotoxic alone or in combination with MNNG, Ara-C, daunorubicin, and melphalan	Cell lines	90
	Cytotoxic alone or in combination with sirtinol, cambinol, and EX527	Cell lines/human primary cells	91
	Cytotoxic (apoptosis)	Cell lines	36c, 58
	Synergism with TRAIL (apoptosis)	Cell lines/human primary cells	92
Non-small-cell lung (NSCL) cancer	Cytotoxic (correlation with EGFR mutation), antitumor activity	Cell lines/xenograft	44c
Gastric cancer	Cytotoxic alone or in combination with fluorouracil	Cell lines	80
	Suppression of gastric cell migration		
	Additive effect in combination with 1-MT	Cell lines/xenograft	44a
Neuroblastoma	Cytotoxic (autophagy), synergism with etoposide/cisplatin/chloroquine (necrosis)	Cell lines	43b
Bladder cancer	Additive effect in combination 1-MT	Cell lines/xenograft	44a
Mammary carcinoma	Cytotoxic (apoptosis)	Cell lines	93
	Enhancement of radiation sensitivity	Cell lines/allograft	
Liver carcinoma	Cytotoxic (apoptosis)	Cell lines	36c
Renal carcinoma	Antitumoral, antiangiogenic, and antimetastatic activity	Allograft (RENCA model)	94
Cervix adenocarcinoma	Cytotoxic in HeLa cells	Cell lines	12, 43b
Glioma	Cytotoxic (cell cycle arrest)	Cell lines	12, 95
	Increase MMS and MX cytotoxicity in temozolomide chemoresistant T98G glioblastoma cell line	Cell lines	96
Lymphoma	Cytotoxic in human lymphoma B-cells in combination with FX11, increased antitumor activity of FX11	Cell lines/xenograft	97
Pancreatic cancer	Cytotoxic in combination with FX11	Cell lines/xenograft	97
	Additive effect in combination with FX11 in xenograft		
Ovarian Cancer	Cytotoxic effect in combination with NA (increase the therapeutic potential in NaPRT-lacking cancers)	Cell lines/xenograft	98
	Antitumoral activity (decreased tumor volume and [^{18}F]FLT uptake)	Xenograft	44d
Colon cancer	Cytotoxic (reduction of TCA and glycolysis)	Cell lines	99
Melanoma	Not cytotoxic	Cell lines	54

[a]Literature was retrieved using the string "FK866 or APO866" and cancer. Further evidence in the literature exists with other NAMPT inhibitors, mainly **2**, and on other diseases, mainly in the inflammation field.

the beginning of past century, with Otto Warburg, a Nobel Prize winner, as a central figure in this hypothesis.[27] In brief, Otto Warburg suggested that tumor cells switch from oxidative phosphorylation to aerobic glycolysis for their energy conversion. The glycolytic pathway has a less favorable ratio of ATP produced per NAD(P) molecule reduced. Furthermore, the known importance of sirtuins and PARPs in cancer also might suggest that NAMPT, one of its upstream regulators via production of NAD, might be involved.[5b] Last, it has been amply shown that NAD turnover in cancer or proliferating cells is significantly increased over healthy or nonproliferating cells. These original, maybe speculative, observations on the possible involvement of NAMPT in cancer have now been supported by various approaches in cancer cells or patients. Many of these observations are summarized in Table 1, while the strongest evidence of all, relying on pharmacological tools, is described at the beginning of the medicinal chemistry section and in Table 2. In brief, in most cancer tissues evaluated, the mRNA and/or protein levels of NAMPT have been found to be elevated. Furthermore, when serum or blood levels of eNAMPT were investigated, these were also found to be increased. Interestingly, in a number of tumors a positive correlation between either tissue or circulating levels and stage progression has been reported (see Table 1). Last, in a recent study an elevated level of circulating eNAMPT has been correlated with an increased risk of developing postmenopausal breast cancer.[28]

One of the initial fields of research that focused on circulating NAMPT was human metabolism, given the observation that eNAMPT was secreted by adipocytes[16] and the availability of commercial kits to detect eNAMPT levels. A vast number of studies have been published, most of them showing an increase of eNAMPT in patients presenting metabolic or endocrine disorders. Most studies have focused on diabetes or obesity,[29] but other conditions, including cardiovascular and endocrine[30] have been investigated. Yet contradicting data exist regarding eNAMPT and metabolic diseases, mainly regarding the plasma levels that are reached in diseases, with at times a 100-fold disparity between reports. Furthermore, in some fields, for example, obesity, contradicting results are present on whether eNAMPT increases or decreases. A possible explanation for this is that patients' characteristics are not superimposable in the different studies. A second possibility, as mentioned below, is that eNAMPT is increased in inflammatory states, and in the field of metabolism this may be a strong confounding factor. Third, it has been shown recently that eNAMPT follows a circadian rhythm, with high levels in the morning and low levels at night.[31] As this was not known previously, it might have been an uncontrolled bias in many reports. Last, it has been shown

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that the different kits commercially available (RIA, EIA, ELISA) do not yield superimposable results, and this is likely to contribute to the discrepancy.[32] While it is possible that post-translational modifications occur to allow for secretion of eNAMPT, whether the discrepancy between kits is due to the fact that they have been based on recombinant NAMPT remains to be ascertained.

As mentioned previously, there is growing evidence that iNAMPT and eNAMPT are involved in modulating the immune system. It is therefore not surprising that circulating eNAMPT has been shown increased in numerous inflammatory conditions, including rheumatoid arthritis, lupus, inflammatory bowel disease, sepsis, and infection.[33] Furthermore, NAMPT mRNA and/or protein levels have been shown to be also increased in inflammatory cells such as polymorphonucleate cells (PMNC) and neutrophils.[34] More convincing evidence regarding the involvement of NAMPT in inflammatory diseases has come by the use of NAMPT inhibitors, as highlighted below.

While cancer, metabolism, and inflammatory diseases have been the most investigated, other diseases that not necessarily fall in these conditions have also been studied and may be found in the literature.

MEDICINAL CHEMISTRY OF NAMPT INHIBITORS

The first attempt to identify NAMPT inhibitors dates back to 1972, when a series of nicotinamide analogues were evaluated. While these studies suffered from the limitation of not having the purified enzyme or a detailed understanding of the enzymatic mechanism, this paper did suggest that nicotinamide analogues could act as inhibitors of the enzyme reaction, albeit at high concentrations, an observation that was capitalized on in later years.[35]

FK866 and CHS828. The turning point in the field was the description of the first nanomolar inhibitor of NAMPT in 2002, originally termed FK866 (**1**, (*E*)-*N*-(4-(1-benzoylpiperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide, Figure 2; also known as WK175 or APO866).[36]

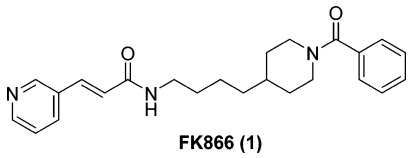

Figure 2. Structure of FK866.

In the original manuscripts,[36b,c] it was shown that FK866, via NAD and subsequent ATP depletion, led to apoptosis. The hallmarks of apoptosis, such as cytochrome *c* release, caspase activation, and mitochondrial depolarization, could all be attributed to NAMPT inhibition and the subsequent decrease in cellular NAD(P) levels. Cell death is delayed and takes place 24−48 h after treatment. Furthermore, FK866 does not have an effect on NaPRT.[36c] In the original report, the mechanism of inhibition was reported to be noncompetitive, although subsequent reports suggested that FK866 and nicotinamide should compete for the same site.[37] FK866 displays an incredibly higher affinity (K_i = 0.3 nM) for the site compared to nicotinamide (K_m = 2 μM), and this may explain the discrepancy.[37] In the same article it was shown that cells that possess an intact and active salvage pathway that can use nicotinic acid are less sensitive to this treatment.

Interestingly, in the same paper it was also emphasized that nicotinamide or nicotinic acid can be viewed as possible antidotes, as they are able to revert the growth inhibiting properties of FK866 (for nicotinic acid, only in those cells that express NaPRT; Figure 1). This finding has also been replicated with other compounds, namely, CHS828 (**2**) (see below).[38]

In a subsequent paper,[37] the X-ray crystal structure of human NAMPT in the presence of FK866 was solved (PDB code 2GVJ). The X-ray structure reveals that NAMPT is a homodimer with a head to tail interaction and a contact surface area of each monomer of ∼4200 Å (Figure 3). There

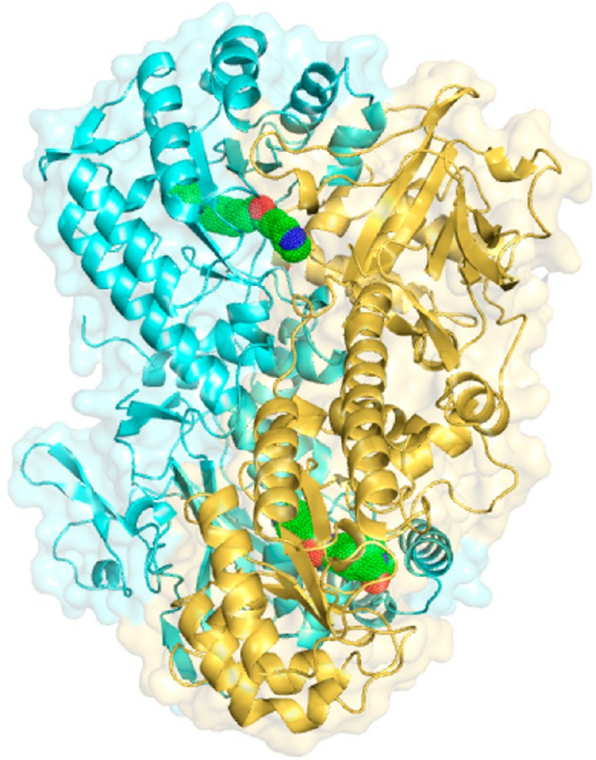

Figure 3. Structure of human NAMPT in complex with FK866 (PDB code 2GVJ). One monomer is shown in blue, the other in gold. Carbon atoms of FK866 are shown in green sphere.

are two binding sites for nicotinamide formed by the union of the two monomers. The structure revealed a long and narrow tunnel (15 Å × 6 Å) at the interface between subunits and in communication with the NMN interaction pocket. The present review will concentrate primarily on the discussion of FK866 interactions, as reviews concentrating on the structural features on NAMPT have been recently published.[39]

The crystal structure of NAMPT bound to FK866 (PDB code 2GVJ) shows that the drug binds in the narrow tunnel at the interface between subunits. Two molecules of FK866 can bind to a dimer, and yet whether both are required to abolish activity has never been investigated. The pyridine of FK866 is sandwiched between the side chains of Phe193 of one monomer and the Tyr18′ (enumeration of amino acids is based on human NAMPT) of the other, occupying the same position where the pyridine of the nicotinamide is located. The main binding interaction is a $\pi-\pi$ offset stacking. The oxygen atom of the amide behaves as a hydrogen bond acceptor interacting with the hydroxyl group of the Ser275 side chain, while the nitrogen atom of the amide is involved in a hydrogen

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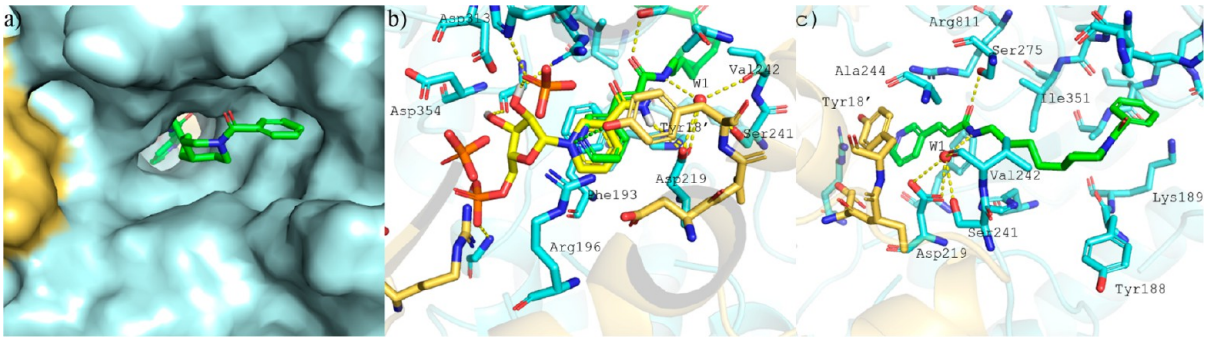

Figure 4. Different details of X-ray crystal structure of human NAMPT in complex with FK866 (PDB code 2GVJ). One monomer is shown in blue, the other in gold. Carbon atoms of FK866 are shown in green sticks. NMN (adapted from PDB code 2GVG) is in yellow sticks, and the retained molecule of water is a red sphere. Hydrogen bond interactions are plotted as yellow dotted lines. (a) Detail of the FK866 tail group binding pocket. (b) Detail of the overlapping of FK866 and NMN binding sites. (c) Binding mode of FK866.

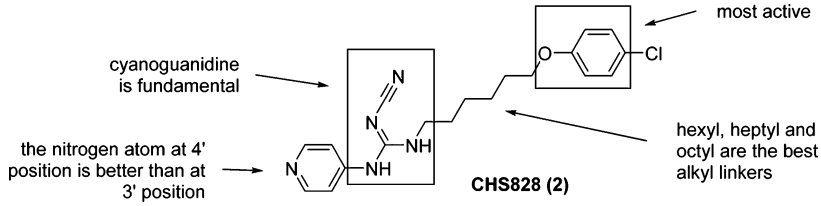

Figure 5. SAR studies on **2**.

bond with a retained molecule of water (W1). The retained molecule of water, stabilized by a network of hydrogen bonds between Asp219, Ser241, and Val242, is conserved among all of the human and rat crystal structures (12 out 17 X-ray crystal structures of NAMPT have this conserved molecule of water).

The tail group plays an important role because of its ability to anchor the molecule because of the interaction of the phenyl group with a hydrophobic cleft formed by Ile309, Pro307, Val350, Ile378, and Ala379. In this case the benzoylpiperidine group is within van der Waals interaction distance of about 10 residues (Tyr188, Lys189, Ala379, Ile378, Asn377, Glu376, Val350, Arg349, Pro307). Although a series of van der Waals interactions between the alkyl chain and the hydrophobic residue of the amino acids of the tunnel can be observed, it is unlikely that the interaction of the alkyl chain with the protein adds much to the affinity and instead serves primarily as a linker between the pyridine cap group and the benzoylpiperidine tail group (Figure 4).

Unfortunately, no manuscript ever appeared on the chemical optimization of this compound and the information that can be gathered by the patent literature is insufficient to yield an exhaustive structure−activity relationship. Nonetheless, this compound has been fundamental in the understanding of the therapeutic potential of NAMPT inhibitors in both cancer and inflammation. While other compounds, namely, **2**, have also participated in the process of elucidating the potential therapeutic role of this class of molecules, FK866 has probably played a more prominent role because it represented a first-in-class and it has been available from commercial and noncommercial sources (i.e., National Institutes of Health) for a number of years. **2** is now also commercially available.

Indeed, a number of authors have shown a strong cytotoxic effect in vitro of FK866 in tumor cell lines (Table 2). Most reported cell types are responsive to this chemotherapic agent. While some authors suggested that NAMPT expression inversely correlates with inhibitor sensitivity,[40] others have not reproduced such finding.[41] While it is beyond the scope of this manuscript to review these data in detail, a number of common themes should be highlighted: (i) FK866 displays low nanomolar potency in both cytotoxicity and enzyme inhibition assays (<10 nM); (ii) while the original report suggested that this agent acted primarily through the induction of apoptosis, autophagy most likely also plays a major role in many cell types;[42] (iii) NAMPT inhibition leads to NAD depletion in a time-dependent fashion, and this in turn brings about the modulation of a number of pathways that act in concert as the executers of cytotoxicity. For example, NAD depletion leads to mitochondrial dysfunction, ATP depletion, and a decreased activity of sirtuins and PARPs,[36c,43] and it is extremely difficult to dissect a single pathway solely responsible for the effects observed. (iv) NAMPT inhibitors, when tested in appropriate models and protocols, strongly synergize with other chemotherapeutic agents or with autophagy inhibitors (see Table 2).

1 has also been used in animal models, and again, it has been shown to be effective in reducing tumor growth, tumor volume, and/or metastatic processes (Table 2). Doses that reduce the tumor burden in rodent models range from 10 to 20 mg/kg given ip.[41,42b,44]

Last, FK866 has also been an invaluable tool to bring about the hypothesis that NAMPT inhibitors may have an important therapeutic role in inflammatory diseases. Indeed, it has been shown to have a striking protective role, among others, in animal models of autoimmune encephalitis, rheumatoid arthritis, sepsis, and spinal cord injury.[43a,45] In one of the key papers that demonstrated the effect of these inhibitors in animal models of rheumatoid arthritis, the magnitude of the effect of FK866 was superimposable to that of etanercept.[33a] In this instance, a significant reduction of cytokines, a reduced activation of the immune system, and a reduced SIRT activity appear to be among the mediators of this effect. Indeed, it would not be surprising if the industrial interest in these

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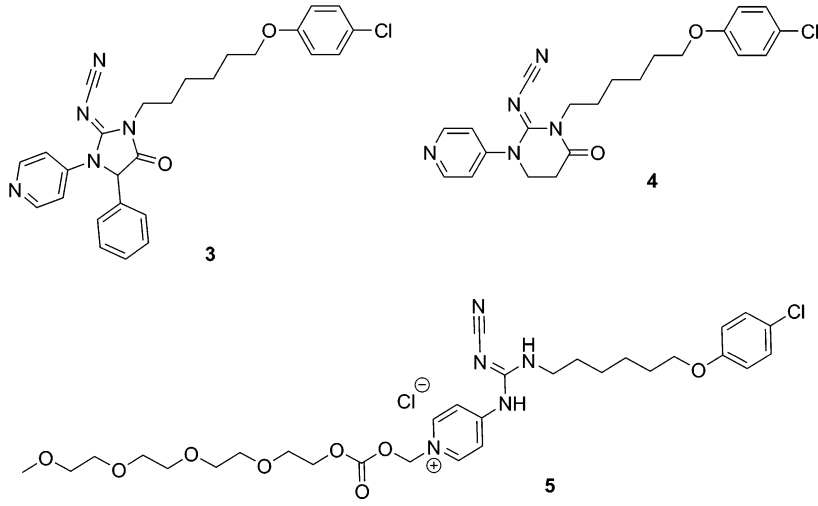

Figure 6. Constrained analogues and water-soluble prodrug of **2**.

molecules will now concentrate on inflammatory or trauma-related diseases.

Another important pillar in the elevation of NAMPT inhibitors to potential therapeutic agents was the characterization of **2** ((*E*)-1-(6-(4-chlorophenoxy)hexyl)-2-cyano-3-(pyridin-4-yl)guanidine, also known as GMX1778), a pyridinylcyanoguanidine. This compound was disclosed by Leo Pharma in 1997, but its mechanism of action was unknown at the time.[46] This molecule can be considered a close analogue of the antihypertensive potassium channel opener pinacidil (*N*-cyano-*N*′-pyridin-4-yl-*N*″-(1,2,2-trimethylpropyl)guanidine), which is not a NAMPT inhibitor. Its potent cytotoxic profile[47] spurred research toward the identification of the mechanism of action, and after almost 10 years of pharmacological efforts, it was demonstrated that NAMPT was the main biological target for the cytotoxic activity of **2** and that the compound is a competitive inhibitor.[38,40]

SAR studies highlighted the importance of the pyridine ring in **2** (Figure 5).[46] In this instance, when the pyridine is substituted in position 4 instead of 3, there is an increase in potency of 10- to 100-fold. Furthermore, substitution of a pyridine with a phenyl ring yields inactive compounds. The length of the alkyl chain is also important for improving the activity. In particular hexyl, heptyl, and octyl are the best linkers while the shorter chain linkers give compounds that display a lower activity. Finally a bulky tail group that protrudes toward the solvent exposed surface is important for determining the potency, with a phenoxy group displaying the best potency.[48] It is interesting that no X-rays or NMR conformational studies on **2** have been reported in order to unveil which conformer of cyanoguanidine prevails in solution and which is the preferred conformation at the binding site.

Interestingly, it has also been suggested that **2** is a substrate for NAMPT and it is phosphoribosylated while binding and inhibiting NAMPT.[40] The phosphoribosylated **2** does not lose its potency of inhibition but, given the positive formal charge, remains trapped in the cell, thereby increasing its concentration at the site of action. Whether other molecules, including FK866, can also be enzyme substrates remains to be ascertained.

Further modifications of **2** have been described (Figure 6).[49] As various modifications of the side chain and the pyridine ring did not lead to more potent compounds compared to **2**, these authors designed and synthesized a series of constrained analogues on the cyanoguanidine core (**3** and **4**). Although some analogues were cytotoxic, they displayed a different spectrum of activity compared to **2** and no proof of their ability to inhibit NAMPT has been reported to date.

A water-soluble prodrug of **2** has also been prepared (**5**, (*E*)-4-(3-(6-(4-chlorophenoxy)hexyl)-2-cyanoguanidino)-1-(3-oxo-2,4,7,10,13,16-hexaoxaheptadecyl)pyridin-1-ium, named GMX1777, EB1627, or teglarinad). This was made in order to prepare a drug that could be administered intravenously. **5** is a pyridinium salt containing an esterase-cleavable carbonate unit and a solubilizing PEG linker. This compound is freely soluble in water up to 97 mg/mL.[50] It is important to stress that the easy to make hydrochloride, hydrobromide, and mesylate salts did not provide compounds with sufficient water solubility.[51]

Cellular chemoresistance to NAMPT inhibitors in in vitro models has been documented. The most relevant studies have been performed using **2**. Continuous exposure of increasing concentrations of NAMPT inhibitors to cell lines has resulted in mutations of the enzyme. In particular, the H191R mutation was detected in two different cell lines; this mutation results in abrogation of inhibitor binding, as suggested by computer modeling. Furthermore, the H191R mutation reduces NAMPT activity by 50%. Moreover, the deletion of Asp93 has been found to be located in cells resistant to both **2** and FK866.[52] In another report, a G217R mutation has been reported in a **2** resistant cell line, once again reducing the binding of **2** to the active site of NAMPT. Contrary to the H191R mutation, NAMPT bearing the G217R mutation maintains enzymatic activity.[40] All these mutations have been found located in either the binding site or dimer interface, and it has been suggested that these mutations may directly or indirectly adjust the shape of the binding site, resulting in an accessible site for Nam or NMN but not for large NAMPT inhibitors. Last, it has been shown that nicotinic acid can protect cells that express NaPRT,[40] and therefore, it could be postulated that up-regulation of NaPRT could be a mechanism of resistance, although we are not aware of data in this direction.

Both FK866 and **2** have entered clinical trials (www.clinicaltrials.gov). While no data are available regarding preclinical toxicology and very little are available regarding preclinical pharmacology, it must be assumed that the results

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were of sufficient reassurance to regulators. Reported phase I/II clinical trials on FK866 used a continuous 96 h infusion every 28 days. Reported phase I/II trials on **2** used an oral administration once or five times a week every 3 or 4 weeks, and a reported phase I trial on **5** used 24 h infusions every 3 weeks.[53] Phase I clinical trials were conducted in patients with either solid tumors or lymphomas.[53] These trials overall suggest that trombocytopoenia and gastrointestinal toxicity (the latter in particular for the oral formulation) are the most severe dose-limiting side effects of this class of drugs. Other toxicities possibly linked to NAMPT inhibitor therapy were anemia, hypoalbuminemia, hyperglycemia, and electrolyte dysfunction.[53] While it might not be expected to obtain significant clinical effects in phase I, given that patients are usually severely compromised, no significant patient responses have been observed with either of the three drugs.[53] Phase II clinical trials have been initiated for FK866 and **5** in the following settings: T-cell lymphoma, refractory chronic lymphocytic leukemia, and advanced melanoma (www.clinicaltrials.gov). Neither the outcome of these nor the preclinical evidence that has led to the development of NAMPT inhibitors in these directions has been disclosed. Indeed, it may well be that these settings were not ideal. For example, it has been recently shown that melanoma cell lines do not respond to FK866.[54] It is therefore likely that these trials were initiated before a more thorough understanding of preclinical models was established, and it is therefore possible that novel trials can capitalize on this knowledge. In particular, for phase II trials, there is now increasing evidence that these drugs should be given in combination with other regimens. To our knowledge, no trial has focused on the inflammatory properties of these drugs. Last, the pharmacokinetics of FK866 in humans appears not necessarily ideal, with a very short half-life and a very fast clearance of the drug in urine in an unmodified form or as the *N*-oxide form.[55] The half-life of **2** appears longer (3−8 h),[56] but it remains to be ascertained whether the dosing schedule is appropriate. As most molecules synthesized in this area of research bear the pyridine ring, the *N*-oxide route of metabolism might represent a common theme and should be evaluated early in the screening process. Yet to our knowledge a thorough description of the pharmacokinetic parameters of these two front-runners (e.g., plasma protein binding, routes of metabolism) has not been published.

Newer NAMPT Inhibitors. A number of academic and industrial groups have explored the possibility of developing novel NAMPT inhibitors. Indeed, industry has been particularly active on this front, with dozens of patents disclosed over the past years. While the rationale for development may not have been necessarily based on FK866 or **2**, and most of the molecules described below have nonobvious novelty features, for simplicity reasons the molecules will be described according to whether the most relevant information, compared to FK866 or **2**, could be derived from (a) replacement or chemical modification of the pyridine ring, (b) replacement of the vinilogous amide group, (c) replacement of the tail group (Figure 7). Most molecules described in the literature present more than one modification, and we have described the articles according to where we felt the most interesting modification was applied from a scientific perspective.

It is interesting that when the compounds we describe below have been tested in both enzymatic assays and cellular assays, the IC_{50} and EC_{50} values have been, in most instances, of the same order of magnitude. Therefore, while off-target effects

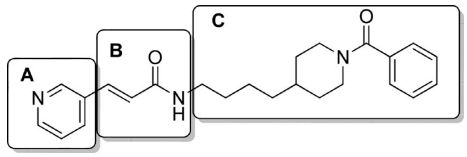

Figure 7. Schematic division of FK866 in three regions of interest for the medicinal chemist: (a) pyridine group; (b) vinylogous amide group; (c) tail group.

cannot be ruled out, it is likely that most compounds synthesized so far are rather specific.

(a) Replacement or Chemical Modification of the Pyridine Group. The rapid clearance of FK866, probably due to the formation of the highly soluble *N*-oxide metabolite, has spurred scientists to try to replace the pyridine ring with other hetereocyclic rings. Yet there is no published report that presents data conclusively assessing that the nitrogen atom of the pyridine presents a very high metabolic liability, and therefore, this hypothesis must still be confirmed.

As the substitution of the pyridine with a phenyl ring leads to inactive compounds,[46] attempts to replace the pyridine ring with other heterocyclic rings containing at least one nitrogen atom (indole, pyrimidine, 1-*H* pyrazole, pyrrole) or a nitrobenzene and 2- and 3-substituted anilines, alone or with a diol group as a ribose mimetic, gave inactive compounds except for the pyrrole derivative (**6**, Figure 8).[57] This compound showed similar cytotoxic effects compared to FK866 in the breast cancer MCF7 cell line (1.29 μM vs 0.68 μM) but was significantly less active in other cancer cell lines where FK866 showed nanomolar potency. Furthermore, the authors stated that their compound had an activity of 1.4 μM on human leukemia cells (K562) while FK866 was inactive at concentrations up to 20 μM. However, there are conflicting reports in the literature, as in another paper[58] it was demonstrated that FK866 had an IC_{50} of 7.2 nM (96 h) on the same cancer cell line. Furthermore, the nanomolar potency of FK866 has been demonstrated by most laboratories working in the field. However, a number of laboratories that rely on commercial sources of FK866 have manifested difficulty in obtaining reliable products (personal communications to A.A.G.). Last, given the clonal nature of cell lines, it is possible that nominally identical cell lines around the world behave differently.

Although not yet registered in the PDB database, the crystal structure of the pyrrole analogue with NAMPT has been described (Figure 9a),[57] and the PDB data were made available to us from the authors (personal communication to A.M.). The compound shares a similar binding pose compared to FK866. The nitrogen atom of the pyrrole is still sandwiched between Phe193 and Tyr18′. The authors suggest that Asp219 could contribute with additional ionic interactions with the pyrrole nitrogen atom. Nonetheless, this interaction would appear unlikely given the impossibility of the pyrrole to be protonated at physiological pH.

Previously, the same authors prepared an analogue (**7**) with a ribose ring attached at the nitrogen atom of the pyridine of FK866 (named ISO001) with the aim to exploit the same hydrogen bond interactions of the ribose of NMN.[59] This analogue was crystallized with NAMPT and showed the same orientation and binding mode of FK866 (Figure 9b,c). Notwithstanding that, the binding affinity was greatly decreased (K_d of 45 μM vs FK866 nanomolar for **1**). A possible

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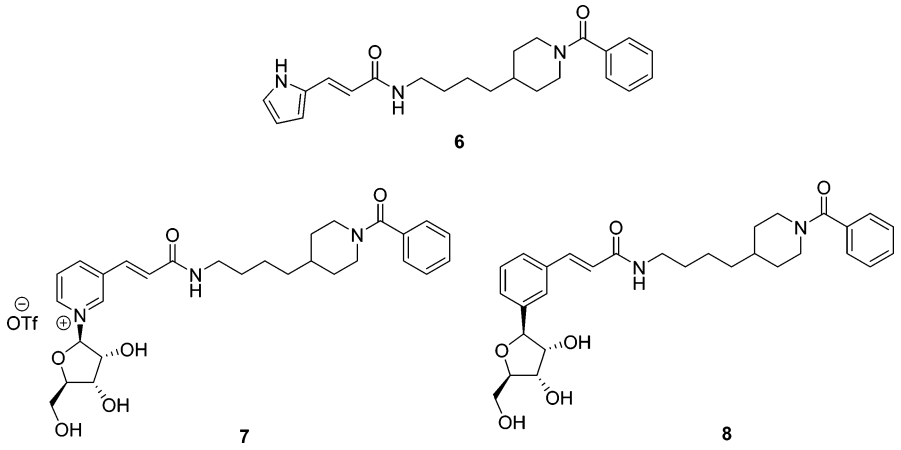

Figure 8. NAMPT inhibitors: replacement or chemical modification of the pyridine ring (I).

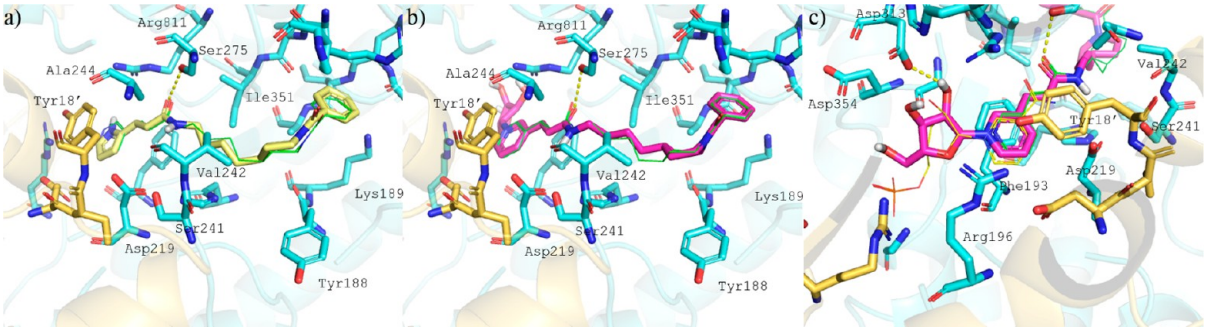

Figure 9. Details of other crystal structures of NAMPT in complex with **6** or **8**. FK866 is shown in green, and NMN is shown in yellow. (a) Binding mode of **6** (PDB code not available; obtained from the authors). Carbon atoms of **6** are shown in light yellow. (b) Binding mode of **8** (PDB code 3G8E). Carbon atoms of **8** are shown in purple. (c) Overlay of **8** and NMN in the NMN binding site.

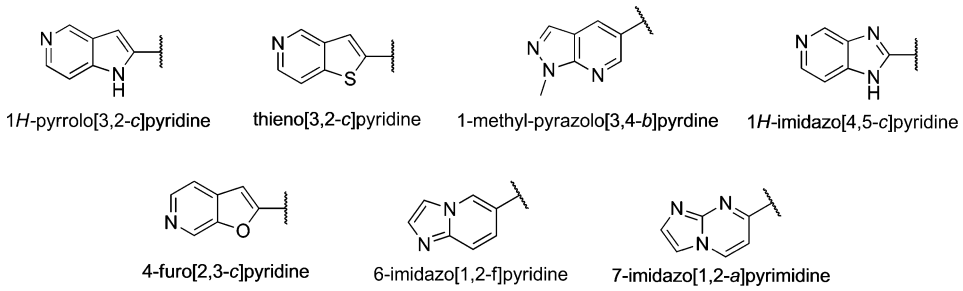

Figure 10. Synoptic scheme of the heterocycles used as cap groups replacing the pyridine.

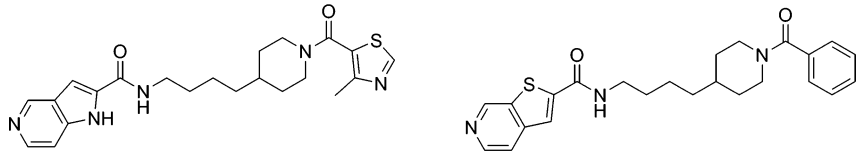

Figure 11. NAMPT inhibitors: replacement or chemical modification of the pyridine ring (II).

explanation for this loss of activity may be the difficulty of the charged ISO001 to pass through the hydrophobic tunnel. Recently, a *C*-iminofuranoside analogue of FK866 has also been prepared (**8**). No pharmacological activity has been reported yet for this compound, but it is likely that the lack of the pyridine ring makes this compound inactive or poorly active.[60]

In two recent patents[61] the pyridine moiety has been replaced with heterocycles in which the nitrogen atom of the pyridine is still present. From a medicinal chemistry point of view these compounds can be considered rigid analogues of the 3-vinylpyridine moiety. In particular, the heterocycles reported in Figure 10 have been used. Although the company did not

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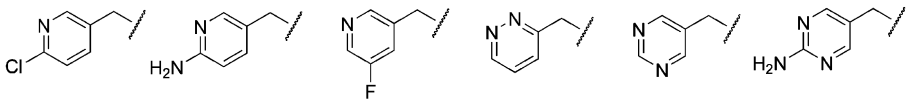

Figure 12. NAMPT inhibitors: replacement or chemical modification of the pyridine ring (III).

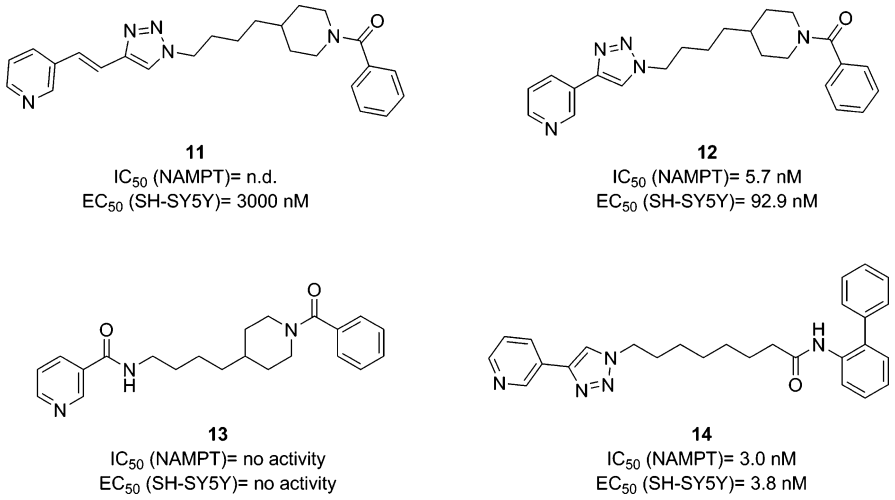

Figure 13. NAMPT inhibitors: replacement of the amide group.

disclose their lead candidates yet, we report two of the most active compounds (**9** and **10**) as NAMPT inhibitors illustrated in the patent (Figure 11).

Recently, a series of substituted pyridines, as well as pyridazine and pyrimidine analogues, have been shown to retain anti-NAMPT activity (Figure 12).[62] Surprisingly, some of them, while potent enzyme inhibitors, fail to elicit cytotoxic effects. A similar effect has been observed by the same authors in a different series of compounds.[63] At present it is unclear which factors may contribute to this phenomenon, although the authors make different hypotheses (cell permeability, protein binding, ability to form the phosphoribosylated adduct, ability to compete with nicotinamide).

An important conclusion is that so far all molecules able to interact with NAMPT at nanomolar levels have to contain a meta or para substituted pyridine, a heterocycle containing a pyridine moiety, a pyridazine, or a pyrimidine. Aromaticity is important to give π offset stacking interactions with Tyr18′ and Phe193, while the role of the nitrogen atom is still puzzling. It is improbable that it forms a hydrogen bond with the phenyl group of Phe193, as the two moieties are on different planes.[64] On the other hand, it could contribute to fine-tune the offset π stacking interaction and to mimic nicotinamide to give the drug−phosphoriboside adduct that stabilizes the enzyme−inhibitor complex.

(b) Replacement of the Vinylogous Amide Group. Recently, a novel inhibitor of NAMPT, exploiting the concept of click chemistry, has been reported (Figure 13).[65] The rationale for the identification of this novel inhibitor was that the replacement of the amide bond of FK866 with a triazole might have yielded a nonclassic bioisosteric substitution.[66] A previous isosteric replacement of the amide of FK866 with a sulfonamide was attempted, but the compound was totally inactive.[65] When the 1,4-disubstituted triazole (**11**) replaced the vinylogous amide FK866, the compound was shown to be cytotoxic (in a neuroblastoma cancer cell line) with an IC_{50} of 3.0 μM, i.e., 3000 times less active than FK866. On the other hand, the molecule (**12**) with a pyridine ring directly attached to the triazole was shown to be active at 90 nM. The activity of these compounds on cytotoxicity was superimposable to their activity on NAD levels and on NAMPT activity assays, supporting a correlation between these three phenomena.[67] It is interesting that compounds with the amide directly attached to the pyridine ring (e.g., **13**) turned out to be inactive.

These results strengthen the idea that a hydrogen bond acceptor should be at a distance of roughly three carbon atoms from the pyridine. The identification of a compound with nanomolar potency suggested to the authors that the pyridinyltriazole moiety was tolerated in the binding site of NAMPT, maintaining key bonds for the interactions, and a follow-up campaign was immediately started.[65] A total of 181 different pyridinyltriazoles were synthesized by click chemistry, resulting in the identification of **14** as a potent nanomolar NAMPT inhibitor. SAR studies were also performed. Again, the replacement of the pyridine ring with a phenyl or other heterocycles yielded inactive compounds. It is important to stress that, also in this case, the length of the alkyl linker chain was fundamental to allow for a perfect accommodation of both the cap and the tail group. In this work with $n = 6$ the IC_{50} was 56 nM, while with $n = 7$ and $n = 8$ the IC_{50} values were 3.8 and 14.6 nM, respectively. The tail group displayed a dramatic shift in the activity, stressing its fundamental role for imparting biological activity with the phenyl analogue being poorly active and the 2 substituted 1,1′-biphenyl group very potent.

Very recently, putative NAMPT inhibitors containing a pyridinylurea or thiourea were synthesized and were shown to have a strong cytotoxic activity against the A2780 ovarian carcinoma cell line exemplified by compound **15** with an IC_{50} of 0.89 nM. A schematic SAR is provided in Figure 14.

In another patent, rigid analogues of the pyridinylurea have been shown to be NAMPT and/or ROCK inhibitors.[68] Because of the huge number of active products reported, we show a general formula (**16**) of these compounds (Figure 15). In another patent a 3,4-diaminocyclobut-3-ene-1,2-dione was used

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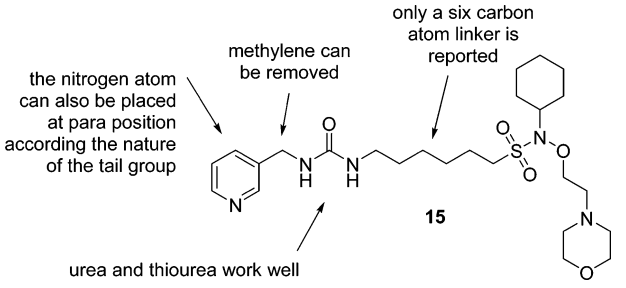

Figure 14. SAR of putative NAMPT inhibitors (I).

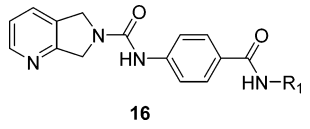

Figure 15. General formula for NAMPT and ROCK inhibitors.

as a hydrogen bond acceptor unit (Figure 16).[69] Compound **17** had an IC_{50} for cytotoxicity of 30 pM on the A2780 ovarian

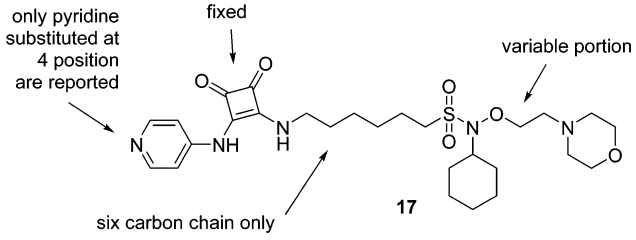

Figure 16. SAR of putative NAMPT inhibitors (II).

carcinoma cell line. Compound **17** is only an example of a selection of potent compounds described in this patent. For this class of molecules, a six-carbon linker appears to be important for the activity, while the tail group can be modified. Even in this case all the compounds must be considered putative inhibitors of NAMPT, as no direct proof of their mechanism of action was directly reported in the patent. It is likewise difficult to extrapolate a SAR from the patent.

The connecting units between the pyridine and the alkyl chain that have been demonstrated to be accepted by the enzyme are represented in Figure 17. In summary, a hydrogen bond donor or acceptor motif appears to be essential for activity. Furthermore, depending on the groups introduced in this region, the optimal chain length may differ. This might be an area in which the medicinal chemist may explore further structures.

(c) Replacement of the Tail Group. A recent patent disclosed analogues of FK866 with different tail groups instead of the benzoylpiperidine moiety. A series of nanomolar cytotoxic compounds active on the A2789 ovarian carcinoma cell line were presented (Figure 18). Compound **18** showed a

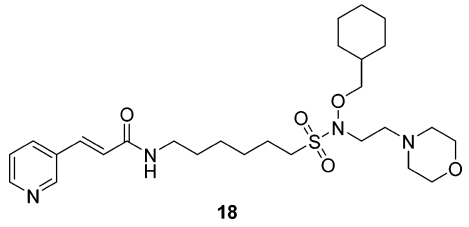

Figure 18. NAMPT inhibitors: tail group modification (I).

cytotoxic activity of 0.16 nM. Once again, no formal proof of their inhibitory activity on NAMPT was reported, and these compounds, although at first sight sharing key features with NAMPT inhibitors, at the moment must be considered putative NAMPT inhibitors. Caution is indeed required before enzymatic inhibition studies are performed, as in our laboratory we have found extremely cytotoxic compounds sharing key features with NAMPT inhibitors but devoid of inhibitory activity on the enzyme.[65]

Analogues of **2**, modified at the tail group have also been reported (Figure 19). In particular, compound **19** (TP201565)

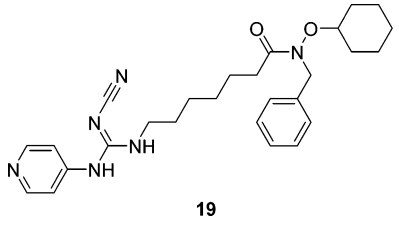

Figure 19. NAMPT inhibitors: tail group modification (II).

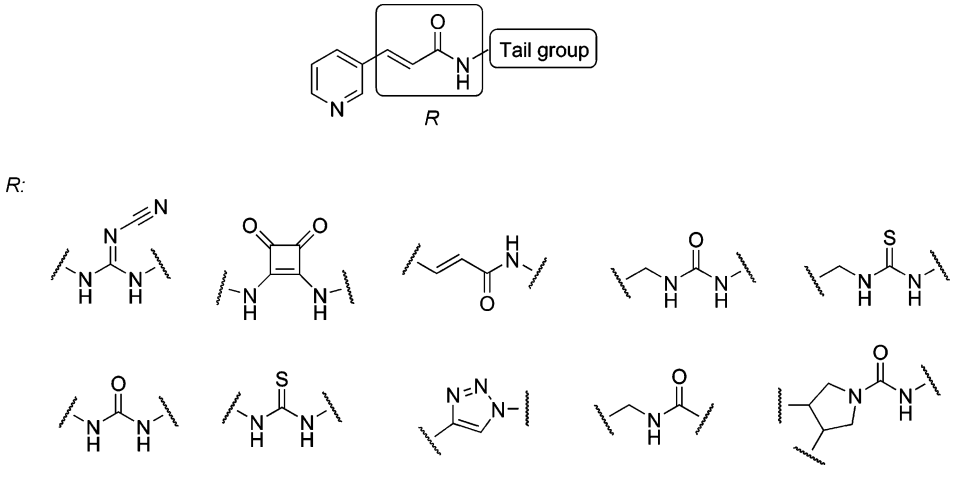

Figure 17. Synoptic scheme of the connecting units accepted by NAMPT.

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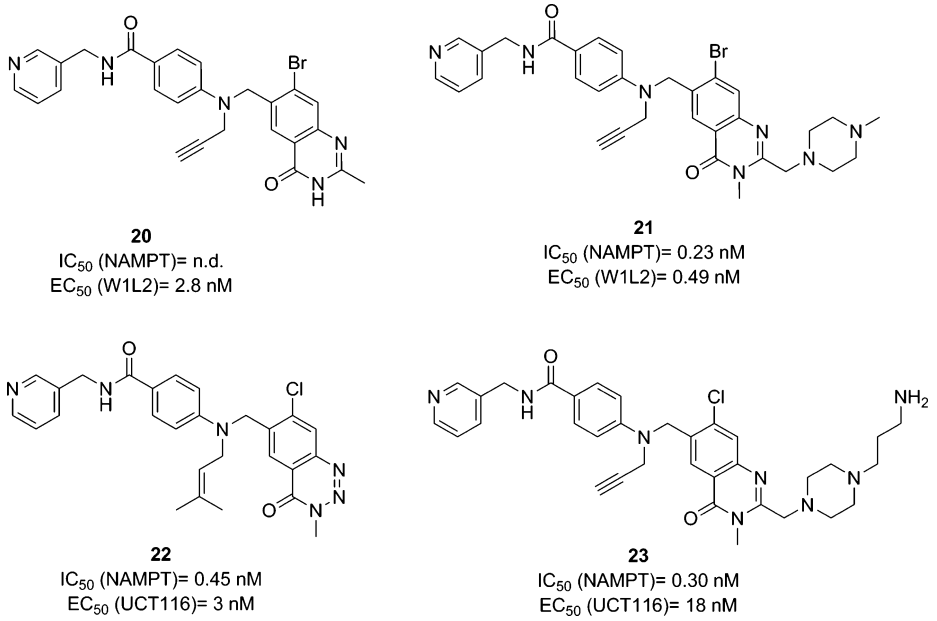

Figure 20. NAMPT inhibitors: tail group modification (III).

appears to be 85 times more cytotoxic than its related compound with a potency in the picomolar range, but other potent analogues with different tail groups have been reported in the same patent (small-cell lung cancer NYH cell line: FK866 1.5 nM, **2** 1.7 nM, **19** 0.02 nM).[52,70] This study supports the notion that the modification of the tail group is a good strategy to identify more potent analogues compared with the parent compound.

Serendipitously, NAMPT inhibitors have also been developed starting from thymidylate synthase (TS) inhibitor scaffolds (Figure 20). In this instance, a discrepancy between cytotoxicity and TS inhibition brought further detailed work that led to the finding that CB-30865 (**20**) was a novel inhibitor of NAMPT. Its water-soluble analogue (**21**, MPI-0486348) has also been synthesized (cytotoxicity in the human lymphoblastoid cell line: W1L2 EC_{50} of 0.49 nM; NAMPT enzyme inhibition of 0.23 nM).[71] An extensive SAR study was undertaken on **20**.[72] In particular, the 3-substituted pyridine was intolerant to any modifications, whereby the propargilic group can also be replaced with other lipophilic moieties. The best tail groups were a quinazolin-4-one or a 1,2,3-benzotriazin-4-one. Although the authors did not explicitly highlight their lead compounds, the most interesting analogues appear to be compounds **22** and **23**.

The replacement of the long alkyl chain with a benzene ring (Figure 21) has been exploited in a patent[73] and recently published.[63] Briefly, a virtual screening based on a pharmacophoric model designed by the authors unraveled a compound (**24**) with nanomolar anti-NAMPT activity. From this lead compound, an impressive number of analogues displaying high cytotoxic activity have been reported in the patent (for example, **25** and **26**). A lead candidate (**25**) has been disclosed in the subsequent article,[63] which showed in vivo antitumor activity when dosed orally in an ovarian tumor xenograft model. The same group has recently raised the issue that the pyridine in this series of NAMPT inhibitors may be a structural determinant for CYP2C9 inhibition. Different pyridine substituted analogues have been synthesized and tested, leading to the discovery of compound **27** which maintains NAMPT

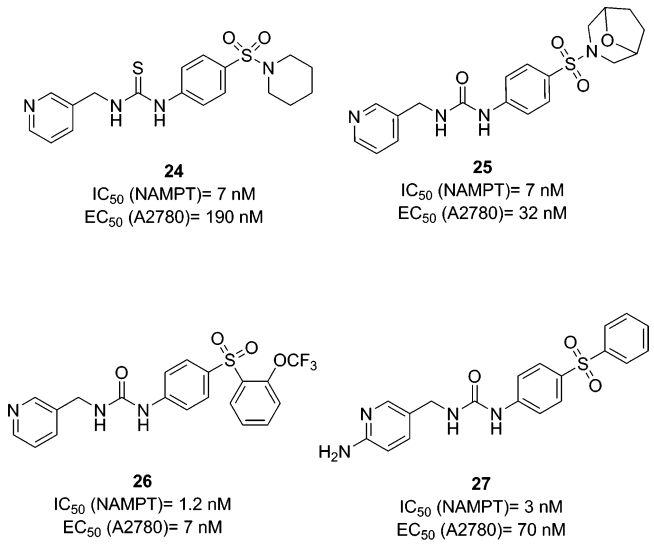

Figure 21. NAMPT inhibitors: tail group modification (IV).

inhibitory activity and antitumoral activity in xenografts without displaying inhibition of CYP2C9.[62] It is interesting that, also in this case, many of substituted pyridines retain NAMPT-inhibitory properties but fail to be cytotoxic.

The same company described a diphenylsulfone tail group (**28**, Figure 22), which was also present in most of the structures described above, that bears as a cap group the pyridinylcyanoguanidine of **2**.[74] Finally, in a recent patent,[75] the

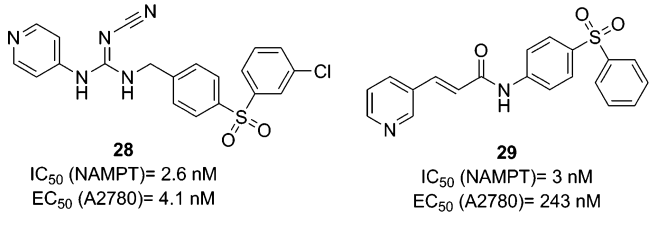

Figure 22. NAMPT inhibitors: tail group modification (V).

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authors disclosed a chimeric compound formed by joining the 3-pyridinylacrylamide of FK866 with the phenylsulfone tail group. Also in this case, we report only one example of this class of compounds (**29**).

The benzoylpiperidine moiety of FK866 has also been replaced with adamantyl or carborane groups (Figure 23).[76]

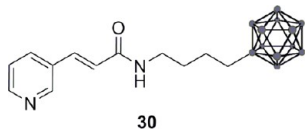

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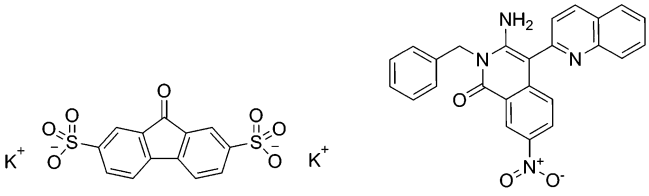
Figure 23. Structure of a carborane derivative of FK866.

The adamantyl derivative was 50 times less potent than FK866 as a cytotoxic agent on a lung cancer cell line (A549), while the carborane derivatives were more active than FK866. In particular, compound **30** showed an IC_{50} of 0.41 nM for cytotoxicity (A549) and exhibited an approximately 100-fold higher inhibitory activity compared with FK866.

The first virtual screening work appeared in 2011.[77] The authors used the crystal structure information of FK866 and NMN bound to NAMPT and performed a docking simulation of compounds contained in a chemical library. This procedure yielded six compounds, which were experimentally tested. Two of these compounds (**31** and **32**) displayed cytotoxicity on U87 cancer cell line (human glioblastoma) comparable to that of FK866, and one of them (**32**) was shown to be a NAMPT inhibitor (Figure 24). Nonetheless, this activity appears to be at least 1000-fold lower for FK866 than that reported by most authors.



31
IC_{50} (NAMPT)= no inhibition
EC_{50} (U87)= 335 μM

32
IC_{50} (NAMPT)= <5 μM
EC_{50} (U87)= 325 μM

Figure 24. Structures of compounds selected by virtual screening.

In conclusion, the tail group modifications have yielded a great diversity of very active compounds, and other modifications are most likely possible and should be attempted. Yet it remains to be established whether these will impinge only on the pharmacodynamic profile of the drug or whether pharmacokinetic improvements will also be possible.

PHARMACOLOGICAL CONSIDERATIONS, FUTURE DIRECTIONS, AND CONCLUSIONS

According to the structures identified to date as potent inhibitors of NAMPT, it is possible to sketch a pharmacophoric working model in order to help the people involved in this field, or newcomers, design novel/innovative NAMPT inhibitors (Figure 25). In brief, it would appear that a NAMPT inhibitor is linear in shape, with a pyridine (substituted or not) or a heterocycle containing a pyridine moiety as a cap group, with the presence of a connecting unit at the 3 or 4 position of pyridine and with a hydrogen bond acceptor at a distance

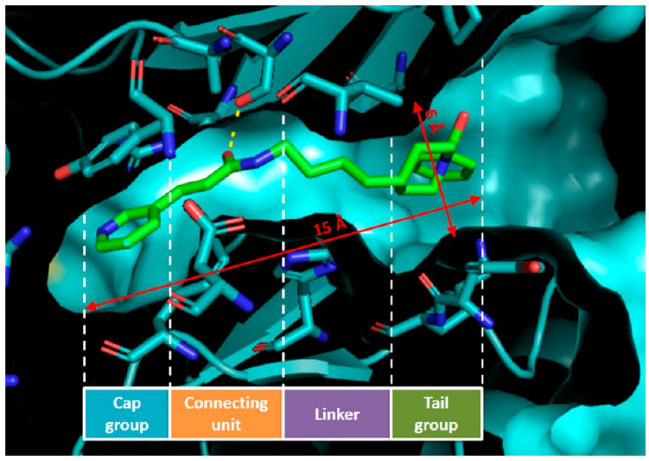


Figure 25. Roadmap to the synthesis of novel NAMPT inhibitors.

between 5.6 and 7.5 Å from the nitrogen atom of the pyridine and an alkyl chain linker with a minimum length of 7.6 Å, which corresponds approximately to five C−C bonds, in order for a hydrophobic group to protrude outward to be able to interact with the hydrophobic amino acids of the rim of the enzyme. Nonetheless, it has not escaped our attention that a similar model has been proposed for other enzymes. For example, histone deacetylase (HDAC) inhibitors possess a hydroxamic acid or other zinc-chelating warhead in the active site of the enzyme, a linker in the long and narrow tunnel, and a hydrophobic cap group that protrudes outside. A potential strategy therefore could be to capitalize on the numerous HDAC libraries present and to substitute the zinc-chelating warhead with a pyridine to evaluate their activity.

In conclusion, there are ample data suggesting that NAMPT inhibitors may have an important therapeutic role in cancer, and this target enzyme appears to display some features that would make it attractive. It is overexpressed in target issues. An antidote exists (nicotinic acid or nicotinamide). Proof-of-principle experiments in rodents have ascertained its potential, and lead compounds with sufficient potency have been described. Less is known about its potential in inflammatory diseases.

Yet novel compounds are urgently warranted to allow for the field to proceed speedily. Compounds should be able to display distinct pharmacokinetic properties compared to the most established compounds, as this seems to be an area that requires improvement, should be able to inhibit those mutated enzymes which have been described, and should be characterized for selectivity. Indeed, while most of the compounds described above discriminate between NAMPT and NaPRT, very little is known regarding their overall specificity, for example, with respect to other enzymes that use NAD. Last and most important, new compounds able to discriminate between iNAMPT and eNAMPT are urgently warranted both because they might have a different usefulness in the clinic and because they will be able to allow us to understand the role of these two forms.

Apart from medicinal chemistry, much remains to be done on these compounds. In the laboratory, it will be fundamental to understand more thoroughly the involvement of NAMPT in health and disease. For example, it has been suggested by others that cancer, inflammation, and metabolism have strong ties, and NAMPT might be an important link.[2b] In translational research, furthermore, it will be important to understand

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which combinations of drugs that include NAMPT inhibitors are more likely to work in the clinic, both regarding cancer and inflammation. Last, NAMPT inhibition brings up a number of practical issues that will need to be resolved for therapy to be effective. For example, what impact will the diet have on therapy (as a source of NAD precursors) or what impact will the time of day in which therapy is administered have?

Notwithstanding all the areas of research that still present uncertainties in the NAMPT field, it has been suggested that basic metabolism may be an important strategy in combating cancer,[2a] and NAMPT still appears to be one of the most promising targets. The potential in inflammatory diseases remains instead to be ascertained.

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Notes

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ABBREVIATIONS USED

1-MT, 1-methyltryptophan; ADPR, ADP-ribose; AMP, adenosine monophosphate; ART, mono (ADP-ribose) transferase; ER, endoplasmatic reticulum; IDO, indoleamine 2,3-dioxygenase; MMS, methyl methanesulfonate; MNNG, methylnitronitrosoguanidine; MX, methoxyamine; NA, nicotinic acid; NAAD, nicotinic acid adenine dinucleotide; NADS, nicotinamide adenine dinucleotide synthetase; Nam, nicotinamide; NAMN, nicotinic acid mononucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NaPRT, nicotinic acid phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NMNAT, nicotinamide mononucleotide adenylyltransferase; NR, nicotinamide riboside; NRK, nicotinamide riboside kinase; PARP, poly (ADP-ribose) polymerase; PMNC, polymorphonucleate cell; PPi, pyrophosphate; PRPP, phosphoribosyl pyrophosphate; QA, quinolinic acid; QAPRT, quinolinic acid phosphoribosyltransferase; SCF, stem cell factor; TCA, tricarboxylic acid; TDO, tryptophan 2,3-dioxygenase; TRAIL, tumor necrosis factor related apoptosis inducing ligand; TS, thymidylate synthase

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