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Optimization of a urea-containing series of nicotinamide phosphoribosyltransferase (NAMPT) activators

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ABSTRACT

NAD^+ is a crucial cellular factor that plays multifaceted roles in wide ranging biological processes. Low levels of NAD^+ have been linked to numerous diseases including metabolic disorders, cardiovascular disease, neurodegeneration, and muscle wasting disorders. A novel strategy to boost NAD^+ is to activate nicotinamide phosphoribosyltransferase (NAMPT), the putative rate-limiting step in the NAD^+ salvage pathway. We previously showed that NAMPT activators increase NAD^+ levels *in vitro* and *in vivo*. Herein we describe the optimization of our NAMPT activator prototype (SBI-0797812) leading to the identification of 1-(4-((4-chlorophenyl)sulfonyl) phenyl)-3-(oxazol-5-ylmethyl)urea (**34**) that showed far more potent NAMPT activation and improved oral bioavailability.

Nicotinamide adenine dinucleotide (NAD^+) is involved in diverse cellular processes that govern human health and disease.[1] NAD^+ has long been known as a redox enzyme cofactor. More recently, NAD^+ was shown to be a co-substrate for sirtuins and poly-ADP-ribose polymerases (PARPs) which has unveiled pivotal roles in cell signaling, DNA repair, cell division, and epigenetics.[2,3] Increased tissue levels of NAD^+ have been shown to mediate beneficial effects in a variety of preclinical disease models.[4] These findings have spurred keen interest in pharmacological and nutraceutical strategies that boost intracellular NAD^+ levels.[5,6]

NAD^+ is consumed in cells due to the actions of sirtuins, PARPs and NADases.[7] Hence, cellular NAD^+ biosynthetic pathways are obligatory to preserve the NAD^+ levels which are required for cell viability. The dominant NAD^+ synthetic route in most mammalian cells is the nicotinamide (NAM) salvage pathway involving sequential actions of nicotinamide phosphoribosyltransferase (NAMPT) and NMN adenylyltransferase (NMNAT). NAMPT forms nicotinamide mononucleotide (NMN) and pyrophosphate (PP) from NAM (produced by sirtuins and PARPs) and α-D-5-phosphoribosyl-1-pyrophosphate (PRPP). In turn, NMNAT produces NAD^+ from NMN.

NAMPT, a homodimeric type II phosphoribosyltransferase, is the putative rate-limiting step in the NAM salvage pathway.[8] Enzymology[9–11] and X-ray crystallograph[12–15] studies have yielded a detailed understanding of NAMPT catalytic activity. NAMPT has also been shown to be the dominant route for NAD^+ synthesis in the heart and kidney[16], with preclinical data suggesting that NAD^+ repletion strategies have the potential to aid in the treatment of cardiac and kidney injuries, possibly by maintaining sirtuin activity.[5] Additionally, early evidence may show beneficial effects of NAM supplementation in some forms of eye disease.[17,18] Conversely, potent and highly selective NAMPT inhibitors have been developed and evaluated as a treatment for cancer.[19]

We recently reported the discovery of SBI-0797812 (**2**), a small molecule NAMPT activator which was generated from the HTS hit SBI-0136892 (**1**; Fig. 1).[20] Interestingly, SBI-0797812 (**2**) was recently shown to exert an antiviral effect due to its ability to modulate innate immunity.[21] While SBI-0797812 displayed acceptable potency, its rodent pharmacokinetic (PK) properties, including oral bioavailability

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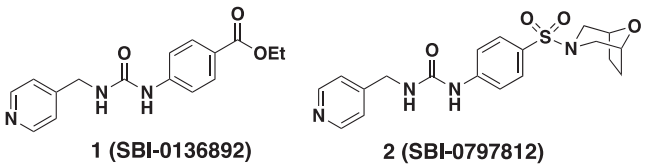


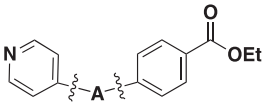
Fig. 1. Previously described NAMPT activators.

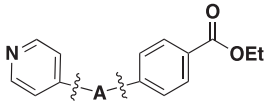
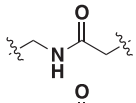
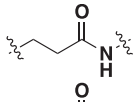
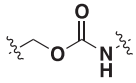
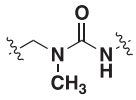
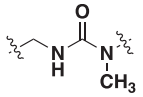
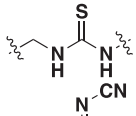
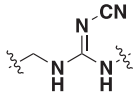
were inadequate. The results of our medicinal chemistry campaign aimed at optimizing SBI-0797812 are described in this report.

The NAMPT activity assay measuring NMN production was used to detect improved NAMPT activator potency.[20] The results of our efforts are summarized in Tables 1–4. Prior work had highlighted the importance of the 4-pyridyl group for NAMPT activation. We initially modified the urea linkage (Table 1) to ascertain the key elements required for NAMPT activator activity. As shown in the table, only the urea moiety was active. One amide linkage (**3**) was inactive, while the other isomer (**4**) and the carbamate (**5**) were both weak NAMPT inhibitors. Methylation of the urea nitrogens (**6**, **7**) abolished NAMPT activator activity. Interestingly other linkers that have been previously used in NAMPT inhibitors,[22,23] including the thiourea (**8**) and cyanoguanidine (**9**) also yielded weak NAMPT inhibitors. This SAR indicated that the structural requirements for NAMPT activation appear to be narrower than for NAMPT inhibition.

We next turned our attention to the pyridyl group. Our previous work showed that the 4-pyridyl ring mediated NAMPT activation whereas the 2-pyridyl group yielded inactive compounds and the 3-pyridyl group yielded NAMPT inhibitors.[20] The 3-pyridyl group is the most

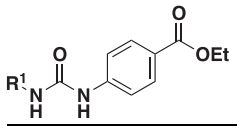
Table 1
SAR elucidation of the urea linker.

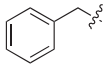
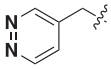
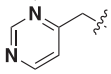
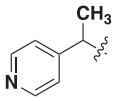
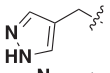
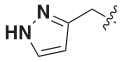
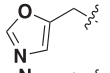
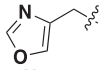
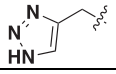


Cmpd	A	hNAMPT EC_{50} (μM)[a]	Emax[a]
1		3.25	98%
3		>100	ND
4		15.9	−13%
5		10.8	−25%
6		>100	ND
7		>100	ND
8		9.77	−15%
9		41.4	−30%

[a] Values are means of at least four experiments, standard deviations were within 20% of reported values. ND = Not determined. Negative values represent inhibition.

Table 2
SAR elucidation around the pyridyl group.



Cmpd	R^1	NAMPT EC_{50} (μM)[a]	Emax[a]
10		1.37	−36%
11		>100	ND
12		13.6	−37%
13		2.28	−44%
14		1.85	158%
15		69	−23%
16		6.90	194%
17		27.6	−39%
18		7.75	−26%

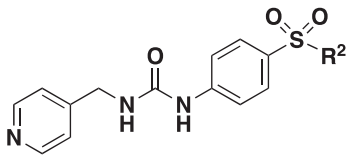
[a] Values are means of at least four experiments, standard deviations were within 20% of reported values. ND = Not determined. Negative values represent inhibition.

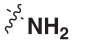
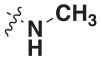
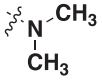
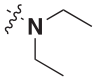
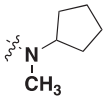
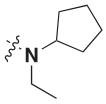
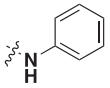
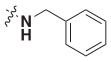
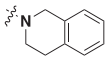
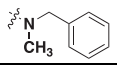
widely used group in NAMPT inhibitor SAR, although a relatively wide range of pyridine-like groups is tolerated. Further exploration of this group is shown in Table 2. Phenyl (**10**), pyridazinyl (**11**) or pyrimidinyl (**12**) gave inactive compounds or weak NAMPT inhibitors, indicating relatively narrow SAR in this region. Indeed, adding a methyl group to the methylene linker (**13**) switched the compound from an activator to a weak inhibitor. We next explored a series of 5-membered heterocyclic rings as potential substitutes for the 4-pyridyl ring. Interestingly, a 4-pyrazoyl group (**14**) mediated strong NAMPT activation while a 3-pyrazoyl group (**15**) produced a weak inhibitor. Likewise, a 5-oxazolyl group (**16**) gave strong NAMPT activation while a 4-oxazolyl (**17**) gave weak NAMPT inhibition, as did a 4-triazolyl (**18**) group. The structural basis for this toggling between NAMPT activation and inhibition is currently under investigation.

We next examined the SAR around a series of phenyl sulfonamide analogs that deviated from the oxa-azabicyclooctane group in SBI-0797812 (Table 3). Previous work on NAMPT inhibitors indicated a very wide range of groups is tolerated in this position.[24] This turns out to be the case for NAMPT activators as well. In general, unsubstituted (**19**) or methyl substituted analogs (**20**, **21**) showed weaker potency. Stepwise introduction of incrementally larger groups such as diethyl (**22**) and disubstituted cyclopentyl (**23**, **24**) moieties yielded improved NAMPT activator potency. Lastly, while phenyl (**25**) showed little improvement, benzyl (**26**), tetrahydroisoquinoline (**27**) and methyl benzyl (**28**) gave potent NAMPT activators. Indeed, **28** displayed a > 10 fold improvement in potency. Both **27** and **28** were examined for their rodent PK properties. While both compounds displayed reasonable bioavailability, exposure after oral dosing was modest (*vide infra*).

Due to the modest exposure after oral dosing of the sulfonamides, we pursued other linker groups in place of the sulfonamide. As previously reported for NAMPT inhibitors[25] a sulfone linker has been shown to

Table 3
SAR elucidation around the sulfonamide.



Cmpd	R^2	NAMPT EC_{50} (μM)[a]	Emax[a]
2		0.279	173%
19		14.3	131%
20		3.23	138%
21		3.11	133%
22		0.384	179%
23		0.123	130%
24		0.060	160%
25		0.410	87%
26		0.102	96%
27		0.142	130%
28		0.023	153%

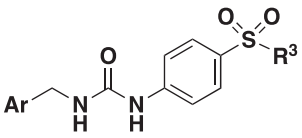
[a] Values are means of at least four experiments, standard deviations were within 20% of reported values.

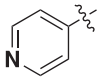
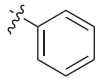
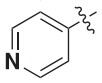
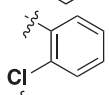
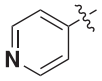
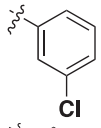
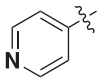
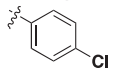
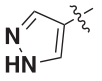
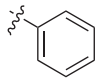
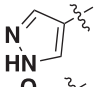
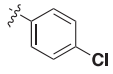
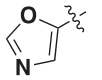
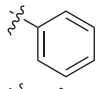
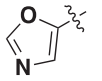
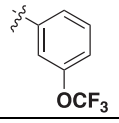
improve PK properties. We therefore examined a series of sulfone linked analogs, while also incorporating the 4-pyridyl head-group replacements (Table 4). An unsubstituted phenyl (**29**) gave 4-fold improved NAMPT activator potency (compare to **25**). A wide range of substitution were introduced for the phenyl group, with the 2-, 3-, and 4-chloro (**30**, **31**, **32**) analogs shown in the table. In most cases, substitution on this ring did not significantly affect NAMPT activator potency, indicating that this group could be modified to improve other properties (i.e. PK). We were delighted to find that incorporation of 4-pyrazoyl (**33**, **34**) and 5-oxazolyl (**35**, **36**) yielded potent NAMPT activators, which in most cases showed significant improvements in exposure after oral dosing (Table 5). In particular **34** showed low plasma clearance, good exposure and excellent oral bioavailability (%F = 80).

Treatment of A549 lung epithelial cells with **34** resulted in a marked increase of both NMN and NAD (Fig. 2). These effects were similar to those observed previous with SBI-0797812.[20]

Compound **34** was mainly clean when evaluated in a Eurofins/Ricerca Hit Profiling screen against 35 targets; however, **34** did show moderate activity against CYP2C9 (0.060 μM), 2D6 (0.41 μM) and 2C19 (0.59 μM). CYP activity displayed by **34** was corroborated in internal assays (see Table 6). Additional *in vitro* ADME screening (Table 6) showed that **34** had moderate solubility, good stability in human liver microsomes but poor permeability. Further optimization to obviate the CYP activity and other limitations will be reported in subsequent publications.

Table 4
Additional SAR elucidation.



Cmpd	Ar	R^3	NAMPT EC_{50} (μM)[a]	Emax[a]
29			0.085	152%
30			0.079	96%
31			0.069	90%
32			0.126	102%
33			0.028	190%
34			0.023	214%
35			0.077	172%
36			0.070	208%

[a] Values are means of at least four experiments, standard deviations were within 20% of reported values.

Table 5
Selected mouse PK parameters for compounds **27**, **28**, **33**, **34** and **36**.[a]

Compds	Cl_p[b](mL/min/kg)	Vd[b](L/kg)	C_{max}[c](ng/mL)	AUC[c] (ng.hr/mL)	$t_{1/2}$[c](hr)	%F
27	20	0.60	7860	4266	1.0	47
28	43	1.37	4953	4508	0.6	>100
33	8	1.12	2230	9344	2.5	45
34	3	0.61	9550	54,463	3.2	80
36	4	0.58	15,313	45,203	2.3	>100

[a] Compounds dosed 1 mpk iv and 10 mpk po.
[b] iv
[c] po.

The majority of the compounds described above were synthesized as outlined in the general schemes below.[26] A representative synthesis for **1** is shown in Scheme 1. Treatment of aniline **37** with phenylchloroformate gave carbamate **38** in good yield. This was then reacted with pyridin-4-ylmethanamine to give the desired product. Compounds **10**–**18** were synthesized in a similar fashion using the appropriate amine.

The sulfonamides in Table 3 were synthesized as outlined in Scheme 2. 4-Nitrophenylsulfonyl chloride **39** was reacted with diethylamine to give **40**. **40** was reduced using hydrogen and Pd/C to give aniline **41**. This was then reacted with phenylchloroformate to give intermediate **42**, which was reacted with pyridin-4-ylmethanamine to give **22**. Compounds **19**–**28** were in general synthesized using this approach.

Lastly, the sulfones in Table 4 were synthesized in general as outlined in Scheme 3. 4-Chlorothiophenol (**43**) was reacted with 4-

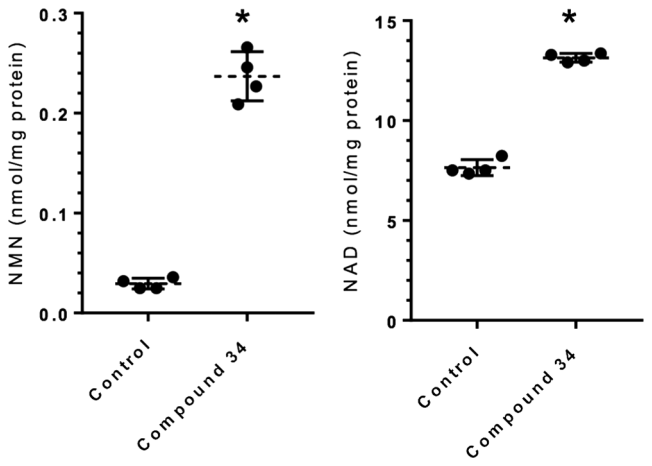
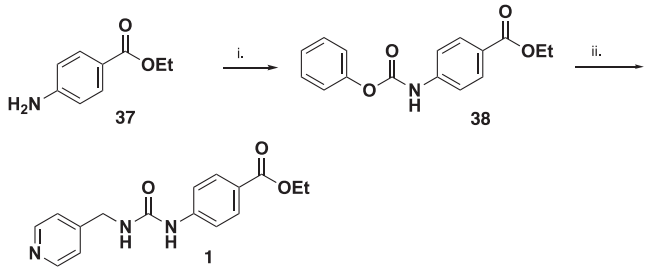


Fig. 2. A549 Cells were treated with DMSO (control) or **34** (10 μM) for 4 hr. Cells were harvested and assayed for NMN (left panel) or NAD^+ (right panel) by LC-MS/MS.[17] Data is normalized to cell protein. *, $P < 0.001$ vs. control.

Table 6
Physicochemical and *in vitro* ADME properties of **34**.

LogD[a]	PAMPA permeability (pH 5.0, pH 7.4) (nm/s)	Solubility (pH 1.2, 6.8) (μg/mL)	Metabolic stability[b] (human, mouse) (%)	CYP DI[c] (1A2/2C9/2D6/3A4)
3.1	<0.1/0.4	110/8.5	87/18	65/96/95/0

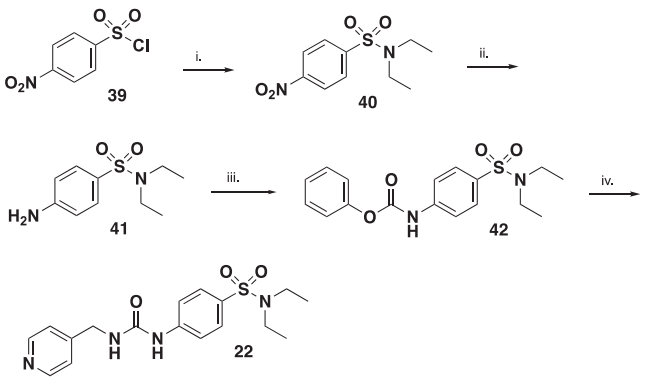
[a] The distribution coefficient (Log D) was measured between 1-octanol and phosphate buffered saline (pH 7.4).
[b] The % remaining value at 1 μM concentration of compounds reacted with human or mouse microsomes for 30 min.
[c] The % inhibition value at 10 μM concentration of compounds reacted with corresponding CYP isoforms for 10 min.



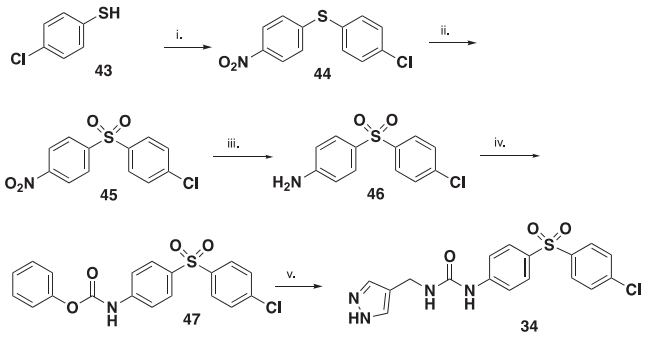
Scheme 1. Synthesis of **1**. i. Phenylchloroformate, Et_3N, CH_2Cl_2, 20–93% ii. pyridin-4-ylmethanamine, DMAP, CH_3CN, 41–89%

chloronitrobenzene to give compound **44**, which was oxidized using mCPBA to sulfone **45**. The nitro group of **45** was reduced via hydrogenation to provide **46**, which was reacted as above with phenylchloroformate to give intermediate **47**. **47** was reacted with (1H-pyrazol-4-yl)methanamine to provide **34**. Compounds **29–36** were in general synthesized using this approach.

In conclusion, we have developed a potent series of NAMPT activators that produce a robust increase in NAD^+ levels in cultured cells. A systematic SAR examination around our urea-based scaffold elucidated the key groups necessary for robust enzyme activation, including the critical nature of the head group, wherein small structural changes between pyridyl or pyrazole isomers led to NAMPT inhibitors. Modification of other areas of the scaffold, in particular incorporating a sulfonamide or sulfone linker, gave compounds that displayed excellent rodent PK properties, which is a significant improvement over the initial



Scheme 2. Synthesis of **22**. i. Diethylamine, Et_3N, CH_2Cl_2, 89% ii. H_2, Pd/C, EtOAc, 90% iii. Phenylchloroformate, Et_3N, CH_2Cl_2, 82% iv. pyridin-4-ylmethanamine, DMAP, CH_3CN, 35%



Scheme 3. Synthesis of **34**. i. 4-Chloronitrophenol, K_2CO_3, DMF, 89% ii. *m*-CPBA, CH_2Cl_2, 92% iii. H_2, Pd/C, MeOH, 90% iv. Phenylchloroformate, Et_3N, CH_2Cl_2, 78%. v. (1H-pyrazol-4-yl)methanamine, Et_3N, CH_3CN, 43%

hit compounds. Our further investigation of this novel NAD^+ booster class across a broad range of disease models, as well as additional compound optimization, will be disclosed in subsequent reports.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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References

1 Garten A, Schuster S, Penke M, Gorski T, de Giorgis T, Kiess W. *Nat Rev Endocrinol.* 2015;11:535–546.
2 Imai S, Guarente L. *Trends Cell Biol.* 2014;24:464–471.
3 Gibson BA, Kraus WL. *Nat Rev Mol Cell Biol.* 2012;13:411–424.
4 Rajman L, Chwalek K, Sinclair DA. *Cell Metab.* 2018;27:529–547.
5 Yoshino J, Baur JA, Imai SI. *Cell Metab.* 2018;27:513–528.
6 Liu L, Su X, Quinn 3rd WJ, et al. *Cell Metab.* 2018;27:1067–1080.
7 Petrelli R, Felczak K, Cappellacci L. *Curr Med Chem.* 2011;18:1973–1992.
8 Revollo JR, Grimm AA, Imai S. *J Biol Chem.* 2004;279:50754–50763.
9 Burgos ES, Ho MC, Almo SC, Schramm VL. *PNAS.* 2009;106:13748–13753.
10 Burgos ES, Schramm VL. *Biochemistry.* 2008;47:11086–11096.
11 Burgos ES, Vetticatt MJ, Schramm VL. *J Am Chem Soc.* 2013;135:3485–3493.
12 Wang T, Zhang X, Bheda P, Revollo JR, Imai S, Wolberger C. *Nat Struct Mol Biol.* 2006;13:661–662.
13 Khan JA, Tao X, Tong L. *Nat Struct Mol Biol.* 2006;13:582–588.
14 Kim MK, Lee JH, Kim H, et al. *J Mol Biol.* 2006;362:66–77.
15 Oh A, Ho YC, Zak M, et al. *ChemBioChem.* 2014;15:1121–1130.
16 Hershberger KA, Martin AS, Hirschey MD. *Nat Rev Nephrol.* 2017;13:213–225.

17 Klimova N. *Kristian Neurochem Res.* 2019;44:2280–2287.
18 Williams PA, Harder JM, Foxworth NE, Cardozo BH, Cochran KE, John SWM. *Front Neurosci.* 2017;11:232.
19 Sampath D, Zabka TS, Misner DL, O'Brien T, Dragovich PS. *Pharmacol Ther.* 2015; 151:16–31.
20 Gardell SJ, Hopf M, Khan A, et al. *Nat Comm.* 2019;10:3241.
21 Heer CD, Sanderson DJ, Voth LS, et al. *J Biol Chem.* 2020.
22 Zheng X, et al. *J Med Chem.* 2013;56:4921–4937.
23 Watson M, et al. *Mol Cell Biol.* 2009;29:5872–5888.
24 Zak M, et al. *Bioorg Med Chem Lett.* 2015;25:529–541.
25 Zak M, Yuen P, Liu X, et al. *J Med Chem.* 2016;59:8345–8368.
26 PCT WO 2018132372 Gardell, S.; Pinkerton, A. B.; Sergienko, E.; Sessions, H.