Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate-limiting step of the nicotinamide adenine dinucleotide (NAD⁺) salvage pathway. Because NAD⁺ plays a pivotal role in energy metabolism and boosting NAD⁺ has positive effects on metabolic regulation, activation of NAMPT is an attractive therapeutic approach for the treatment of various diseases, including type 2 diabetes and obesity. Herein we report the discovery of 1-(2-phenyl-1,3-benzoxazol-6-yl)-3-(pyridin-4-ylmethyl)urea 12c (DS68702229), which was identified as a potent NAMPT activator. Compound 12c activated NAMPT, increased cellular NAD⁺ levels, and exhibited an excellent pharmacokinetic profile in mice after oral administration. Oral administration of compound 12c to high-fat diet-induced obese mice decreased body weight. These observations indicate that compound 12c is a promising anti-obesity drug candidate.

Key words anti-obesity; nicotinamide phosphoribosyltransferase activator; benzoxazole; nicotinamide adenine dinucleotide (NAD⁺); body weight reduction

Introduction

Obesity is a global health concern, with its worldwide prevalence nearly tripling since 1975.¹ In addition to its well-known association with disorders such as type 2 diabetes (T2D), hepatic steatosis, and atherosclerosis, obesity has been implicated in other diseases, such as colon, prostate, and hematologic malignancies, osteoarthritis, worsening autoimmune conditions, and lung diseases.²–⁴ Weight gain is generally considered to be the result of an imbalance between total energy intake and expenditure. Although weight loss can be temporarily achieved through diet restriction and/or increased physical activity, many individuals tend to regain the weight they have lost over the long term because weight loss causes compensatory changes in energy expenditure.⁵–⁷

Nicotinamide adenine dinucleotide (NAD⁺) is a biologically important cellular factor implicated in many metabolic processes in cells.⁸–¹² NAD⁺ is known to have two modes of action: (1) as an electron-carrying coenzyme for oxidoreductases; and (2) as an ADP-donating cosubstrate for many enzymes. As a coenzyme, the nicotinamide moiety of NAD⁺ accepts hydride equivalents to form reduced nicotinamide adenine dinucleotide (NADH); both NAD⁺ and NADH have vital roles in many energy-producing processes, including glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation.⁸–¹² As a cosubstrate, NAD⁺ donates its ADP-ribose to certain enzymes, such as sirtuins (SIRTs), poly(ADP-ribose) polymerases (PARPs), and cADPR-ribosyl (cADPR) synthases (CD38 and CD157).⁸–¹² In these reactions, the linkage between nicotinamide (NAM) and the ADP-ribosyl moieties of NAD⁺ is hydrolyzed, thus necessitating continuous replenishment of NAD⁺.¹⁰ In mammals, the dominant NAD⁺ synthetic pathway is the salvage pathway, which converts NAM back to NAD⁺. The first step in this pathway, the conversion of NAM to NAM mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT), is the rate-limiting step of the salvage pathway.⁸–¹²

Given its role as an intrinsic regulator of cell bioenergetics, increasing NAD⁺ levels has been shown to have several beneficial physiological effects similar to those achieved by diet or exercise.⁸,⁹ In particular, NAMPT is considered an important enzyme in the regulation of NAD⁺ levels, with several preclinical studies suggesting that decreases in NAMPT are associated with metabolic disorders.⁹,¹³,¹⁴ In one study, NAMPT overexpression in the skeletal muscle of C57BL6/J mice elicited increases in skeletal muscle NMN and NAD⁺ levels, which led to higher exercise endurance capacity compared with wild-type mice, along with an improvement in metabolic abnormalities.¹⁵ Extrapolating from these results, pharmacological activation of NAMPT would be expected to modulate metabolism by increasing NAD⁺ levels. Therefore, NAMPT activators may be a promising approach for the treatment of metabolic disorders, including obesity and T2D.

We have previously described the acquisition of a series of urea-containing NAMPT activators, such as 2 derived from the high-throughput screening (HTS) hit 1. These compounds possess a (pyridin-4-ylmethyl)urea moiety and a triazolopyridine core structure (Fig. 1). Compound 2 showed potent NAMPT activating activity in an NAMPT enzyme assay and increased intracellular NAD⁺ levels in cell-based assays.¹⁸ Compound 2 also exhibited attenuated direct inhibition (DI) of CYP, which is an issue that has also been observed with other triazolopyridine derivatives.¹⁶ Although its low logD was beneficial for attenuation of CYP DI, compound 2 was not suitable for oral administration due to low membrane permeability. We therefore focused our structure–activity relationship (SAR) studies on screening bicyclic ring structures
to find an alternative core structure. Herein we report further optimization of the series to develop an orally available potent NAMPT activator, DS68702229, in which benzoxazole with a (pyridin-4-ylmethyl)urea moiety was embedded.

Chemistry

The retrosynthesis of benzoxazole derivatives possessing the (pyridin-4-ylmethyl)urea moiety is shown in Fig. 2. The target molecules were synthesized from the corresponding 5-amino-benzoxazoles via several urea-forming methods. The intermediate 5-amino-benzoxazole derivatives were provided by using either one of two methods, namely palladium-catalyzed direct 2-arylation of the benzoxazoles (Method A) or condensation of 2-aminophenol derivatives with benzoyl chloride followed by cyclization under acidic conditions (Method B).

Following the retrosynthesis, the compounds in Table 1 were prepared (Charts 1, 2). Chart 1 shows the synthesis of benzoxazole intermediates (benzoxazole intermediates 4a–c were commercially available). 2-Arylated compounds 4d and 4e were prepared from compound 3 via palladium-catalyzed direct 2-arylation via a deprotonative cross-coupling process (Method A). As for benzoxazole intermediates with substituents (F, Me, Cl) at 5-position (4f–h), the 6-position of 5f–h was nitrated to give 6f–h, followed by reduction of the NO2 group to an NH2 group by hydrogenation to afford 4f–h. Synthesis of oxazolo[4,5-b]pyridine intermediate 4i was achieved using Method B; commercially available 7 was condensed with benzoic acid in polyphosphoric acid at 160 °C to give 6i, followed by NO2 reduction to afford 4i. Regarding the benzoxazole intermediate with a nitrile group at 4-position (4k), commercially available 8 was brominated to give 9ka, followed by the construction of a benzoxazole structure (6ka) via Method B, a three-step procedure involving condensation of 9ka with benzoyl chloride, demethylation of the OMe group, and subsequent cyclization under acidic conditions. Subsequently, the nitrile group was introduced to 6ka via Pd-catalyzed coupling to give 6k, the NO2 group of which was reduced using iron powder to give the intermediate 4k. As for 4j and 4l, which possess a methyl group or an amide group at the 4-position of the benzoxazole ring, the corresponding amino-benzoxazoles (9j, 9la) underwent a three-step procedure analogous to 9ka to provide benzoxazoles 6j and 6la. The methyl ester of 6la was converted to N,N-dimethylamide 6i via hydrolysis and amidation. Subsequently, the 6-bromine of 6i was converted to an NHBoc group by Pd-catalyzed amination to give 4j and 4l.

Chart 2 delineates the urea formation reactions of the benzoxazole intermediates to afford the final compounds 12a–l. Three methods were used for urea formation. The first was the carbamate method (Chart 2(a)). tert-Butoxycarbonyl (Boc)-protected 4e, 4j, and 4l were deprotected in acidic conditions and reacted with 4-nitrophenyl [(pyridin-4-yl)methyl]carbamate to provide 12e, 12j, and 12l. Similarly, compounds 4a–c, 4f, and 4k were converted to urea 12a–c, 12i, and 12k.

The second method was the triphosgene method (Chart 2(b)). Boc-protected 4d was deprotected and the aniline intermediate obtained was treated with triphosgene and coupled with the corresponding amine to provide 12d. Similarly, aniline derivatives 4c and 4f–h were converted to the corresponding urea derivatives 12cb, 12cc, 12cd, 12f, 12g, and 12h.

In the third method, the pyrazole derivative 12ca was synthesized by reacting the aniline 4c with commercially available 4-nitrophenyl chloroformate to afford the carbamate intermediate 14, which was coupled with 1H-pyrazol-4-ylmethylamine to give the final product 12ca (Chart 2(c)).

Results and Discussion

We initially focused our SAR studies on screening bicyclic ring structures (region B) in a search for an alternative core structure (Table 1). Our initial attempt started with the 2-phenyl-1H-benzoimidazole analog 12a, which, unfortunately, resulted in decrease of activity in the NAMPT enzyme assay compared to our previous lead compound 2. Conversion of the central ring to a benzoxazole (12b) led to recovery of activity in both NAMPT enzyme and cell-based assays. Notably, benzoxazole compound 12c, in which the positions of the oxygen and nitrogen atoms of the benzoxazole ring were exchanged compared with 12b, demonstrated higher NAMPT activation activity compared to 2. Compounds 12b and 12c...
were further profiled to assess their physicochemical and \textit{in vitro} ADME (absorption, distribution, metabolism, and excretion) properties (Table 2). These compounds exhibited high membrane permeability compared to the prior triazolopyridine lead compound 2, which would be expected to be beneficial towards oral absorption. Of note, compound 12c showed a lower tendency of CYP D1 compared to 12b, which was an issue widely observed in our previous triazolopyridine series.\textsuperscript{16)} Accordingly, we decided to fix the central region B to the benzoxazole ring of 12c for further derivatization.

We decided to explore region A, B, and C within the benzoxazole template with an aim to consider whether further improvement of NAMPT activation activity, while concomitantly possessing a favorable ADME profile for oral absorption, was

| Cpd | A | B | C | NAMPT enzyme assay | Cell-based assay (HEK293A) |
|-----|---|---|---|-------------------|--------------------------|
| 2   |   |   |   | 0.18 µM/132%     | 71%\textsuperscript{b)} |
| 12a |   |   |   | 3.2 µM/165%      | 17%\textsuperscript{b)} |
| 12b |   |   |   | 0.41 µM/106%     | 0.54 µM/140%           |
| 12c |   |   |   | 0.046 µM/153%    | 0.19 µM/158%          |
| 12d |   |   |   | 0.20 µM/92%      | 111%\textsuperscript{b)} |
| 12e |   |   |   | 0.13 µM/87%      | 0.44 µM/160%          |
| 12ea|   |   |   | 0.10 µM/166%     | 0.18 µM/169%          |
| 12eb|   |   |   | 0.11 µM/100%     | 134%\textsuperscript{b)} |
| 12cc|   |   |   | 0.092 µM/84%     | 81%\textsuperscript{b)} |
| 12cd|   |   |   | 0.55 µM/85%      | 51%\textsuperscript{b)} |
| 12f |   |   |   | 0.014 µM/106%    | 100%\textsuperscript{b)} |
| 12g |   |   |   | 0.29 µM/93%      | 58%\textsuperscript{b)} |
| 12h |   |   |   | 0.17 µM/64%      | 87%\textsuperscript{b)} |
| 12i |   |   |   | ND\textsuperscript{b)} | 0.68 µM/107%          |
| 12j |   |   |   | 0.059 µM/155%    | 0.63 µM/167%          |
| 12k |   |   |   | 0.075 µM/110%    | 0.40 µM/141%         |
| 12l |   |   |   | 3.4 µM/90%       | NT\textsuperscript{b)} |

\textsuperscript{a)} Percentages indicate the magnitude of the increase in NAD\textsuperscript{+} achieved with 1 µM of the compound relative to that achieved with 30 µM compound 1. \textsuperscript{b)} Not determined. The NAMPT enzyme assay could not be performed because of the fluorescence of the compound. \textsuperscript{c)} Not tested.
First, SAR exploration of the benzoxazole template was directed towards modification of the right-hand region C (Table 1). Installing substituents on the phenyl ring did not have a significant effect on NAMPT activation activity (data not shown), consistent with the results observed with our previous triazolopyridine series. We subsequently screened several heteroaryl groups. Although installing a pyridine ring (12d) did not lead to significant improvements, installing an N-methylpyrazole structure (12e) resulted in comparable increases in intracellular NAD^+ to those seen in our previously described triazolopyridine series. Furthermore, various aliphatic groups were explored within this region, but generally resulted in impaired activity and suffered from instability in acidic medium.

We then focused on the left-hand region A, the exposed pyridine ring. An intensive exploration around this left-hand region has been conducted previously; among the various heterocycles, 4-pyrazole and 5-oxazole were the only heterocycles that activated NAMPT, with the others exhibiting inhibitory or no activity. 4-Pyrazole and 5-oxazole (12ca and 12cb) were also tolerated with the benzoxazole template, exhibiting equipotent NAMPT activation to the 4-pyridyl group. Interestingly, the benzoxazole template tolerated a slightly wider variation of heterocycles at this region, with 5-thiazole and 4-imidazole derivatives (12cc and 12cd) also exhibiting NAMPT activation.

Finally, we shifted our efforts to investigation of substitu-
ents on the central benzoxazole core in region B. Substitutions on the 5-position demonstrated that F substitution \((\text{12f})\) was tolerated in terms of NAMPT activation, but slightly larger substituents, such as methyl \((\text{12g})\) or Cl \((\text{12h})\), resulted in impaired activity, which suggested steric limitations around this position. In contrast, the 4-position seemed to be relatively tolerant of modification. For example, replacing the carbon atom with nitrogen was tolerated, with oxazolo[4,5-b]pyridine analog \(\text{12i}\) being the most potent of the three possible oxazolopyridine isomers,\(^{20}\) exhibiting comparable cellular activity.

![Chart 2. Reagents and Conditions](attachment:image.png)
Substitution was also tolerated; methyl (12j)- and nitrile (12k)-substituted analogs were equipotent to the non-substituted lead compound (12c), whereas a larger dimethylamide group (12l) had an adverse effect on potency.

The benzoxazole analogs with optimal activity in the cell-based assay were examined for their physicochemical and in vitro ADME properties (Table 2). Many of the benzoxazole compounds (12e, 12i, 12j) retained the low tendency for CYP DI observed with 12c. Moreover, the benzoxazole compounds widely showed a higher membrane permeability compared to the prior triazolopyridine lead compound. Being aware of the low solubility at neutral pH observed with 12c, we investigated to see if the improvement of solubility was possible within this template. Incorporation of a pyrazole group in the right-hand side region C (12e) led to decrease in logD, which improved solubility at neutral pH and resulted in moderate membrane permeability. However, incorporation of a pyrazole ring in the left-hand side region A (12ca) was not beneficial, resulting in low solubility at both acidic and neutral pH, along with lower murine metabolic stability. Substitution on the central benzoxazole core structure (12i and 12j) showed a similar tendency of ADME properties as 12c being poorly soluble at neutral pH, whereas the nitrile analog (12k) additionally showed a relatively low solubility at acidic pH.

Several selected analogs were also examined for their in vivo pharmacokinetic properties in mice (Table 3). To consider the influence of low solubility at neutral pH on the absorption, oral suspensions of evaluation compounds were prepared using 0.5% methylcellulose without addition of any solubilizing agents and administered to mice. As a result, benzoxazole lead compound 12c exhibited the highest exposure as seen in the area under curve [AUC]. Compound 12c had a shorter half-life (t½) than 12c, thus resulting in lower AUC than 12c. Moreover, compounds 12l, j had a lower maximum drug concentration (Cmax) in addition to shorter t½, resulting in a lower AUC than compound 12c. Obtained results suggested that low solubility of 12c at neutral pH (0.1 µg/mL in pH 7.4) was unlikely the critical issue of oral absorption, and 12c is highly likely well dissolved under the acidic condition (640 µg/mL in pH 1.2) in the stomach and subsequently absorbed in the intestine.

Encouraged by the satisfactory overall profile of compound 12c, we evaluated the ability of 12c to boost NAD⁺ levels in mice (Fig. 3). Administration of a single oral dose (30 or 100 mg/kg) of compound 12c to diet-induced obese (DIO) mice elicited a significant increase in liver, gastrocnemius, and soleus tissue NAD⁺ levels compared with the vehicle control group.

Next, we examined the effect of compound 12c on body weight in order to assess its therapeutic potential as a treat-
ment for obesity (Fig. 4). Oral administration of 30 mg/kg compound 12c once a day for 21 d resulted in a continuous and significant decrease in body weight compared with the vehicle control group. The compound was well tolerated, with no overt toxicity during the study. Because there was no difference in cumulative food intake between the two groups (data not shown), we hypothesize that the increases in NAD⁺ levels elicited by compound 12c led to enhanced energy metabolism, culminating in a reduction in body weight.

**Conclusion**

In summary, optimization of our prior lead compound 2 led to the discovery of a series of benzoazole derivatives as potent NAMPT activators, culminating in the identification of compound 12c (DS68702229) as a promising lead compound. Compound 12c activated NAMPT *in vitro* and had an excellent pharmacokinetic profile in mice. This corresponded to a significant increase in tissue NAD⁺ levels in mice after the oral administration of compound 12c. Chronic oral dosing with compound 12c led to a robust decrease in body weight in DIO mice, demonstrating its use as a tool to explore the *in vivo* effects of pharmacological NAMPT activation. These results indicate that the use of NAMPT activators could be a promising approach for anti-obesity drugs.

**Experimental**

**General** Unless noted otherwise, materials were obtained from commercial suppliers and used without further purification. ¹H-NMR spectra were recorded on a Unity Mercury Plus 400 or 500 spectrometer (Varian) and chemical shifts are given in ppm from tetramethylsilane (TMS) as an internal standard. Low-resolution mass spectroscopy was conducted on an Agilent Infinity 1260 LC/MS system. TLC was performed on Merck precoated TLC glass sheets with silica gel 60F254. Compounds were separated by column chromatography using Chromatorex Q-pack silica gel (Fuji Sylissia Chemical, 30 μm). High-resolution (HR) MS was performed using an LC/MS system comprising a Waters Xevo Q-ToF MS and an Acquity UHPLC system. Elemental analyses were performed on a Microcorder JM10 and a Dionex ICS-1500 system. The purity of compounds was confirmed as >95% based on a diode array detector (DAD) signal area obtained using an Agilent Infinity 1260 LC/MS system. Conditions were; column, Develosil Combi-RP-5 2.0 mm ID ×50 mm L; gradient elution, 0.1% HCO₂H–H₂O/0.1% HCO₂H–MeCN = 98/2 to 0/100 (v/v); flow rate, 1.2 mL/min; UV detection, 254 nm; column temperature, 40°C; ionization: atmospheric pressure chemical ionization (APCI)/electrospray ionization (ESI).

**General Procedure for Urea Formation (12a, b, c, e, i, j, k, l)** (general Procedure A; Carbamate Method) Aniline (0.30 mmol) and (4-nitrophenyl)-N-(4-pyridylmethyl)carbamate (92 mg, 0.33 mmol) were suspended in 1,4-dioxane (2 mL), and then N,N-disopropylethylamine (0.058 mL, 0.33 mmol) was added to the suspension. The mixture was stirred at 50°C for 2 h. After cooling to r.t., the precipitated solid was collected by filtration and washed with diethyl ether to give the title compound (12a, b, c, e, i, j, k, l) as a solid.

1-(2-Phenyl-1H-benzimidazol-6-yl)-3-(pyridin-4-ylmethyl)urea (12a)

Prepared according to general procedure A (yield: 25%). ¹H-NMR (dimethyl sulfoxide (DMSO)-d₆) δ: 4.36 (d, J = 6.2 Hz, 2H), 6.70 (t, J = 6.2 Hz, 1H), 7.05 (br s, 1H), 7.32 (d, J = 5.9 Hz, 2H), 7.43–7.57 (m, 4H), 7.88 (br s, 1H), 8.12 (d, J = 7.0 Hz, 2H), 8.52 (d, J = 5.9 Hz, 2H), 8.74 (br s, 1H), 12.69 (br s, 1H). ¹³C-NMR (DMSO-d₆) δ: 41.8, 114.3, 122.0, 126.1, 128.9, 129.4, 135.6, 149.4, 149.7, 150.6, 155.6; Anal. Calcd for C₂₀H₁₇N₅O: C, 63.37; H, 5.44; N, 18.46. HRMS (ESI): Calcd for C₂₀H₁₇N₅O
Prepared according to general procedure A (yield: 65%). 1H-NMR (DMSO-δ6): δ = 4.36 (d, J = 6.3 Hz, 2H), 6.80 (t, J = 5.9 Hz, 1H), 7.30–7.37 (m, 3H), 7.57–7.69 (m, 4H), 7.96 (d, J = 2.0 Hz, 1H), 8.14–8.23 (m, 2H), 8.50–8.55 (m, 2H), 8.92 (s, 1H); 13C-NMR (DMSO-δ6): δ = 41.9, 108.4, 110.5, 116.6, 121.9, 126.5, 127.1, 131.2, 123.1, 137.6, 141.8, 145.3, 149.5, 149.9, 155.5, 162.6; Anal. Cacld for C21H16N5O2·0.247H2O: C, 67.47; H, 4.18; N, 18.37. Found: C, 67.74; H, 4.34; N, 18.47. HRMS (ESI): Calcld for C21H15N5O2: [M + H]+: 370.1304. Found 370.1311.

Dimethyl-2-phenyl-1H-pyrazol-5-yl)-1,3-benzoxazol-6-yl[carbamoyl] amino]-1,3-benzoxazole-4-carboxamide hydrochloride was obtained from tert-butyl (4-methyl-2-phenyl-1,3-benzoxazol-6-yl)[carbamoyl] amino]-1,3-benzoxazole-4-carboxamide hydrochloride obtained above (yield: 84% over two steps). 1H-NMR (DMSO-δ6): δ = 2.89 (s, 3H), 3.09 (s, 3H), 4.37 (d, J = 6.1 Hz, 2H), 6.92 (t, J = 6.1 Hz, 1H), 7.27–7.37 (m, 3H), 7.55–7.67 (m, 3H), 8.06 (d, J = 2.0 Hz, 1H), 8.17 (brd, J = 7.9, 1.6 Hz, 2H), 8.52 (d, J = 6.1 Hz, 2H), 9.16 (s, 1H); 13C-NMR (DMSO-δ6): δ = 34.6, 38.8, 41.9, 100.5, 113.9, 122.0, 126.3, 127.1, 128.2, 132.1, 137.4, 140.3, 145.2, 149.5, 150.4, 155.2, 161.8, 166.7; Anal. Cacld for C23H17N3O3·H2O: C, 63.39; H, 5.38; N, 16.07. Found C, 64.59; H, 5.47; N, 15.94. HRMS (ESI): Calcld for C23H17N3O3·H2O: [M + H]+: 416.1723. Found 416.1722.

General Procedure for Urea Formation (12d, ch, cc, cd, f, g, h) (General Procedure B; Triphosgene Method)
Aniline (0.30 mmol) and triethylamine (0.13 mL, 0.90 mmol) were dissolved in dichloromethane (3 mL) and cooled to 0°C. Triphosgene (31 mg, 0.11 mmol) was then added to the solution.
tion. The mixture was stirred for 10 min. Arylmethylamine (0.33 mmol) was then added, and the mixture was stirred for 1 h. The reaction solution was poured into water (30 mL), followed by partition using ethyl acetate. The organic phase was washed with brine, then dried over magnesium sulfate and filtered. Then, the solvent was distilled off under reduced pressure to give the crude title compound. The crude product was purified by silica gel column chromatography to give the title compound (12c, ee, cd, d, f, g, h) as a solid.

1-(1,3-Oxazol-5-ylmethyl)-3-(2-phenyl-1,3-benzoxazol-6-yl)urea (12b)

Compound 12b was prepared according to general procedure B (yield: 66%). 1H-NMR (CD3OD) δ: 4.50 (s, 2H), 7.06 (s, 1H), 7.18 (dd, J = 8.6, 2.0Hz, 1H), 7.53–7.61 (m, 4H), 8.03 (d, J = 2.0Hz, 1H), 8.16 (s, 1H), 8.17–8.22 (m, 2H); 13C-NMR (DMSO-d6) δ: 33.9, 99.8, 115.6, 119.5, 122.9, 126.6, 129.2, 131.4, 135.7, 138.5, 150.3, 150.7, 151.5, 154.9, 161.3; Anal. Calcd for C14H11N2O·0.117H2O: C, 64.26; H, 4.26; N, 15.74. HRMS (ESI): Calcd for C14H11N2O·0.117H2O [M + H]+: 214.1267. Found 214.1258.

1-(5-Chloro-2-phenyl-1,3-benzoxazol-6-yl)-3-(pyridin-4-ylmethyl)urea (12f)

Compound 12f was prepared according to general procedure B (yield: 52%). 1H-NMR (CD3OD) δ: 2.35 (s, 3H), 4.39 (d, J = 5.9Hz, 2H), 7.31–7.36 (m, 3H), 7.56–7.64 (m, 4H), 8.07 (s, 1H), 8.11–8.19 (m, 2H), 8.29 (s, 1H), 8.53 (d, J = 5.9Hz, 2H); 13C-NMR (DMSO-d6) δ: 18.4, 41.9, 102.0, 120.1, 122.1, 124.5, 126.7, 126.8, 129.2, 131.4, 136.1, 136.2, 149.0, 149.3, 149.6, 155.5, 161.4; Anal. Calcd for C14H11ClN2O·0.429H2O·C, 68.89; H, 5.19; N, 15.30. Found C, 68.79; H, 5.20; N, 15.36. HRMS (ESI): Calcd for C14H11ClN2O·0.429H2O·C [M + H]+: 359.1508. Found 359.1487.

1-(5-Chloro-2-phenyl-1,3-benzoxazol-6-yl)-3-(pyridin-4-ylmethyl)urea (12h)

Compound 12h was prepared according to general procedure B (yield: 26%). 1H-NMR (DMSO-d6) δ: 4.40 (d, J = 6.1Hz, 2H), 7.33–7.35 (m, 2H), 7.59–7.65 (m, 3H), 7.71 (t, J = 6.1Hz, 1H), 7.95 (s, 1H), 8.15–8.19 (m, 2H), 8.43 (s, 1H), 8.51–8.57 (m, 3H); 13C-NMR (DMSO-d6) δ: 41.8, 102.5, 118.8, 119.6, 122.0, 126.2, 127.1, 129.3, 131.9, 134.2, 136.3, 148.9, 149.3, 149.6, 155.0, 162.7; HRMS (ESI): Calcd for C14H11ClN2O·0.317H2O·C [M + H]+: 379.0962. Found 379.0956.

1-(2-(Pyridin-3-yl)-1,3-benzoxazol-6-yl)-3-(pyridin-4-ylmethyl)urea (12d)

Crude 2-(pyridin-3-yl)-1,3-benzoxazol-6-amine hydrochloride was obtained from tert-butyl [2-(pyridin-3-yl)-1,3-benzoxazol-6-yl]carbamate (4d) following the same procedure as for the synthesis of crude 2-(1-methyl-1H-pyrazol-5-yl)-1,3-benzoxazol-6-amine hydrochloride described in the synthesis of 12e.

The title compound was prepared according to general procedure B from crude 2-(pyridin-3-yl)-1,3-benzoxazol-6-amine hydrochloride obtained above (yield: 85% over two steps). 1H-NMR (400 MHz, DMSO-d6) δ: 4.37 (d, J = 6.0Hz, 2H), 6.87 (t, J = 6.0Hz, 1H), 7.26 (dd, J = 8.6, 2.0Hz, 1H), 7.29–7.33 (m, 2H), 7.60–7.66 (m, 1H), 7.70 (d, J = 8.6Hz, 1H), 8.12 (d, J = 2.0Hz, 1H), 8.49 (dt, J = 7.8, 2.2Hz, 1H), 8.51–8.54 (m, 2H), 8.77 (dd, J = 4.7, 1.6Hz, 1H), 9.13 (s, 1H), 9.31 (dd, J = 2.3, 0.8Hz, 1H); 13C-NMR (DMSO-d6) δ: 41.8, 99.7, 115.8, 119.7, 121.9, 123.0, 124.2, 132.4, 135.4, 139.0, 147.6, 149.4, 149.5, 150.8, 151.8, 155.3, 159.2; Anal. Calcd for C14H11N2O·0.741H2O·C, 63.62; H, 4.63; N, 19.52. Found C, 63.38; H, 4.36; N, 19.36. HRMS (ESI): Calcd for C14H11N2O·0.741H2O·C [M + H]+: 356.1304. Found 346.1274.

1-(2-Phenyl-1,3-benzoxazol-6-yl)-3-(1H-pyrazol-4-ylmethyl)urea (12ca)

To a solution of 2-phenylbenzoxazol-6-ylamine (12e) (0.28g, 1.3 mmol) in toluene (5 mL), 4-nitrophenyl chloroformate (0.26g, 1.3 mmol) was added. The suspension was stirred at 80°C for 1 h. The precipitate was filtered off to give the crude 4-nitrophenyl (2-phenyl-1,3-benzoxazol-6-yl)carbamate (14) (0.52g), which was used in the next reaction without further purification.

Crude 4-nitrophenyl (2-phenyl-1,3-benzoxazol-6-yl)carbamate (14) (0.15g) was added to a solution of 1H-pyrazol-4-ylmethylamine dihydrochloride (0.14g, 0.80mmol) and N,N-disopropyl ethylamine (0.28mL, 1.6mmol) in methanol (3mL) at 0°C. The reaction mixture was warmed to 50°C and stirred at that temperature for 30 min. After cooling to r.t., the precipitate was filtered off and washed with ethyl acetate and methanol to afford the title compound (94mg, 71% over two steps) as a solid.

1H-NMR (DMSO-d6) δ: 4.19 (d, J = 5.5Hz, 2H), 6.43 (t, J = 5.5Hz, 1H), 7.17 (dd, J = 8.8, 2.0Hz, 1H), 7.46 (brs, 1H), 7.55–7.69 (m, 5H), 8.10 (d, J = 2.0Hz, 1H), 8.13–8.19 (m, 2H), 8.78 (s, 1H), 12.67 (brs, 1H); 13C-NMR (DMSO-d6) δ: 33.7, 99.5, 115.4, 118.4, 119.5, 126.7, 126.8, 129.2, 131.4, 135.5, 138.8, 150.8, 155.0, 161.1; Anal. Calcd for...
t-Butyl [2-(Pyridin-3-yl)-1,3-benzoxazol-6-y]carbamate (4d)  t-Butyl N-(1,3-benzoxazol-6-yl)carbamate (3) (0.11 g, 0.47 mmol), 4,6-bis(diphenylphosphino)phenoxazine (40 mg, 0.072 mmol), and palladium (II) acetate (38 mg, 0.20 mmol) were added to the solution, and the mixture was stirred at r.t. overnight. Then, 5-bromo-1-methylnium chromatography (silica gel, ethyl acetate : hexane = 20 : 100 to 60 : 40) to give the title compound (0.13 g, 48%) as a solid.

5-Chloro-2-phenyl-1,3-benzoxazol-6-amine (4i)  5-Chloro-6-nitro-2-phenyl-1,3-benzoxazole (5f) (0.35 g, 1.5 mmol) following the same procedure as for the synthesis of 4f.

6-Nitro-2-phenyl[1,3]oxazolo[4,5-b]pyridin-6-amine (6i)  2-Amino-5-nitropyridin-3-ol (7) (0.30 g, 1.9 mmol) and benzoic acid (0.24 g, 1.9 mmol) were dissolved in polyphosphoric acid (3 mL), and the solution was heated at 160 °C for 4 h. The reaction solution was poured into ice water (20 mL), followed by partition using dichlormethane. The organic phase was washed with brine, then dried over sodium sulfate and filtered. Then, the solvent was distilled off under reduced pressure to give the crude title compound. The residue obtained was purified by silica gel column chromatography (ethyl acetate : hexane = 20 : 60 to 60 : 40) to give the title compound (43 mg, 30%) as a solid.

6-Chloro-2-phenyl[1,3]oxazolo[4,5-b]pyridin-6-amine (6j)  2-Methoxy-5-nitropyridine (8) (0.20 g, 1.2 mmol) in DMF (2 mL) was stirred at 50 °C for 1 h. The reaction solution was filtered through Celite. Then, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (silica gel, ethyl acetate : hexane = 1 : 10 to 1 : 100) to give the title compound (0.13 g, 48%) as a solid.

1H-NMR (CDCl 3) δ: 7.54–7.68 (m, 3H), 7.91 (s, 1H), 8.19 (s, 1H), 8.25–8.31 (m, 2H).

5-Chloro-2-phenyl-1,3-benzoxazol-6-amine (4h)  5-Chloro-6-nitro-2-phenyl-1,3-benzoxazole (6h) (0.32 g, 1.2 mmol) was dissolved in ethanol (3 mL) and ethyl acetate (3 mL). Platinum (IV) oxide (30 mg) was added to this solution. After purging of the reaction container with hydrogen, the mixture was stirred at 50 °C for 1 h. The reaction solution was filtered through Celite. Then, the filtrate was concentrated under reduced pressure to give the crude title compound. A small amount of ethyl acetate: dichlormethane (1 : 1) was then added. Then, the precipitated solid was collected by filtration to give the title compound (0.13 g, 48%) as a solid.

1H-NMR (CDCl 3) δ: 4.23 (brs, 2H), 6.97 (s, 1H), 7.45–7.53 (m, 3H), 7.66 (s, 1H), 8.13–8.20 (m, 2H).

2-Phenyl[1,3]oxazolo[4,5-b]pyridin-6-amine (4i)  The title compound (0.15 g, 67%) was obtained from 6-nitro-2-phenyl-1,3-benzoxazole (5f) (0.35 g, 1.5 mmol) following the same procedure as for the synthesis of 4f. 1H-NMR (CDCl 3) δ: 7.32 (d, J = 2.9 Hz, 1H), 7.52–7.62 (m, 4H), 7.97 (d, J = 2.3 Hz, 1H). MS (ESI): m/z 242 [M + H]+.

2-Phenyl[1,3]oxazolo[4,5-b]pyridin-6-amine (4j)  The title compound (0.15 g, 67%) was obtained from 6-nitro-2-phenyl-[1,3]oxazolo[4,5-b]pyridine (6i) (0.26 g, 1.1 mmol) following the same procedure as for the synthesis of 4f. 1H-NMR (CD 2OD) δ: 7.32 (d, J = 2.9 Hz, 1H), 7.52–7.62 (m, 4H), 7.97 (d, J = 2.3 Hz, 1H). MS (ESI): m/z 242 [M + H]+.

2-Methoxy-5-nitropyridine (8) (0.20 g, 1.2 mmol) in DMF (2 mL) was stirred in ice bath, and then NBS (0.23 g, 1.3 mmol) was added. The solution was stirred at r.t. for 3 h. The reaction solution was poured into saturated aqueous ammonium chloride solution (20 mL), followed by partitioning using ethyl acetate. The organic phase was washed with brine, then dried over magnesium sulfate and filtered. The solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography (silica gel, ethyl acetate : hexane = 0 : 100 to 100 : 0) to give the title compound (0.26 g, 56%) as a solid.
with ethyl acetate. The combined organic phase was washed
reaction was quenched with water (10 mL) and then extracted (0.022 g, 0.088 mmol) were suspended in toluene (10 mL). The
filtration to give the title compound (0.13 g, 36% over three
steps) as a solid.

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filtration to give the title compound (0.13 g, 36% over three
steps) as a solid.
over two steps) as a solid.

$^1$H-NMR (CDCl$_3$) δ: 3.01 (s, 3H), 3.24 (s, 3H), 7.49–7.62 (m, 4H), 7.79 (d, J = 1.7Hz, 1H), 8.22–8.28 (m, 2H). MS (ESI): m/z 345 [M + H]$^+$. 

**tert-Butyl [4-(Dimethylcarbamoyl)-2-phenyl-1,3-benzoxazol-6-yl]carbamate (4)** The title compound (0.12 g, 76%) was obtained from 6-bromo-$N,N$-dimethyl-2-phenyl-1,3-benzoxazole-4-carboxamide (6) (0.15 g, 0.42 mmol) following the same procedure as for the synthesis of 4j.

$^1$H-NMR (CDCl$_3$) δ: 1.55 (s, 9H), 3.02 (s, 3H), 3.23 (s, 3H), 6.82 (brs, 1H), 7.17 (d, J = 2.0Hz, 1H), 7.45–7.57 (m, 3H), 8.04–8.11 (m, 1H), 8.18–8.26 (m, 2H). MS (ESI): m/z 382 [M + H]$^+$. 

**Biology. 1. Measurement of NAMPT Enzyme Activating Effect (In Vitro Cell-free Enzyme Assay)** The NAMPT enzyme assay was conducted in accordance with the method of Formentini et al. by chemically converting nicotinamide mononucleotide (NMN) produced by the NAMPT enzyme to a fluorescent substance and using the fluorescence intensity of this fluorescent substance as an index for the amount of NMN produced. Hereinafter, the procedures will be briefly described. The NAMPT enzyme reaction was carried out using polypropylene 384 well V shaped black plate (Greiner Bio One International GmbH). The NAMPT activity was measured in assay buffer containing 50 mM Tris-KCl, pH 7.5, 5 mM MgCl$_2$, 1 mM TCEP, 0.1% Prionex, 0.005% Tween 20, 0.12 mM ATP, 5 µM nicotinamide (NAM), 6.25 µM phospho-ribose pyrophosphate (PRPP), 0.04 U/mL pyrophosphatase and 2ng/mL human NAMPT enzyme in the presence or absence of test compounds. After the 1-h incubation at 25 °C, the enzyme reaction was terminated by the addition of 50 µL of 2M KOH and 5 µL of a 20% acetonphenol solution. Then, 22 µL of 88% formic acid was added and the mixture was further incubated for 30 min in the dark. The NAMPT enzyme activity was calculated as the difference between fluorescence intensity (Ex 380 nm/Em 450 nm) from the reaction of a test compound treatment group and fluorescence intensity from control reaction free from the NAMPT enzyme.

$$\text{[NAMPT enzyme activity]} = \text{[Fluorescence intensity of the test substance treatment group]} - \text{[Mean fluorescence intensity of the control reaction free from the NAMPT enzyme]}$$

The EC$_{50}$ of the tested compounds were calculated using GraphPad Prism (GraphPad Software, Inc.), and the Ex$_{50}$ were calculated as percentage of the maximal activity achieved with 30 µM of compound I.

**2. Study on Effect of NAMPT Activator on the Intracellular NAD$^+$ Level (In Vitro Cell-Based Assay)** HEK293A cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM (high glucose)) containing 10% fetal bovine serum (FBS), non-essential amino acid (NEAA), and antibiotic-antimycotic [reaction medium]. On the day before the test, HEK293A cells were inoculated at a ratio of $3.0 \times 10^4$ cells/80 µL to 96 well poly-Ø-lysine coated plate and cultured overnight in a CO$^2$ incubator. On the day of the test, each test compound diluted with reaction medium to a concentration of 5 times the final concentration was added at a ratio of 20 µL/well, and the cells were further cultured for 3 h. After removal of the medium, the cells were lysed in 50 µL/well of Bicarbonate Base Buffer (100 mM sodium carbonate, 20 mM sodium bicarbonate, 100 mM nicotinamide, 20 mM Triton X-100, 1% dodecyl trimethyl-ammonium bromide (DTAB)). The NAD$^+$ content in the cell extract was measured using NAD$^+/$/NADH-Glo assay kit according to the manufacturer’s instructions. Hereinafter, the procedures will be briefly described. 20 µL of the lysate was separated, and 10 µL of 0.4 N HCl was added thereto, followed by incubation at 60 °C for 15 min and subsequently at 25 °C for 10 min. The mixture was neutralized by the addition of 10 µL of 0.5 M Trizma base solution and then diluted 10-fold with distilled water. Fifteen microliters of the diluted sample was mixed with an equal amount (15 µL) of NAD$^+/$/NADH-Glo reagent in 384 well black plate, and the mixture was incubated for 30 min in the dark. Subsequently, the chemiluminescence intensity was measured.

**3. Evaluation of NAMPT Activator on Tissue NAD$^+$ Level in Diet-Induced Obese (DIO) Mice** Compound 12c prepared with 0.5% methylcellulose was orally administered at 30 or 100 mg/kg to 47-week-old male C57BL/6N DIO mice (high-fat loading obesity mouse models) having a pathological obese condition induced by purified high-fat diet (D12451) from 7 weeks of age. Twenty-four hours after the administration, each mouse was sacrificed by exsanguination. The liver, gastrocnemius, soleus, and brown adipose tissues were harvested. Each tissue was homogenized with a 10- or 100-fold amount of 2 M perchloric acid per its weight. Then, the samples were centrifuged, and the supernatant was neutralized with 5 M sodium hydroxide. After removal of potassium perchlorate, the NAD$^+$ concentration in the supernatant of sample was measured using LC/MS.

**4. Evaluation of Anti-obesity Effect of NAMPT Activator (DIO Mice)** Forty-five-week-old male C57BL/6N DIO mice were each acclimatized for 1 wk under individual housing. Then, the mice were divided into 2 groups each involving 6 mice with their weights, amounts of weight change, and food intakes during the acclimatization period as indexes. Each mouse was freely fed a purified high-fat diet (D12451) for 3 wk under individual rearing. Compound 12c prepared with 0.5% methylcellulose was orally administered at 30 mg/kg once a day for 3 wk from the start day of the test (a vehicle group was treated with 0.5% methylcellulose in the same way as above). The weight was compared between the test groups.

**PAMPA, LogD, Solubility, and Metabolic Stability Assay** Each assay was performed as previously described.

**CYP Direct Inhibition Assay** The reversible inhibition by compounds on the specific activities of 4 CYP isoforms (CYP1A2, CYP2C9, CYP2D6 and CYP3A4) were examined in the reaction mixture containing human liver microsome and a NADPH generating system. The microsomal protein concentration in the assay was 0.1 mg/mL. After CYP substrate was incubated at 37 °C for 10 min in the absence or presence of compounds (10 µmol/L), concentration of metabolites from CYP substrate was semi-quantitatively analyzed by LC-MS/MS and % inhibition was calculated by the following formula;

$$\text{[% inhibition]} = \frac{100 - \text{[metabolite concentration with compound]}}{\text{[metabolite concentration without compound]}} \times 100$$
Pharmacokinetic Evaluation in Mice  The test compounds suspended with vehicle (0.5% (w/v) methyl cellulose 400 solution (Wako Pure Chemical Corporation, Osaka, Japan) were orally administered to 7-week-old male C57BL/6J mice at a dose of 10mg/kg. Plasma samples were collected from the peripheral vein at several time points after dosing. The plasma concentration was determined by liquid chromatography-tandem mass spectrometry (LC-MS) to obtain PK parameters via non-compartmental analysis.

Conflict of Interest  The authors declare no conflict of interest.

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