HETEROCYCLES, Vol. 104, No. 1, 2022, pp. 94 - 122. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 27th September, 2021, Accepted, 15th October, 2021, Published online, 21st October, 2021
DOI: 10.3987/COM-21-14559

OPTIMIZATION OF NAMPT (NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE) ACTIVATORS: DISCOVERY OF *N,N*-DIETHYL-1,2-BENZOXAZOLE-3-CARBOXAMIDE DERIVATIVES AS POTENT NAMPT ACTIVATORS WITH MITIGATED MUTAGENIC RISKS

Mayuko Akiu,[a]* Takashi Tsuji,[a] Yoshitaka Sogawa,[a] Koji Terayama,[a] Mika Yokoyama,[a] Daigo Asano,[a] Tomohiro Honda,[a] Tomomichi Ishizaka,[a] Tomoko Hasegawa,[a] Anthony B. Pinkerton,[b] and Tsuyoshi Nakamura[a]

[a]R&D Division, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b]Sanford Burnham Prebys Medical Discovery Institute, 10901 North Torrey Pines Road, La Jolla, California 92037, USA
[*]Corresponding author: akiu.mayuko.bs@daiichisankyo.co.jp

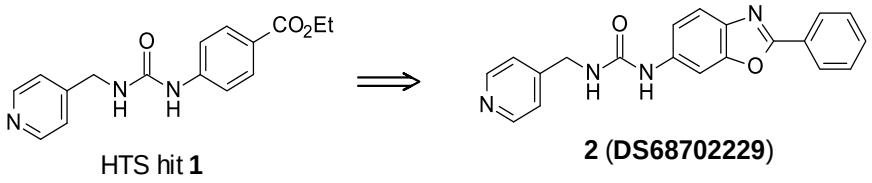
Abstract – **DS68702229**, a potent NAMPT activator developed from HTS followed by a hit-to-lead campaign, is a promising candidate compound that significantly reduced body weight when orally administered to mice with high fat diet-induced obesity. However, *in vitro* toxicology profiling of **DS68702229** revealed bacterial mutagenicity using *Salmonella typhimurium* TA98 and TA100 strains upon S9 activation. Hypothesizing that DNA intercalation is the likely cause, we employed several approaches to disrupt the putative DNA intercalation, including modulation of the molecular shape. Our efforts culminated in the discovery of compounds **20k** and **20l**, which increased intracellular NAD^+ levels in a cell-based assay without inducing mutagenicity, along with acceptable plasma exposure in mice after oral administration.

INTRODUCTION

Nicotinamide adenine dinucleotide (NAD^+) is a ubiquitously expressed biomolecule involved in energy-producing processes such as mitochondrial oxidative phosphorylation, β-oxidation, glycolysis, and the citric acid cycle.[1-5] NAD^+ is also utilized as a cosubstrate by enzymes such as poly-ADP-ribose

polymerases, sirtuins, and cADPR synthases.[1-5] Cleavage of the NAD^+ ribose–nicotinamide bond occurs during the process of these enzymatic reactions, thus consequently liberating nicotinamide (NAM).[1-5] Therefore, replenishment of NAD^+ is necessary for cell survival. In mammalian cells, the dominant pathway for NAD^+ biosynthesis is the NAD^+ salvage pathway, which recycles NAM to NAD^+.[1-5] Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the first step of the recycling of NAM to NAD^+, which is the condensation of NAM with 5-phosphoribosyl-1-pyrophosphate (PRPP), resulting in the formation of nicotinamide mononucleotide (NMN).[1-5] Given that NAMPT is the rate-limiting enzyme in this NAD^+ salvage pathway, NAMPT activation is expected to enhance the biosynthesis of NAD^+ and consequently increase cellular NAD^+ levels, which is suggested to be beneficial for treating a wide range of diseases, including type 2 diabetes mellitus and obesity.[5,6]

In our recent publication, we described the development of potent NAMPT activators from high-throughput screening (HTS) hit **1**, which led to our candidate compound **2** (**DS68702229**) (Figure 1).[7] Compound **2** potently increased cellular NAD^+ levels and exhibited an excellent pharmacokinetic profile in mice after oral administration. A single oral dose (30 or 100 mg/kg) of compound **2** to high-fat diet-induced obese (DIO) mice increased NAD^+ levels in various tissues such as the liver, gastrocnemius, and soleus. Moreover, compound **2** exhibited continuous and significant body weight reduction when orally administered (30 mg/kg) to DIO mice once a day for 21 days. Encouraged by these results, compound **2** was further profiled to assess its *in vitro* toxicology, which unfortunately increased mutagenic risks in a six-well Ames test (a miniaturized version of the standard Ames test). This test was strongly positive upon metabolic activation, showing more than 2-fold increase of revertant counts in histidine-depleted *Salmonella typhimurium* strains TA98 and TA100 at all evaluated compound concentrations when activated with rodent liver extract (S9) (Figure 1).[8] We therefore continued optimization around this series to remove the risk. Herein, we report our medicinal chemistry effort to overcome the mutagenicity risk, culminating in the discovery of compounds **20k** and **20l**, which were devoid of mutagenicity while concomitantly exhibiting favorable potency, ADME, and pharmacokinetic profiles.



6-well Ames test	S9-	S9+
TA 98	weakly positive (1000)[a]	positive (1.95)[a]
TA 100	negative	positive (1.95)[a]

Figure 1. Result of the six-well Ames test (TA98 and TA100) of compound **2** (**DS68702229**). "Positive" indicates a more than 2-fold increase of the number of revertant colonies compared with the vehicle in addition to a concentration-specific increase. [a]Minimum concentration (μg/well) that increased the number of revertant colonies by more than 2-fold versus the vehicle.

RESULTS AND DISCUSSION

The retrosynthesis of derivatives possessing the (pyridin-4-ylmethyl)urea moiety is depicted in Figure 2. The target molecules were synthesized from the corresponding aniline intermediates via several urea-forming methods. The aniline intermediates with various 6, 5-ring systems were mainly provided by reducing the NO_2 group of the corresponding NO_2 derivatives, or by the Pd-catalyzed amination of the corresponding Br derivatives.

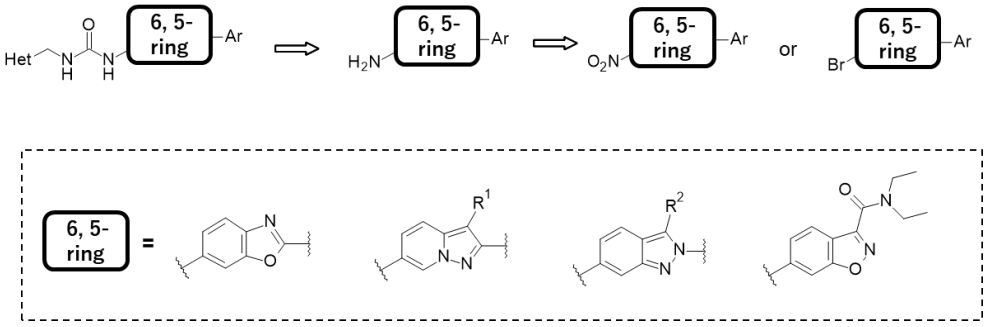


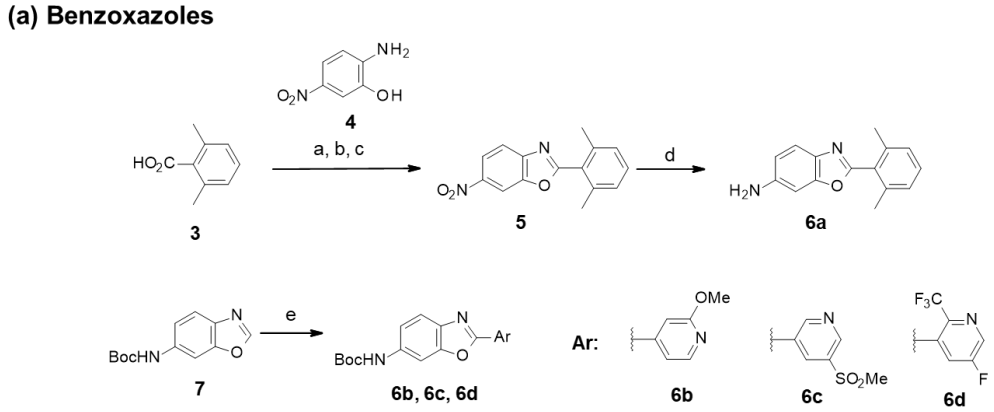
Figure 2. Retrosynthesis of the compounds possessing (pyridin-4-ylmethyl)urea moiety

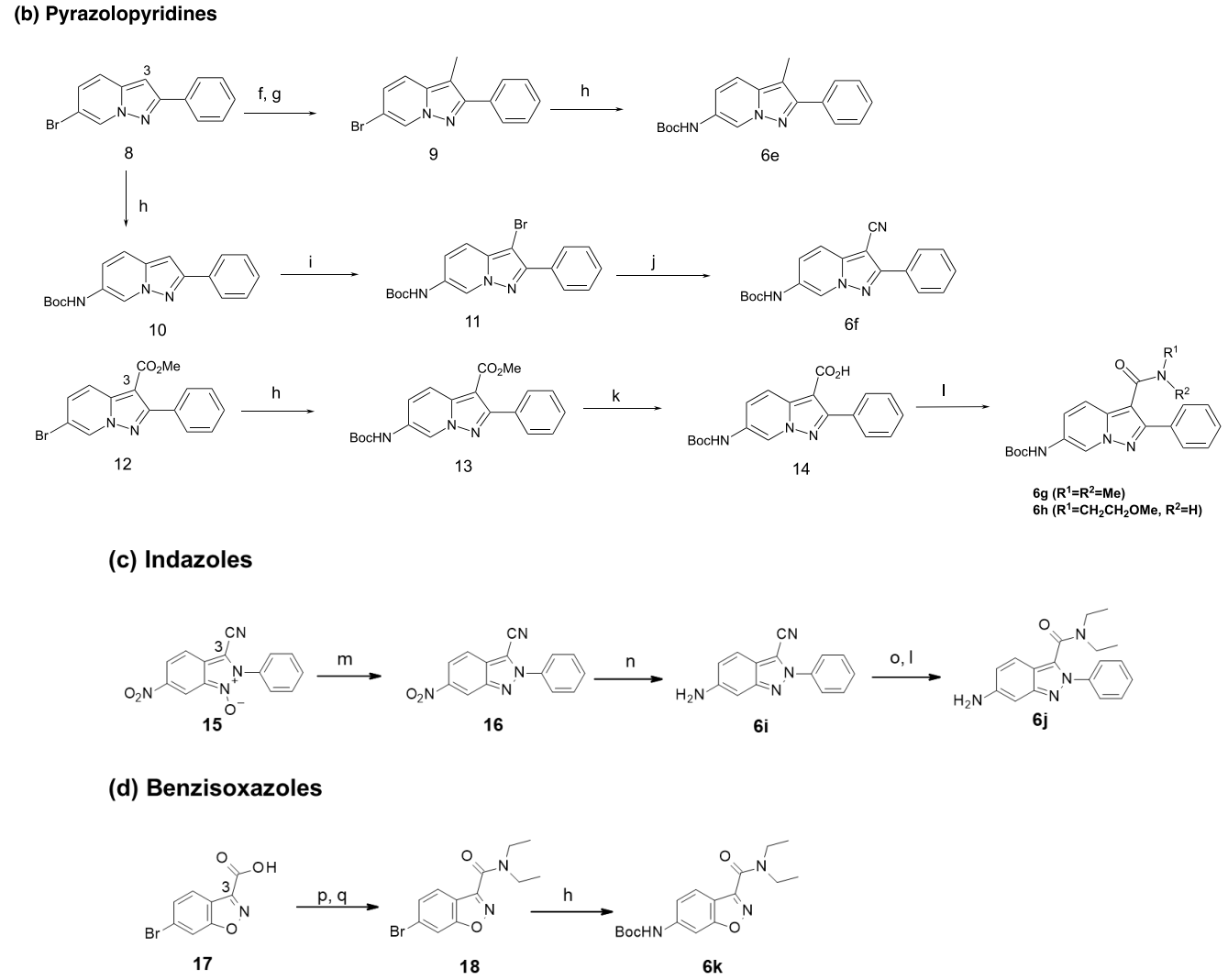
Following the retrosynthesis, the compounds in Table 1 were prepared (Schemes 1 and 2). Scheme 1 depicts the preparation of the aniline intermediates **6a-m**. Concerning benzoxazole intermediate **6a**, the carboxyl group of commercially available **3** was converted to the acyl chloride, which was reacted with 2-amino-5-nitrophenol **4** and cyclized under acidic conditions to afford compound **5**. Subsequently, the nitro group of **5** was reduced by hydrogenation to afford the benzoxazole intermediate **6a**. 2-Arylated benzoxazole compounds **6b-d** were prepared from compound **7**[9] by palladium-catalyzed direct 2-arylation via a deprotonative cross-coupling process.[10]

As for the pyrazolopyridine intermediate **6e** with a methyl group substituted at the 3-position, compound **8**[11] was methylated at the 3-position to afford **9** via a two-step procedure reported by Igawa et al.,[12] which involves formylation at the 3-position followed by reduction of the formyl group. The bromine of **9** was converted to NHBoc via Pd-catalyzed amination[13] to afford the pyrazolopyridine intermediate **6e**. The pyrazolopyridine intermediate with a 3-cyano group (**6f**) was synthesized via the Pd-catalyzed amination of **8**, followed by subsequent bromination at the 3-position and conversion of the resulting bromine to a cyano group. Regarding the pyrazolopyridine intermediate with an amide at the 3-position, the bromine of compound **12**[11] was converted to NHBoc by Pd-catalyzed amination[13] followed by basic hydrolysis of the methyl ester to give the carboxylic acid **14**. Compound **14** was subjected to condensation with the corresponding amines to furnish the intermediates **6g** and **6h**.

The indazole intermediates **6i** and **6j** were synthesized starting from 6-nitro-1-oxido-2-phenylindazole derivative **15**,[14] which was reduced with triphenylphosphine to yield compound **16.** Compound **16** was subjected to subsequent reduction of nitro group to afford the cyano intermediate **6i**, or its cyano group was hydrolyzed to give the corresponding carboxylic acid and then coupled with diethylamine to afford the intermediate **6j**.

Aiming to synthesize the benzisoxazole intermediate **6k**, the carboxyl group present in 6-bromo-1,2-benzoxazole-3-carboxylic acid **17** was converted to diethylamide through a two-step procedure involving the formation of acyl chloride to furnish **18**. Compound **18** was then subjected to Pd-catalyzed amination to give the intermediate **6k**.



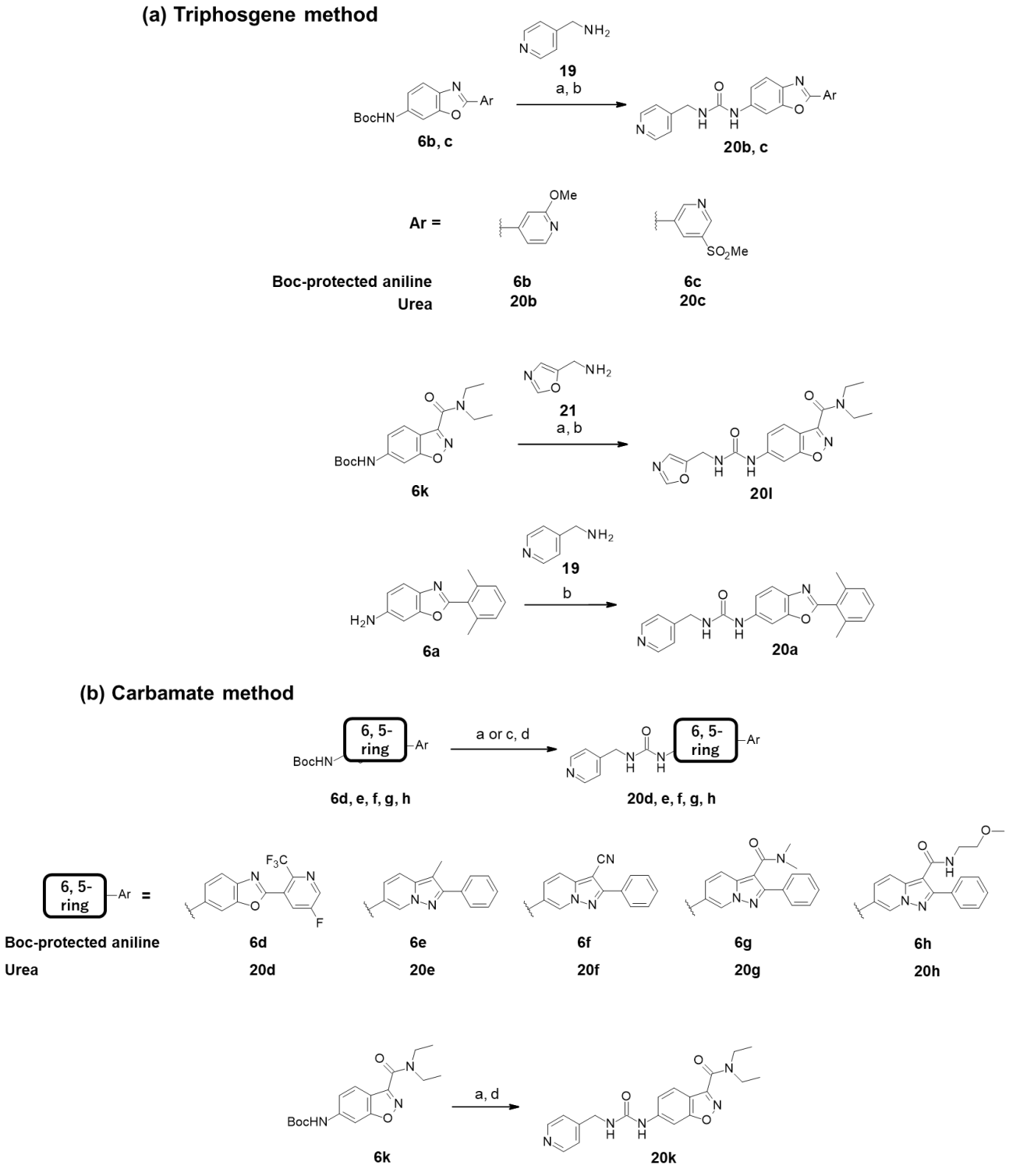


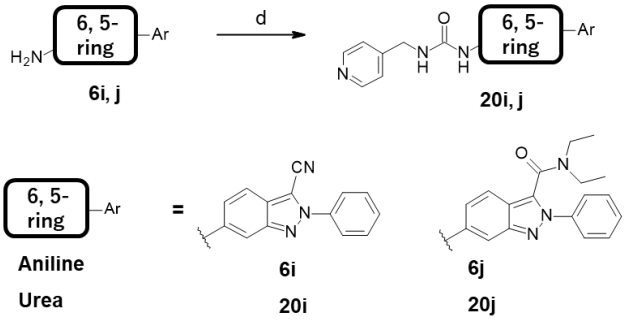
Scheme 1. Reagents and conditions: (a) thionyl chloride, toluene, 100 °C; (b) **4**, Et_3N, CH_2Cl_2; (c) *p*-TsOH, toluene, 100 °C, 40% over three steps; (d) H_2, 10% Pd/C, EtOH, AcOEt, 87%; (e) aryl bromide, NiXantphos, $Pd(OAc)_2$, Na(O*t*-Bu), 1,2-dimethoxyethane, 40 °C, 24%-quant.; (f) (chloromethylene)dimethyliminium chloride, MeCN; (g) triethylsilane, TFA, 55% over two steps; (h) *t*-butyl carbamate, *t*-butylXPhos, Pd_2dba_3, Na(O*t*-Bu), toluene, 57-85%; (i) NBS, DMF, 52%; (j) zinc cyanide, $Pd(PPh_3)_4$, DMF, 100 °C, 58%; (k) 5M NaOHaq, THF, MeOH, 55 °C, 63%; (l) amine, EDCI, HOAt, Et_3N, DMF, 82-85% (**6g**, **6h**), 81% over two steps (**6j**); (m) PPh_3, EtOH, 81%; (n) iron, NH_4Cl, EtOH, H_2O, 80 °C, 68%; (o) 5M NaOHaq, THF, MeOH, 100 °C; (p) oxalyl chloride, DMF, CH_2Cl_2; (q) Et_2NH, pyridine, CH_2Cl_2, 89% over two steps.

Scheme 2 illustrates the urea formation reactions of the aniline intermediates to afford the final compounds **20a**–**l**. Two methods were employed for the urea formation.

First is the triphosgene method (Scheme 2(a)). Boc-protected **6b-c** and **6k** were deprotected, and the obtained aniline intermediate was treated with triphosgene and coupled with the corresponding amine **19**

or **21** to provide **20b–c** or **20l**, respectively. Similarly, the aniline derivative **6a** was converted to the corresponding urea derivative **20a**. Second is the carbamate method (Scheme 2(b)). The Boc-protected aniline intermediates **6d–h** and **6k** were deprotected under acidic conditions and reacted with 4-nitrophenyl [(pyridin-4-yl)methyl]carbamate[15] to provide **20d–h** and **20k**, respectively. Similarly, compounds **6i** and **6j** were converted to the urea compounds **20i** and **20j**, respectively.





Scheme 2. Reagents and conditions: (a) 4M HCl in dioxane, CH_2Cl_2; (b) triphosgene, Et_3N, **19** or **21**, CH_2Cl_2, 85% (**20a**), 18-49% over two steps (**20b, c, l**), (c) TFA, CH_2Cl_2; (d) 4-nitrophenyl [(pyridin-4-yl)methyl]carbamate, Et_3N, 1,4-dioxane, 80 °C, 8-62% over two steps (**20d-h**, **m**), 27-72% (**20i**, **j**).

Since compound **2** was strongly positive in the six-well Ames test in the presence of S9, we suspected that an oxidative metabolite might be the potential cause of the mutagenicity. Accordingly, we conducted a structural analysis of metabolites by treating 1 mg/mL mouse or human microsomes with 10 μM compound **2** for 2 h. Although the major metabolite observed in both microsomes was identified as oxygenation occurring at pyridin-4-ylmethyl moiety (**A**), an additional ring-opened metabolite (**B**), which involves the hydrolysis of the benzoxazole ring, was also identified following treatment in human microsomes (Figure 3). As this metabolite **B** contains a para-dianiline structure, which could possibly form an electrophilic quinone diimine-like compound upon further oxidation, we hypothesized that this structure may covalently bind to DNA.[16,17]

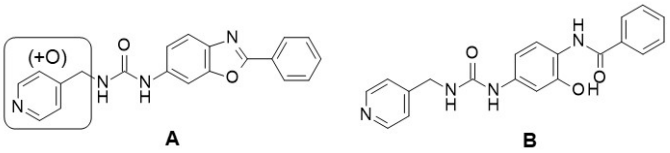
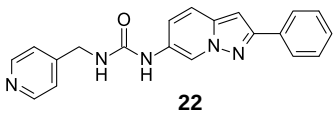


Figure 3. Structure of possible metabolites of compound **2**

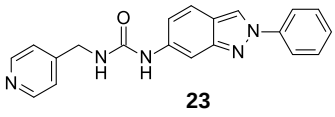
Based on the hypothesis that the benzoxazole ring might be the toxicophore, we decided to evaluate the mutagenicity of previously synthesized bicyclic NAMPT activators possessing alternative core structures[18] (Figure 4). Contrary to our expectations, indazole **22**[18] and pyrazolopyridine **23**,[18] both of which contain bicyclic structures that are less prone to ring opening via hydrolysis, were also six-well Ames-positive upon S9 activation. Given that the reported DNA intercalators are generally polycyclic, aromatic, and planar ligands, therefore being able to insert into the space between two adjacent base pairs,[19,20] we suspected that our series of compounds, possessing multiple consecutive aromatic rings with

a relatively planar structure, could possibly intercalate into DNA. Hence, we decided focus on overcoming the putative DNA intercalation liability within these series of compounds.



22

6-well Ames test	S9-	S9+
TA 98	negative	positive (1.95)[a]
TA 100	negative	positive (1.95)[a]



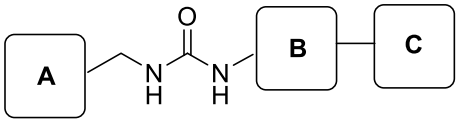
23

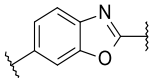
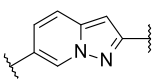
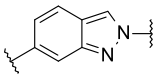
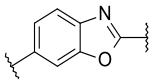
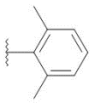
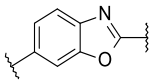
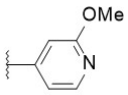
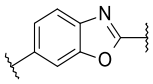
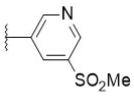
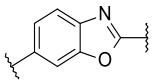
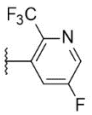
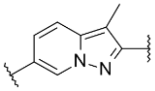
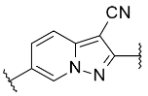
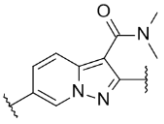
6-well Ames test	S9-	S9+
TA 98	negative	positive (1.95)[a]
TA 100	negative	positive (1.95)[a]

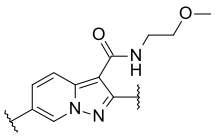
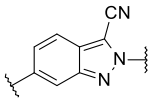
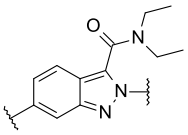
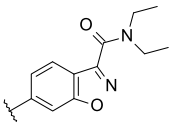
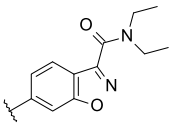
Figure 4. Results of the six-well Ames test (TA98 and TA100) for compounds **22** and **23**. "Positive" indicates that more than 2-fold increase of the revertant colony count compared with the vehicle was observed in addition to a concentration-specific increase. [a]Minimum concentration (μg/well) that induced a more than 2 -fold increase of the revertant colony count compared with the vehicle.

In general, structural modifications are effective for disrupting DNA intercalation as strategies for reducing mutagenic risk;[12,20,21] for example, the introduction of substituents disrupts the planarity of the compound to disfavor the stabilizing of π-π interaction with DNA, and modulation of the two-dimensional shape to a less-linear compound disrupts the fit of molecules into DNA. Being aware of these strategies, we first investigated the modification of the phenyl ring situated on the right-hand side of benzoxazole derivatives, pursuing alteration of the molecular shape (Table 1). To screen the mutagenic risk of the synthesized compounds efficiently, the six-well Ames test was conducted utilizing two test strains, namely TA98 and TA100, with metabolic activation by S9. Although introducing a 2, 6-dimethylphenyl group (**20a**) with the purpose of increasing steric bulk was unsuccessful, installment of a pyridine ring on the right-hand side (**20b**) proved to be partially successful, as the increase in the number of revertant colonies was less than 2-fold in the TA100 strain at all assessed concentrations. Additionally, the introduction of electron-withdrawing substituents into the pyridine ring effectively alleviated the mutagenic risk. Compound **20c** with a methylsulfonyl group on the pyridine ring tended to decrease mutagenicity, with 250 μg/well being the minimum concentration needed to increase revertant colony counts. Ultimately, compound **20d,** with an electron-withdrawing trifluoromethyl group at the ortho position and a fluorine atom substituted on the pyridine ring, successfully alleviated mutagenic risk. We assume that the *ortho*-CF_3 altered the planarity of the molecule, which, combined with its electron-withdrawing effect, led to the alleviation of mutagenic risk.

Table 1. EC_{50} (μM)/Emax (%) values of NAMPT enzyme assay[22] and mutagenicity risk of compounds **2**, **22**, **23**, and **20a–l**



Cpd	A	B	C	NAMPT enzyme assay	Six-well Ames[a] (S9+)	
					TA98	TA100
2				0.046 μM/153%	Positive[b] (1.95)[c]	Positive[b] (1.95)[c]
22				0.012 μM/135%	Positive[b] (1.95)[c]	Positive[b] (1.95)[c]
23				0.040 μM/155%	Positive[b] (1.95)[c]	Positive[b] (1.95)[c]
20a				0.30 μM/130%	Positive[b] (15.6)[c]	Positive[b] (62.5)[c]
20b				0.086 μM/123%	Positive[b] (1.95)[c]	Negative
20c				1.3 μM/119%	Positive[b] (250)[c]	Negative
20d				0.25 μM/89%	Negative	Negative
20e				0.067 μM/131%	Positive[b] (3.91)[c]	Positive[b] (1.95)[c]
20f				0.047 μM/142%	Positive[b] (1.95)[c]	Positive[b] (1.95)[c]
20g				0.11 μM/130%	Equivocal	Negative

Cpd	A	B	C	NAMPT enzyme assay	Six-well Ames[a] (S9+)	
					TA98	TA100
20h				0.39 μM/162%	Negative	Negative
20i				0.11 μM/225%	Positive[b] (0.49)[c]	Positive[b] (0.49)[c]
20j				0.054 μM/190%	Negative	Negative
20k			-	0.24 μM/140%	Negative	Negative
20l			-	0.18 μM/149%	Negative	Negative

[a]*Test concentration range of **2**, **22**, **23**, **20b**, **20c**, **20e**, **20f**, and **20g**: 1.95–1000 μg/well; **20a**, **20d**, **20h**, **20i**, **20j**, **20k**, and **20l:** 0.49–250 μg/well.*
[b]*More than 2-fold increase of the revertant colony count compared with the vehicle was observed in addition to a concentration-dependent increase.*
[c]*Minimum concentration (μg/well) that induced a more than 2-fold increase of the revertant colony count compared with the vehicle.*

In parallel to the aforementioned work, we also investigated the modification of alternative central core structures (Table 1). We first introduced substituents at the 3-position of the pyrazolopyridine ring of **22** to modify the two- and three-dimensionality of the molecule. Overall, the introduction of substituents at the 3-position was tolerated in terms of NAMPT activation activity, exhibiting potent activity of EC_{50} < 0.1 μM. Regarding mutagenicity, the introduction of a methyl (**20e**) or nitrile group (**20f**) was not beneficial, and both compounds were six-well Ames-positive in both the TA98 and TA100 strains. However, compound **20g** with a dimethylamide group displayed lower mutagenicity, resulting in negativity in the TA100 mutagenicity test. Notably, elongation of the amide substituent by introducing a methoxyethyl side chain (**20h**) successfully led to alleviation of the mutagenic risk in both the TA98 and TA100 strains.

Encouraged by the effects of substituents at the 3-position, we subsequently explored other bicyclic core structures with substituents at the corresponding 3-position (Table 1). Indazoles displayed a similar trend as pyrazolopyridines. Specifically, 3-cyano substitution resulted in six-well Ames positivity (**20i**), whereas the diethylamide derivative **20j** exhibited lower mutagenic risk while concomitantly displaying

potent NAMPT activation activity. Interestingly, the phenyl ring on the right-hand side was unnecessary for potent NAMPT activation activity in the presence of the 3-diethylamide motif (unlike our previous SAR with 6, 5-ring systems);[7,18] for example, the benzisoxazole derivative **20k** lacking the terminal phenyl ring exhibited potent activity and negativity in both the TA98 and TA100 assays. In line with our previous observations, a 5-ozaxoyl group at region A (**20l**) retained potent NAMPT activation, and resulted in six-well Ames test negativity in the TA98 and TA100 strains.

Compounds that potently increased NAMPT activity and alleviated the risk of mutagenesis were further characterized in a cell-based assay,[23] and their physicochemical and *in vitro* ADME properties were evaluated (Table 2). Compounds **20k** and **20l** were particularly potent in the cell-based assay, and both compounds exhibited favorable ADME properties including high membrane permeability and metabolic stability and an acceptable level of CYP direct inhibition (DI). Compounds **20k** and **20l** also exhibited adequate solubility in acidic and neutral media, together with a substantial free fraction (18%) in mouse plasma. Considering their potency and drug-like properties, compounds **20k** and **20l** were selected for further evaluation.

Table 2. EC_{50} (μM)/Emax (%) values in the cell-based assay[23] and physicochemical and *in vitro* ADME properties of compounds **20d**, **20h 20j**, **20k**, and **20l**

Cpd	Cell-based assay (HEK293A)	log*D*[a]	PAMPA permeability (pH 5.0, 7.4) (nm/s)	Solubility (pH 1.2, 6.8) (μg/mL)	Metabolic stability[b] (h, mou) (%)	CYP DI[c] (1A2/2C9/2D6/3A4) (%)	Plasma protein unbound[d] (%) (mou)
20d	1.1 μM/148%	3.5	>50, >50	840, 0.3	74, 68	0/17/7/16	2.4
20h	12%[e]	2.4	0.3, 1	860, 45	41, 26	15/48/23/42	NT[f]
20j	90%[e]	2.8	5.8, 18.7	800, 62	23, 1	19/50/44/78	NT[f]
20k	0.45 μM/152%	2.8	18.9, 43.7	>710, 93	74, 47	36/44/26/45	18
20l	0.85 μM/192%	2.1	19.5, 21.8	260, 150	83, 54	70/27/8/2	21

[a]*The distribution coefficients (logD) were measured between 1-octanol and phosphate-buffered saline (pH 7.4).*

[b]*The percent remaining value after the compound (1 μM) was incubated with human or mouse microsomes for 30 min.*

[c]*The percent inhibition after the compound (10 μM) was incubated with corresponding CYP isoforms for 10 min.*

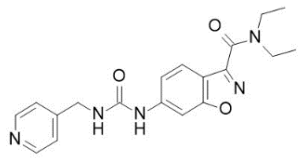
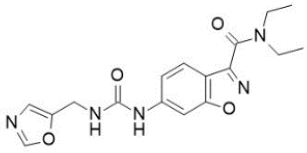
[d]*The percent unbound compound in mouse plasma.*

[e]*Percentage indicates the amount of NAD^+ increase achieved with 1 μM of the compound compared with that achieved with 30 μM compound* ***1.***
[f]*Not tested.*

Compounds **20k** and **20l** were subsequently evaluated in the standard Ames test utilizing five bacterial strains, including four *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and one *Escherichia coli* strain (WP2*uvrA*), both with and without S9 activation. Compounds **20k** and **20l** were found to be negative in all assay conditions, inducing a less than 2-fold increase of the revertant colony count compared with the vehicle at all test concentrations (9.77–5000 μg/plate).[24]

The results of pharmacokinetic studies are summarized in Table 3. The favorable ADME properties of compounds **20k** and **20l** successfully translated into acceptable plasma exposure in mice after oral administration, and we therefore selected **20k** and **20l** as lead compounds. Further *in vivo* evaluation of **20k** and **20l** will be reported in due course.

Table 3. Pharmacokinetic parameters of compounds **20k** and **20l** (C57BL/6N mice, *p.o.* administration,[a] 10 mg/kg)

Cpd	Structure	$T_{1/2}$ (h)	Cmax (μM)	$AUC^b_{0–24}$ (μM·h)
20k		0.7	7.2	12
20l		0.7	15	16

[a]*Compounds were administered as suspension in 0.5% methylcellulose.* [b]*Area under the curve.*

In summary, we have described the evolution of a urea-containing series of NAMPT activators to develop potent compounds with minimal mutagenic risk. Presuming that DNA intercalation was the cause of mutagenicity, we conducted a derivatization using a structure-based design hypothesis including modulation of the molecular shape. Increasing the steric bulk and introducing an electron-withdrawing substituent were found to be beneficial, and applying these methods to the benzoxazole, pyrazolopyridine, and benzindazole templates led to the acquisition of **20d**, **20h**, and **20j** with mitigated mutagenicity.

Moreover, the 3-diethylamide motif of **20j** enabled us to eliminate the phenyl ring in right-hand region C, which culminated in the discovery of the non-mutagenic benzisoxazole compounds **20k** and **20l**. Compounds **20k** and **20l** potently increased intracellular NAD^+ levels in the cell-based assay and displayed favorable ADME properties, which translated into acceptable oral exposure. Further *in vivo* evaluations of **20k** and **20l** will be reported in due course.

EXPERIMENTAL

General

Unless noted otherwise, materials were obtained from commercial suppliers and used without further purification. ^{1}H NMR spectra were recorded on a Unity Mercury Plus 400 or 500 spectrometer (Varian), and chemical shifts were given in ppm from tetramethylsilane as an internal standard. Low-resolution mass spectroscopy (MS) was conducted on an Agilent Infinity 1260 liquid chromatography (LC)/mass spectrometry (MS) system. Thin-layer chromatography (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 Silysia Chemical, 30 μm). High-resolution MS was performed using an LC/MS system comprising a Waters Xevo Q-ToF MS system 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 the diode array detector signal area obtained using an Agilent Infinity 1260 LC/MS system. The conditions were as follows: column, Develosil Combi-RP-5 2.0 mm ID × 50 mm L; gradient elution, 0.1% HCO_2H–H_2O/0.1% HCO_2H–MeCN = 98/2 to 0/100 (v/v); flow rate, 1.2 mL/min; UV detection, 254 nm; column temperature, 40 °C; and ionization, atmospheric pressure chemical ionization/electrospray ionization (ESI).

General procedure for urea formation (20a, b, c, l) (General procedure A: Triphosgene method)

Aniline (0.30 mmol) and triethylamine (0.13 mL, 0.90 mmol) were dissolved in CH_2Cl_2 (3 mL) and cooled to 0 °C. Triphosgene (31 mg, 0.11 mmol) was then added to the solution. 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 partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude title compound. The crude product was purified by silica gel column chromatography to give the title compound (**20a, b, c, l**) as a solid.

1-[2-(2,6-Dimethylphenyl)-1,3-benzoxazol-6-yl]-3-(pyridin-4-ylmethyl)urea (20a) Prepared according

to general procedure A (yield: 85%). ^{1}H NMR (DMSO-d_6) δ ppm 2.23 (s, 6 H) 4.36 (d, J = 6.0 Hz, 2 H) 6.85 (t, J = 6.0 Hz, 1 H) 7.26–7.20 (m, 3 H) 7.33–7.29 (m, 2 H) 7.34–7.43 (m, 1 H) 7.69 (d, J = 8.6 Hz, 1 H) 8.08 (d, J = 2.0 Hz, 1 H) 8.46–8.53 (m, 2 H) 9.07 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 19.9, 41.9, 99.9, 115.4, 119.5, 121.9, 127.6, 127.8, 130.3, 135.0, 137.9, 138.5, 149.4, 149.5, 150.5, 155.3, 160.8; Anal. Calcd for $C_{22}H_{20}N_4O_2 \cdot 0.559H_2O$: C, 69.08; H, 5.57; N, 14.65. Found C, 69.17; H, 5.55; N, 14.62; HRMS (ESI): Calcd for $C_{22}H_{20}N_4O_2$ $[M+H]^+$: 373.1665 found 373.1661.

1-[2-(2-Methoxypyridin-4-yl)-1,3-benzoxazol-6-yl]-3-(pyridin-4-ylmethyl)urea (20b) *tert*-Butyl [2-(5-methylpyridin-3-yl)-1,3-benzoxazol-6-yl]carbamate (**6b**) (104 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (4 mL), and a 4 M solution of hydrochloric acid in 1,4-dioxane (1.0 mL, 4.0 mmol) was added. The mixture was stirred at rt for 2 h. Then, the solvent was distilled under reduced pressure to give the crude 2-(2-methoxypyridin-4-yl)-1,3-benzoxazol-6-amine hydrochloride, which was used in the next reaction without further purification.

1-[2-(2-Methoxypyridin-4-yl)-1,3-benzoxazol-6-yl]-3-(pyridin-4-ylmethyl)urea (**20b**) was prepared according to general procedure A from crude 2-(2-methoxypyridin-4-yl)-1,3-benzoxazol-6-amine hydrochloride (yield: 49% over two steps). ^{1}H NMR (DMSO-d_6) δ ppm 3.94 (s, 3 H) 4.37 (d, J = 6.1 Hz, 2 H) 6.89 (t, J = 6.1 Hz, 1 H) 7.28 (dd, J = 8.5, 2.0 Hz, 1 H) 7.31 (d, J = 5.9 Hz, 2 H) 7.42 (s, 1 H) 7.65 (dd, J = 5.4, 1.5 Hz, 1 H) 7.73 (d, J = 8.8 Hz, 1 H) 8.13 (d, J = 1.7 Hz, 1 H) 8.39 (dd, J = 5.4, 0.7 Hz, 1 H) 8.51 (d, J = 5.9 Hz, 2 H) 9.17 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 41.8, 53.6, 99.6, 107.3, 113.9, 116.1, 120.1, 121.9, 135.2, 136.5, 139.6, 148.3, 149.4, 149.5, 150.9, 155.2, 158.9, 164.3; Anal. Calcd for $C_{20}H_{17}N_5O_3 \cdot 0.273H_2O$: C, 63.16; H, 4.65; N, 18.42. Found C, 63.09; H, 4.74; N, 18.45; HRMS (ESI): Calcd for $C_{20}H_{17}N_5O_3$ $[M+H]^+$: 376.1410 found 376.1401.

1-{2-[5-(Methylsulfonyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}-3-(pyridin-4-ylmethyl)urea (20c) Crude 2-[5-(methylsulfonyl)pyridin-3-yl]-1,3-benzoxazol-6-amine hydrochloride was obtained from *tert*-butyl {2-[5-(methylsulfonyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}carbamate (**6c**) following the same procedure used to synthesize crude 2-(1-methyl-1*H*-pyrazol-5-yl)-1,3-benzoxazol-6-amine hydrochloride described in the synthesis of **20b**.

1-{2-[5-(Methylsulfonyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}-3-(pyridin-4-ylmethyl)urea (**20c**) was prepared according to general procedure A from crude 2-[5-(methylsulfonyl)pyridin-3-yl]-1,3-benzoxazol-6-amine hydrochloride (yield: 22% over two steps). ^{1}H NMR (DMSO-d_6) δ ppm 3.46 (s, 3 H) 4.38 (d, J = 6.0 Hz, 2 H) 6.89 (t, J = 6.0 Hz, 1 H) 7.28–7.35 (m,

3 H) 7.75 (d, *J* = 8.8 Hz, 1 H) 8.15 (d, *J* = 1.7 Hz, 1 H) 8.52 (d, *J* = 5.9 Hz, 2 H) 8.87 (t, *J* = 2.4 Hz, 1 H) 9.18 (s, 1 H) 9.24 (d, *J* = 2.2 Hz, 1 H) 9.58 (d, *J* = 2.0 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 41.8, 43.5, 99.7, 116.2, 120.0, 121.9, 123.4, 133.0, 135.2, 137.5, 139.6, 149.4, 149.4, 149.5, 151.0, 151.5, 155.2, 157.8; Anal. Calcd for $C_{20}H_{17}N_5O_4S \cdot 0.793H_2O$: C, 54.88; H, 4.28; N, 16.00. Found C, 54.82; H, 4.19; N, 15.95; HRMS (ESI): Calcd for $C_{20}H_{17}N_5O_4S$ $[M+H]^+$: 424.1080 found 424.1086.

***N*,*N*-Diethyl-6-{[(1,3-oxazol-5-ylmethyl)carbamoyl]amino}-1,2-benzoxazole-3-carboxamide (20l)** Crude 6-amino-*N*,*N*-diethyl-1,2-benzoxazole-3-carboxamide hydrochloride was obtained from *tert*-butyl [3-(diethylcarbamoyl)-1,2-benzoxazol-6-yl]carbamate (**6k**) following the same procedure used to synthesize crude 2-(1-methyl-1*H*-pyrazol-5-yl)-1,3-benzoxazol-6-amine hydrochloride described in the synthesis of **20b**.

***N*,*N*-Diethyl-6-{[(1,3-oxazol-5-ylmethyl)carbamoyl]amino}-1,2-benzoxazole-3-carboxamide** (**20l**) was prepared according to general procedure A from crude 6-amino-*N*,*N*-diethyl-1,2-benzoxazole-3-carboxamide hydrochloride (yield: 18% over two steps). ^{1}H NMR (CD_3OD) δ ppm 8.13–8.18 (m, 1 H) 1.24 (t, *J* = 7.1 Hz, 3 H) 1.30 (t, *J* = 7.1 Hz, 3 H) 3.58 (q, *J* = 7.1 Hz, 2 H) 3.64 (q, *J* = 7.1 Hz, 2 H) 4.50 (s, 2 H) 7.05 (s, 1 H) 7.20 (dd, *J* = 8.5, 1.7 Hz, 1 H) 7.68 (d, *J* = 8.5 Hz, 1 H) 8.07 (d, *J* = 1.7 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 12.7, 14.6, 33.9, 42.8, 96.4, 113.8, 116.4, 122.3, 123.0, 143.3, 150.0, 151.5, 152.7, 154.6, 159.3, 163.4; Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found C, 57.25; H, 5.58; N, 19.81; HRMS (ESI): Calcd for $C_{17}H_{19}N_5O_4$ $[M+H]^+$: 358.1515 found 358.1519.

General procedure for urea formation (20d, e, f, g, h, i, j, k) (General procedure B: 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*-diisopropylethylamine (0.058 mL, 0.33 mmol) was added to the suspension. The mixture was stirred at 50 °C for 2 h. After cooling to rt, the precipitated solid was collected by filtration and washed with Et_2O to give the title compound (**20d, e, f, g, h, i, j, k**) as a solid.

1-(3-Cyano-2-phenyl-2*H*-indazol-6-yl)-3-(pyridin-4-ylmethyl)urea (20i) Prepared according to general procedure B (yield: 72%).^{1}H NMR (DMSO-d_6) δ ppm 4.38 (d, *J* = 6.0 Hz, 2 H) 6.91 (br t, *J* = 6.0 Hz, 1 H) 7.27–7.38 (m, 3 H) 7.59–7.73 (m, 3 H) 7.82 (d, *J* = 9.2 Hz, 1 H) 7.86–7.95 (m, 2 H) 8.18 (br d, *J* = 4.3 Hz, 1 H) 8.49–8.55 (m, 2 H) 9.12 (s, 1 H); Anal. Calcd for $C_{21}H_{16}N_6O \cdot 0.299H_2O$: C, 67.48; H, 4.48; N,

22.48. Found C, 67.47; H, 4.57; N, 22.42. HRMS (ESI): Calcd for $C_{21}H_{16}N_6O$ $[M+H]^+$: 369.1463 found 369.1468.

***N,N*-Diethyl-2-phenyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}-2*H*-indazole-3-carboxamide (20j)** Prepared according to general procedure B (yield: 27%). ^{1}H NMR ($CDCl_3$) δ ppm 0.83 (t, *J* = 7.1 Hz, 3 H) 1.19 (t, *J* = 7.1 Hz, 3 H) 2.93–3.09 (m, 2 H) 3.51–3.82 (m, 2 H) 4.30 (d, *J* = 5.9 Hz, 2 H) 5.92–6.05 (m, 1 H) 6.92–7.01 (m, 1 H) 7.12 (d, *J* = 5.9 Hz, 2 H) 7.31–7.56 (m, 5 H) 7.59–7.71 (m, 3 H) 8.45 (d, *J* = 5.9 Hz, 2 H); Anal. Calcd for $C_{25}H_{26}N_6O_2 \cdot 0.143H_2O$: C, 67.46; H, 5.95; N, 18.88. Found C, 67.45; H, 5.98; N, 18.80. HRMS (ESI): Calcd for $C_{25}H_{26}N_6O_2$ $[M+H]^+$: 443.2195 found 443.2204.

1-{2-[5-Fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}-3-(pyridin-4-ylmethyl)urea (20d) *tert*-Butyl {2-[5-fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}carbamate (**6d**) (0.72 g, 1.8 mmol) was dissolved in CH_2Cl_2 (16 mL), and TFA (5.0 mL) was added. The mixture was stirred at rt for 4 h. The reaction solution was poured into saturated aqueous sodium hydrogen carbonate solution (30 mL), followed by partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude 2-[5-fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-amine (as trifluoroacetic acid salt), which was used in the next reaction without further purification.

1-{2-[5-Fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}-3-(pyridin-4-ylmethyl)urea (**20d**) was prepared according to general procedure B from crude 2-[5-fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-amine (trifluoroacetic acid salt) (yield: 60% over two steps). ^{1}H NMR (DMSO-d_6) δ ppm 4.37 (d, *J* = 6.1 Hz, 2 H) 6.91 (t, *J* = 6.1 Hz, 1 H) 7.24–7.35 (m, 3 H) 7.78 (d, *J* = 9.2 Hz, 1 H) 8.17 (d, *J* = 1.6 Hz, 1 H) 8.52 (d, *J* = 6.1 Hz, 2 H) 8.60 (dd, *J* = 8.7, 2.6 Hz, 1 H) 9.00 (d, *J* = 2.7 Hz, 1 H) 9.19 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 41.8, 99.4, 116.2, 120.3, 121.9, 124.5, 126.9, 127.1, 134.9, 139.5, 139.7, 149.3, 149.4, 151.2, 155.2, 155.7, 158.7, 160.8; Anal. Calcd for $C_{20}H_{13}F_4N_5O_2$: C, 55.69; H, 3.04; N, 16.24; F, 17.62. Found C, 55.71; H, 3.12; N, 16.30; F, 17.78. HRMS (ESI): Calcd for $C_{20}H_{13}F_4N_5O_2$ $[M+H]^+$: 432.1084 found 432.1093.

***N,N*-Dimethyl-2-phenyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}pyrazolo[1,5-*a*]pyridine-3-carboxamide (20g)** Crude 6-amino-*N*,*N*-dimethyl-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxamide trifluoroacetic acid salt was obtained from *tert*-butyl (3-methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate (**6g**) following the same procedure used to synthesize crude 2-[5-fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-amine trifluoroacetic acid

salt described in the synthesis of **20d**.

***N*,*N*-Dimethyl-2-phenyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}pyrazolo[1,5-*a*]pyridine-3-carboxamide** (**20g**) was prepared according to general procedure B from crude 6-amino-*N*,*N*-dimethyl-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxamide trifluoroacetic acid salt (yield: 52% over two steps). ^{1}H NMR (DMSO-d_6) δ ppm 2.68 (br s, 3 H) 3.01 (br s, 3 H) 4.37 (d, *J* = 5.9 Hz, 2 H) 6.97 (t, *J* = 6.1 Hz, 1 H) 7.25 (dd, *J* = 9.5, 1.9 Hz, 1 H) 7.32 (d, *J* = 5.9 Hz, 2 H) 7.37–7.54 (m, 4 H) 7.67–7.74 (m, 2 H) 8.52 (d, *J* = 6.0 Hz, 2 H) 8.98 (s, 1 H) 9.06–9.11 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 41.9, 66.3, 104.0, 116.3, 116.8, 121.0, 122.0, 127.2, 128.4, 128.6, 128.7, 132.7, 135.9, 149.1, 149.3, 149.5, 155.3, 164.8; HRMS (ESI): Calcd for $C_{23}H_{22}N_6O_2$ $[M+H]^+$: 415.1882 found 415.1883.

***N*-(2-Methoxyethyl)-2-phenyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}pyrazolo[1,5-*a*]pyridine-3-carboxamide (20h)** Crude 6-amino-*N*-(2-methoxyethyl)-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxamide trifluoroacetic acid salt was obtained from *tert*-butyl {3-[(2-methoxyethyl)carbamoyl]-2-phenylpyrazolo-[1,5-*a*]pyridin-6-yl}carbamate (**6h**) following the same procedure used to synthesize crude 2-[5-fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-amine trifluoroacetic acid described in the synthesis of **20d**.

***N*-(2-Methoxyethyl)-2-phenyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}pyrazolo[1,5-*a*]pyridine-3-carboxamide** (**20h**) was prepared according to general procedure B from crude 6-amino-*N*-(2-methoxyethyl)-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxamide trifluoroacetic acid salt (yield: 62% over two steps). ^{1}H NMR ($CDCl_3$) δ ppm 3.17 (s, 3 H) 3.28–3.34 (m, 2 H) 3.43 (q, *J* = 5.2 Hz, 2 H) 4.42 (d, *J* = 5.5 Hz, 2 H) 5.96–6.04 (m, 1 H) 6.11 (t, *J* = 5.5 Hz, 1 H) 6.92–6.97 (m, 1 H) 7.19 (d, *J* = 6.0 Hz, 2 H) 7.45–7.52 (m, 3 H) 7.61–7.66 (m, 2 H) 7.68–7.74 (m, 1 H) 8.06 (d, *J* = 9.6 Hz, 1 H) 8.50 (d, *J* = 6.0 Hz, 2 H) 9.02 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 38.4, 41.9, 57.8, 70.4, 105.1, 116.1, 117.7, 121.1, 122.0, 128.3, 128.4, 128.6, 128.8, 132.5, 136.8, 149.3, 149.5, 150.5, 155.3, 163.1; Anal. Calcd for $C_{24}H_{24}N_6O_3 \cdot 0.208H_2O$: C, 64.31; H, 5.49; N, 18.75. Found C, 64.37; H, 5.48; N, 18.74. HRMS (ESI): Calcd for $C_{24}H_{24}N_6O_3$ $[M+H]^+$: 445.1988 found 445.1997.

1-(3-Methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)-3-(pyridin-4-ylmethyl)urea (20e) Crude 3-methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-amine hydrochloride was obtained from *tert*-butyl (3-methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate (**6e**) following the same procedure used to synthesize **20b**.

1-(3-Methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)-3-(pyridin-4-ylmethyl)urea (**20e**) was prepared according to general procedure B from crude 3-methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-amine hydrochloride (yield: 8% over two steps). ^{1}H NMR (DMSO-d_6) δ (ppm) 2.40 (s, 3H) 4.36 (d, *J* = 6.1 Hz, 2H) 6.87–6.93 (m, 1H) 7.06 (dd, *J* = 9.6, 1.8 Hz, 1H) 7.29–7.33 (m, 2H) 7.36–7.41 (m, 1H) 7.44–7.51 (m, 2H) 7.60 (d, *J* = 9.4 Hz, 1H) 7.75–7.80 (m, 2H) 8.50–8.53 (m, 2H) 8.96–8.99 (m, 1H) 8.81 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm 8.7, 41.9, 102.4, 116.2, 116.4, 118.4, 122.0, 127.4, 127.5, 127.6, 128.5, 133.8, 136.3, 149.3, 149.4, 149.5, 155.4; Anal. Calcd for $C_{21}H_{19}N_5O \cdot 0.208H_2O$: C, 69.84; H, 5.42; N, 19.39. Found C, 69.78; H, 5.46; N, 19.36. HRMS (ESI): Calcd for $C_{21}H_{19}N_5O$ $[M+H]^+$: 358.1668 found 358.1678.

1-(3-Cyano-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)-3-(pyridin-4-ylmethyl)urea (20f) Crude 6-amino-2-phenylpyrazolo[1,5-*a*]pyridine-3-carbonitrile hydrochloride was obtained from *tert*-butyl (3-cyano-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate (**6f**) following the same procedure used to synthesize **20b**.

1-(3-Cyano-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)-3-(pyridin-4-ylmethyl)urea (**20f**) was prepared according to general procedure B from crude 2-phenylpyrazolo[1,5-*a*]pyridine-3-carbonitrile hydrochloride obtained above (yield: 22% over two steps). ^{1}H NMR (DMSO-d_6) δ ppm 4.37 (d, *J* = 6.1 Hz, 2 H) 7.08 (t, *J* = 6.1 Hz, 1 H) 7.32 (d, *J* = 6.1 Hz, 2 H) 7.47–7.64 (m, 4 H) 7.85 (d, *J* = 9.4 Hz, 1 H) 7.99–8.06 (m, 2 H) 8.52 (d, *J* = 6.1 Hz, 2 H) 9.21 (s, 1 H) 9.24–9.29 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 42.0, 77.4, 115.0, 116.1, 117.3, 122.0, 123.7, 126.8, 129.1, 129.9, 130.4, 130.7, 139.5, 149.2, 149.5, 154.1, 155.1; Anal. Calcd for $C_{21}H_{16}N_6O$: C, 68.47; H, 4.38; N, 22.81. Found C, 68.19; H, 4.57; N, 22.66. HRMS (ESI): Calcd for $C_{21}H_{16}N_6O$ $[M+H]^+$: 369.1464 found 369.1464.

***N*,*N*-Diethyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}-1,2-benzoxazole-3-carboxamide (20k)** Crude 6-amino-*N*,*N*-diethyl-1,2-benzoxazole-3-carboxamide hydrochloride was obtained from *tert*-butyl [3-(diethylcarbamoyl)-1,2-benzoxazol-6-yl]carbamate (**6k**) following the same procedure used to synthesize **20b**.

***N*,*N*-Diethyl-6-{[(pyridin-4-ylmethyl)carbamoyl]amino}-1,2-benzoxazole-3-carboxamide** (**20k**) was prepared according to general procedure B from crude 6-amino-*N*,*N*-diethyl-1,2-benzoxazole-3-carboxamide hydrochloride (yield: 46% over two steps). ^{1}H NMR (CD_3OD) δ ppm 8.48 (d, *J* = 5.9 Hz, 2 H) 8.07 (d, *J* = 1.5 Hz, 1 H) 7.69 (d, *J* = 8.8 Hz, 1 H) 7.41 (d, *J* = 6.3 Hz, 2 H) 7.23 (dd, *J* = 8.8, 1.5 Hz, 1 H), 4.49 (s, 2 H) 3.64 (q, *J* = 7.2 Hz, 2 H) 3.58 (q, *J* = 7.0

Hz, 2 H) 1.30 (t, *J* = 7.2 Hz, 3 H) 1.24 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ ppm 12.7, 14.6, 41.8, 42.8, 95.4, 113.7, 116.4, 121.9, 122.3, 143.4, 149.2, 149.5, 152.7, 155.0, 159.3, 163.4; Anal. Calcd for $C_{19}H_{21}N_5O_3$: C, 62.11; H, 5.76; N, 19.06. Found C, 62.00; H, 5.81; N, 19.01; HRMS (ESI): Calcd for $C_{19}H_{21}N_5O_3$ $[M+H]^+$:368.1722 found 368.1717.

2-(2,6-Dimethylphenyl)-6-nitro-1,3-benzoxazole (**5**) To a solution of 2,6-dimethylbenzoic acid (**3**) (0.55 g, 3.7 mmol) in toluene (4 mL) was added thionyl chloride (0.53 mL, 7.3 mmol). The mixture was stirred at 100 °C for 30 min. After cooling to rt, the solvent was then distilled under reduced pressure to give crude 2,6-dimethylbenzoyl chloride (0.62 g), which was used in the next reaction without further purification.

To a solution of 2-amino-5-nitrophenol (**4**) (0.57 g, 3.7 mmol) and triethylamine (0.61 mL, 4.4 mmol) in CH_2Cl_2 (10 mL) was added crude 2,6-dimethylbenzoyl chloride (0.62 g). The mixture was stirred at rt overnight. Water was added to the reaction solution, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over sodium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude title compound. The obtained residue was partially purified by silica gel column chromatography (AcOEt: hexane = 10:90 to 45:55) to give crude (2-amino-5-nitro-phenyl) 2,6-dimethylbenzoate (0.63 g), which was used in the next reaction without further purification.

A solution of crude (2-amino-5-nitro-phenyl) 2,6-dimethylbenzoate (0.63 g) and *p*-toluenesulfonic acid monohydrate (84 mg, 0.44 mmol) dissolved in toluene (10 mL) was stirred at 100 °C for 4 h. The reaction mixture was poured into water, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over sodium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude title compound. The obtained residue was purified by silica gel column chromatography (AcOEt: hexane = 10:90 to 45:55) to give the title compound (0.41 g, 40% over three steps) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 2.34 (s, 6 H) 7.20 (d, *J* = 7.4 Hz, 2 H) 7.33–7.41 (m, 1 H) 7.93 (d, *J* = 8.6 Hz, 1 H) 8.37 (dd, *J* = 8.8, 2.2 Hz, 1 H) 8.53 (d, *J* = 1.6 Hz, 1 H).

2-(2,6-Dimethylphenyl)-1,3-benzoxazol-6-amine (**6a**) 2-(2,6-Dimethylphenyl)-6-nitro-1,3-benzoxazole (**5**) (0.40 g, 1.5 mmol) was dissolved in EtOH (5 mL) and AcOEt (5 mL), and 10% Pd/C (50% wet) (0.10 g) was added to this solution. After purging the reaction container with hydrogen, the mixture was stirred at rt for 1 h. The reaction solution was filtered through Celite. Then, the filtrate was concentrated under

reduced pressure to give the crude title compound. The residue obtained was purified by silica gel column chromatography (AcOEt: hexane = 10:90 to 45:55) to give the title compound (0.31 g, 87%) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 2.30 (s, 6 H) 3.84 (br s, 2 H) 6.73 (dd, *J* = 8.6, 2.3 Hz, 1 H) 6.88 (d, *J* = 2.3 Hz, 1 H) 7.13 (d, *J* = 7.8 Hz, 2 H) 7.27–7.31 (m, 1 H) 7.57 (d, *J* = 8.2 Hz, 1 H)

***tert*-Butyl [2-(2-methoxypyridin-4-yl)-1,3-benzoxazol-6-yl]carbamate** (**6b**) *tert*-Butyl *N*-(1,3-benzoxazol-6-yl)carbamate[9] (**7**) (0.20 g, 0.85 mmol), 4,6-bis(diphenylphosphino)phenoxazine (72 mg, 0.13 mmol), and palladium(II) acetate (22 mg, 0.085 mmol) in 1,2-dimethoxyethane (4 mL) were stirred at rt under an N_2 atmosphere for 5 min. 4-Bromo-2-methoxypyridine (0.20 g, 1.02 mmol) and sodium *tert*-butoxide (0.20 g, 2.0 mmol) were then added, and the reaction mixture was stirred at 50 °C under an N_2 atmosphere for 3 h and then rt overnight. Then, the solvent was distilled under reduced pressure to give the crude title compound. The obtained residue was purified by silica gel column chromatography (AcOEt: hexane = 10:80 to 50:50) to give the title compound (0.10 g, 35%) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 1.55 (s, 9 H) 4.01 (s, 3 H) 6.69 (br s, 1 H) 7.09 (dd, *J* = 8.5, 2.2 Hz, 1 H) 7.47–7.51 (m, 1 H) 7.63 (dd, *J* = 5.4, 1.5 Hz, 1 H) 7.67 (d, *J* = 8.5 Hz, 1 H) 8.04 (br s, 1 H) 8.32 (dd, *J* = 5.4, 0.7 Hz, 1 H); MS (ESI): *m/z* 342 $[M+H]^+$.

***tert*-Butyl {2-[5-(methylsulfonyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}carbamate** (**6c**) The title compound (0.38 g, quant.) was obtained from *tert*-butyl *N*-(1,3-benzoxazol-6-yl)carbamate[9] (**7**) (0.20 g, 0.85 mmol) following the same procedure used to synthesize **6b**.

^{1}H NMR (DMSO-d_6) δ ppm 1.55 (s, 9 H) 3.46 (s, 3 H) 7.47 (dd, *J* = 8.8, 1.7 Hz, 1 H) 7.77 (d, *J* = 8.5 Hz, 1 H) 8.06 (s, 1 H) 8.88 (t, *J* = 2.1 Hz, 1 H) 9.26 (d, *J* = 2.2 Hz, 1 H) 9.59 (d, *J* = 2.0 Hz, 1 H) 9.78 (s, 1 H); MS (ESI): *m/z* 390 $[M+H]^+$.

***tert*-Butyl {2-[5-fluoro-2-(trifluoromethyl)pyridin-3-yl]-1,3-benzoxazol-6-yl}carbamate** (**6d**) The title compound (53 mg, 24%) was obtained from *tert*-butyl *N*-(1,3-benzoxazol-6-yl)carbamate[9] (**7**) (0.13 g, 0.56 mmol) following the same procedure used to synthesize **6b**.

^{1}H NMR ($CDCl_3$) δ ppm 1.58 (s, 9 H) 6.83 (br s, 1 H) 7.11 (dd, *J* = 8.6, 2.0 Hz, 1 H) 7.71 (d, *J* = 8.6 Hz, 1 H) 8.13 (br s, 1 H) 8.25 (dd, *J* = 8.3, 2.6 Hz, 1 H) 8.67 (d, *J* = 2.5 Hz, 1 H).

6-Bromo-3-methyl-2-phenylpyrazolo[1,5-*a*]pyridine (**9**) To a solution of

6-bromo-2-phenylpyrazolo[1,5-*a*]pyridine[11] (**8**) in DMF (6 mL) was added (chloromethylene)dimethylimium chloride (0.28 g, 2.2 mmol) in DMF (1 mL). The mixture was stirred at rt for 3 h. The reaction mixture was poured into saturated ammonium chloride aqueous solution, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over sodium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude title compound. The residue obtained was partially purified by silica gel column chromatography (AcOEt: hexane = 0:100 to 10:90) to give the crude 6-bromo-2-phenylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (0.21 g), which was used in the next reaction without further purification.

To a solution of crude 6-bromo-2-phenylpyrazolo[1,5-*a*]pyridine-3-carbaldehyde (0.11 g) in TFA (3 mL) was added triethylsilane (1.1 mL, 7.2 mmol). The mixture was stirred at rt for 5 h. The reaction mixture was poured into saturated sodium hydrogen carbonate aqueous solution, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over sodium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude title compound. The residue obtained was partially purified by silica gel column chromatography (AcOEt: hexane = 0:100 to 10:90) to give the title compound (89 mg, 55% over two steps) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 2.43 (s, 3 H) 7.13 (dd, *J* = 9.5, 1.7 Hz, 1 H) 7.34 (dd, *J* = 9.4, 0.8 Hz, 1 H) 7.38–7.42 (m, 1 H) 7.45–7.52 (m, 2 H) 7.74–7.81 (m, 2 H) 8.57 (s, 1 H).

***tert*-Butyl (3-methyl-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate** (**6e**) 6-Bromo-3-methyl-2-phenylpyrazolo[1,5-*a*]pyridine (**9**) (89 mg, 0.31 mmol), *tert*-butyl carbamate (43 mg, 0.37 mmol), 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (53 mg, 0.12 mmol), tris(dibenzylideneacetone)dipalladium(0) (28 mg, 0.031 mmol), and sodium *tert*-butoxide (68 mg, 0.71 mmol) were dissolved in toluene (5 mL) and then stirred at room temperature for 3 h. The reaction solution was poured into water, followed by partitioning using dichloromethane. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure. The residue was purified by silica gel column chromatography (silica gel, AcOEt: hexane = 0:100 to 20:80) to give the title compound (85 mg, 85%) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 1.53 (s, 9 H) 2.42 (s, 3 H) 6.41–6.52 (m, 1 H) 6.98 (br d, *J* = 10.4 Hz, 1 H) 7.30–7.39 (m, 2 H) 7.41–7.53 (m, 2 H) 7.74–7.82 (m, 2 H) 8.77 (br s, 1 H).

***tert*-Butyl (2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate** (**10**) The title compound (0.31 g, 68%) was

synthesized from 6-bromo-2-phenyl-pyrazolo[1,5-*a*]pyridine[11] (**8**) (0.40 g, 1.5 mmol) following the same procedure used to synthesize **6e**.

^{1}H NMR ($CDCl_3$) δ ppm 1.52 (s, 9H) 6.48–6.62 (m, 1H) 6.73 (s, 1H) 6.97 (dd, *J* = 9.6, 1.2 Hz, 1H) 7.30–7.50 (m, 4H) 7.93 (d, *J* = 7.2 Hz, 2H) 8.84 (br s, 1H).

***tert*-Butyl (3-bromo-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate** (**11**) *tert*-Butyl (2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate (**10**) (0.31 g, 1.0 mmol) was dissolved in DMF (5 mL). To the solution was added *N*-bromosuccinimide (0.21 g, 1.2 mmol). The mixture was stirred at room temperature for 21 h. The reaction solution was poured into a mixed solution of water (10 mL) and brine (5 mL), followed by partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure. The residue was purified by silica gel column chromatography (silica gel, AcOEt: hexane = 0:100 to 20:80) to give the title compound (0.20 g, 52%) as an oil.

^{1}H NMR ($CDCl_3$) δ ppm 1.54 (s, 9H) 6.42 (br s, 1H) 7.04 (dd, *J* = 9.4, 1.8 Hz, 1H) 7.35–7.52 (m, 4H) 7.99–8.07 (m, 2H) 8.93 (br s, 1H).

***tert*-Butyl (3-cyano-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate** (**6f**) *tert*-Butyl (3-bromo-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl)carbamate (**11**) (76 mg, 0.20 mmol), zinc cyanide (48 mg, 0.41 mmol), and tetrakis(triphenylphosphine)palladium(0) (22 mg, 0.020 mmol) were dissolved in DMF (2 mL) and then heated at 100 °C for 6 h. Water was added to the reaction solution, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the crude title compound. The residue was purified by silica gel column chromatography (silica gel, AcOEt: hexane = 0:100 to 20:80) to give the title compound (38 mg, 58%) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 1.54 (s, 9H) 6.55–6.67 (m, 1H) 7.23 (dd, *J* = 9.4, 2.0 Hz, 1H) 7.39–7.55 (m, 3H) 7.64 (dd, *J* = 9.4, 0.8 Hz, 1H) 8.06–8.20 (m, 2H) 9.14 (br s, 1H).

Methyl 6-[(*tert*-butoxycarbonyl)amino]-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylate (**13**) The title compound (0.54 g, 85%) was synthesized from methyl 6-bromo-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylate[11] (12) (0.57 g, 1.7 mmol) following the same procedure used to synthesize **6e**.

^{1}H NMR ($CDCl_3$) δ ppm 1.53 (s, 9H) 3.83 (s, 3H) 6.56–6.77 (m, 1H) 7.23 (dd, *J* = 9.4, 2.0 Hz, 1H) 7.38–7.50 (m, 3H) 7.73–7.83 (m, 2H) 8.09 (dt, *J* = 9.5, 0.9 Hz, 1H) 9.03 (br s, 1H).

6-[(*tert*-Butoxycarbonyl)amino]-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid (**14**) Methyl 6-[(*tert*-butoxycarbonyl)amino]-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylate (**13**) (0.35 g, 0.94 mmol) was dissolved in MeOH (2 mL) and THF (2 mL). To the solution was added 5 M aqueous NaOH (0.90 mL, 4.5 mmol). The mixture was stirred at 50 °C for 18 h. After cooling the reaction solution to room temperature, 1 M aqueous HCl solution (4.5 mL, 4.5 mmol) and water (10 mL) were added, followed by partitioning using CH_2Cl_2. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the title compound (0.23 g, 63%) as a solid.

^{1}H NMR (DMSO-d_6) δ ppm 1.51 (s, 9H) 7.41–7.47 (m, 3H) 7.56 (dd, *J* = 9.6, 2.0 Hz, 1H) 7.72–7.79 (m, 2H) 8.06 (dd, *J* = 9.6, 0.7 Hz, 1H) 9.01 (br s, 1H) 9.78 (br s, 1H) 12.34 (br s, 1H); MS (ESI): *m/z* 354 $[M+H]^+$.

***tert*-Butyl [3-(dimethylcarbamoyl)-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl]carbamate** (**6g**) 6-[(*tert*-Butoxycarbonyl)amino]-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid (**14**) (50 mg, 0.14 mmol), dimethylamine (2.0 M solution in THF) (0.084 mL, 0.17 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (32 mg, 0.17 mmol), 3*H*-1,2,3-triazolo[4,5-*b*]pyridin-3-ol (23 mg, 0.17 mmol), and triethylamine (0.047 mL, 0.34 mmol) were dissolved in DMF (2 mL) and stirred at room temperature for 20 h. The reaction solution was poured into water, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure. The residue was purified by silica gel column chromatography (silica gel, AcOEt: hexane = 0:100 to 80:20) to give the title compound (44 mg, 82%) as an oil.

^{1}H NMR ($CDCl_3$) δ ppm 1.52 (s, 9H) 2.61 (br s, 3H) 3.10 (br s, 3H) 6.75–6.94 (m, 1H) 7.05 (dd, *J* = 9.4, 1.8 Hz, 1H) 7.34–7.46 (m, 3H) 7.47–7.57 (m, 1H) 7.69–7.80 (m, 2H) 8.89 (br s, 1H).

***tert*-Butyl {3-[(2-methoxyethyl)carbamoyl]-2-phenylpyrazolo[1,5-*a*]pyridin-6-yl}carbamate** (**6h**) The title compound (69 mg, 85%) was synthesized from 6-[(*tert*-Butoxycarbonyl)amino]-2-phenylpyrazolo[1,5-*a*]pyridine-3-carboxylic acid (**14**) (70 mg, 0.20 mmol) following the same procedure used to synthesize **6g**.

^{1}H NMR ($CDCl_3$) δ ppm 1.52 (s, 9 H) 3.19 (s, 3 H) 3.32–3.40 (m, 2 H) 3.48 (q, *J* = 5.2 Hz, 2 H) 5.99 (br t, *J* = 5.2 Hz, 1 H) 6.75–6.85 (m, 1 H) 7.19 (dd, *J* = 9.6, 2.0 Hz, 1 H) 7.46–7.56 (m, 3 H) 7.63–7.70 (m, 2 H) 8.26 (d, *J* = 9.6 Hz, 1 H) 8.99 (br s, 1 H).

6-Nitro-2-phenyl-2*H*-indazole-3-carbonitrile (**16**) 6-Nitro-2-phenyl-2*H*-indazole-3-carbonitrile 1-oxide[14] (**15**) (0.29 g, 1.0 mmol) and triphenylphosphine (0.27 g, 1.0 mmol) were dissolved in EtOH (25 mL) and then stirred at 80 °C for 4 h. The reaction solution was cooled to rt and then concentrated under reduced pressure to give the crude title compound. The crude product was purified by silica gel column chromatography (silica gel, AcOEt: hexane = 0:100 to 10:90) to give the title compound (0.25 g, 91%) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 7.61–7.71 (m, 3H), 7.89–7.95 (m, 2H), 7.99 (dd, *J* = 9.2, 0.8 Hz, 1H), 8.22 (dd, *J* = 9.2, 2.0 Hz, 1H), 8.92 (dd, *J* = 2.0, 0.8 Hz, 1H).

6-Amino-2-phenyl-2*H*-indazole-3-carbonitrile (**6i**) 6-Nitro-2-phenyl-2*H*-indazole-3-carbonitrile (**16**) (0.25 g, 0.95 mmol) and ammonium chloride (51 mg, 0.95 mmol) were dissolved in EtOH (14 mL) and water (7 mL) and then stirred at 80 °C for 1 min. Iron (0.53 g, 9.5 mmol) was added to the reaction solution. The mixture was stirred at 80 °C for 2.5 h. The reaction solution was filtered through Celite, and the filtrate was concentrated under reduced pressure. Water was added to the residue, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give the title compound (0.21 g, 94%) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 3.97 (br s, 2H), 6.88 (dd, *J* = 9.0, 2.0 Hz, 1H) 6.93–6.95 (m, 1H), 7.48-7.53 (m, 1H), 7.55–7.61 (m, 2H), 7.63 (dd, *J* = 9.0, 0.6 Hz, 1H), 7.82–7.87 (m, 2H).

6-Amino-*N*,*N*-diethyl-2-phenyl-2*H*-indazole-3-carboxamide (**6j**) To 6-amino-2-phenyl-2*H*-indazole-3-carbonitrile (0.14 g, 0.58 mmol) was added a 5 M aqueous NaOH solution (10 mL). The mixture was stirred at 100 °C for 13.5 h. After cooling the reaction solution to rt, 2 M HCl solution (25 mL) and water were added, followed by partitioning using AcOEt. The organic phase was washed with brine, dried over magnesium sulfate, and filtered. Then, the solvent was distilled under reduced pressure to give crude 6-amino-2-phenyl-2*H*-indazole-3-carboxylic acid (0.16 g), which was used in the next reaction without further purification.

The title compound (62 mg, 81% over two steps) was synthesized from crude 6-amino-2-phenyl-2*H*-indazole-3-carboxylic acid (70 mg) following the same procedure used to synthesize **6g**.

^{1}H NMR ($CDCl_3$) δ ppm 0.81 (t, *J* = 7.1 Hz, 3H), 1.15 (t, *J* = 7.1 Hz, 3H), 3.90 (br s, 2H) 6.62–6.70 (m, 1H), 6.80–6.85 (m, 1H), 7.34–7.50 (m, 4H), 7.66–7.73 (m, 2H).

6-Bromo-*N*,*N*-diethyl-1,2-benzoxazole-3-carboxamide (**18**) To a solution of 6-bromo-1,2-benzoxazole-3-carboxylic acid (4.0 g, 16 mmol) in CH_2Cl_2 (150 mL) were added DMF (0.10 mL, 0.82 mmol) and oxalyl chloride (1.8 mL, 20 mmol). The mixture was stirred at room temperature overnight. The reaction solution was concentrated. The obtained crude product (4.3 g) was used in the next reaction without purification.

Crude 6-bromo-1,2-benzoxazole-3-carbonyl chloride (0.50 g) was dissolved in CH_2Cl_2 (30 mL), and to the solution mixture were added pyridine (0.46 mL, 5.8 mmol) and diethylamine (0.40 mL, 3.8 mmol). The mixture was stirred at room temperature overnight. The reaction solution was concentrated. The crude residue was purified by silica gel column chromatography (silica gel, AcOEt: hexane = 0:100 to 25:70) to give the title compound (0.51 g, 1.7 mmol, 89% over two steps) as a solid.

^{1}H NMR ($CDCl_3$) δ ppm 1.34–1.26 (m, 6 H) 3.71–3.61 (m, 4 H) 7.51 (dd, *J* = 8.4, 1.5 Hz, 1 H) 7.82 (d, *J* = 1.0 Hz, 1 H) 7.87 (d, *J* = 8.4 Hz, 1 H); MS (ESI): *m/z* 297 $[M+H]^+$.

***tert*-Butyl [3-(diethylcarbamoyl)-1,2-benzoxazol-6-yl]carbamate** (**6k**) The title compound (67 mg, 60%) was synthesized from 6-bromo-*N*,*N*-diethyl-1,2-benzoxazole-3-carboxamide (**18**) (0.10 g, 0.34 mmol) following the same procedure used to synthesize **6e**.

^{1}H NMR ($CDCl_3$) δ ppm 1.32–1.24 (m, 6 H) 1.46 (s, 9 H) 3.72–3.58 (m, 4 H) 6.75 (br s, 1 H) 7.06 (dd, *J* = 8.5, 1.7 Hz, 1 H) 7.83 (d, *J* = 8.5 Hz, 1 H) 8.06–7.99 (m, 1 H); MS (ESI): *m/z* 334 $[M+H]^+$.

Biology. 1. Measurement of NAMPT activation (*in vitro* cell-free enzyme assay)

The NAMPT activity assay was conducted in accordance with the method of Formentini *et al.*[29] by chemically converting NMN produced by NAMPT to a fluorescent substance and using the fluorescence intensity of this fluorescent substance as an index of the amount of NMN produced. Briefly, the NAMPT enzymatic reaction was conducted using polypropylene 384-well V-shaped black plates (Greiner Bio One

International GmbH). NAMPT activity was measured in assay buffer containing 50 mM HEPES, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM TCEP, 0.1% Prionex, 0.005% Tween 20, 0.12 mM adenosine triphosphate, 5 μM NAM, 6.25 μM PRPP, 0.04 U/mL pyrophosphatase, and 2 ng/mL human NAMPT in the presence or absence of test compounds. After incubation for 1 h at 25 °C, the enzymatic reaction was terminated by adding 5 μL of 2 M KOH and 5 μL of 20% acetophenone. Then, 22 μL of 88% formic acid were added, and the mixture was further incubated for 30 min in the dark. NAMPT activity was calculated as the difference between fluorescence intensity (Ex 380 nm/Em 450 nm) in the reaction of a test compound treatment group and that in a control reaction free of the NAMPT enzyme as follows:

NAMPT enzyme activity = Fluorescence intensity of the test substance treatment group − Mean fluorescence intensity of the control reaction free from the NAMPT enzyme.

The EC_{50} of each test compound was calculated using GraphPad Prism (GraphPad Software, Inc.), and Emax was calculated as percentage of the maximal activity achieved with 30 μM compound **1**.

2. Study of the effect of NAMPT activators on intracellular NAD^+ levels (*in vitro* cell-based assay)

HEK293A cells were cultured in Dulbecco's modified Eagle medium (high glucose) containing 10% fetal bovine serum, non-essential amino acids, and antibiotic–antimycotic (reaction medium). On the day before the test, HEK293A cells were seeded at a density of 3.0×10^4 cells/80 μL into 96-well poly-D-lysine–coated plates and cultured overnight in a CO_2 incubator. On the day of the test, each test compound was diluted with reaction medium by 5-fold and added at a concentration 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 bicarbonate base buffer (100 mM sodium carbonate, 20 mM sodium bicarbonate, 100 mM nicotinamide, 20 mM Triton X-100, 1% dodecyltrimethylammonium bromide). The NAD^+ content in the cell extract was measured using an NAD^+/NADH-Glo assay kit according to the manufacturer's instructions. Briefly, 20 μL of the lysate were separated, and 10 μL of 0.4 N HCl were added, followed by incubation at 60 °C for 15 min and subsequently at 25 °C for 10 min. The mixture was neutralized by adding 10 μL of 0.5 M Trizma base solution and then diluted 10-fold with distilled water. Next, 15 μL of the diluted sample were mixed with an equal amount (15 μL) of NAD^+/NADH-Glo reagent in a 384-well black plate, and the mixture was incubated for 30 min in the dark. Subsequently, the chemiluminescence intensity was measured.

PAMPA, log*D*, solubility, metabolic stability, and protein binding assay

Each assay was performed as previously described.[26-28]

CYP DI assay

The reversible inhibition of the specific activities of four CYP isoforms (CYP1A2, CYP2C9, CYP2D6, and CYP3A4) by each compound was examined in a reaction mixture containing human liver microsomes and an NADPH-generating system. The microsomal protein concentration in the assay was 0.1 mg/mL. After the CYP substrate was incubated at 37 °C for 10 min in the absence or presence of compounds (10 μmol/L), the concentration of metabolites of the CYP substrate was semi-quantitatively analyzed by LC-MS/MS, and the percent inhibition was calculated using the following formula:

% inhibition = (metabolite concentration with compound/metabolite concentration without compound) × 100.

Pharmacokinetic evaluation in mice

The test compounds suspended in vehicle (0.5% (w/v) methylcellulose 400 solution (Wako Pure Chemical Industries) were orally administered to 7-week-old male C57BL/6J mice at a dose of 10 mg/kg. Plasma samples were collected from the peripheral vein at several time points after dosing. The plasma concentration was determined by LC-MS/MS to obtain pharmacokinetic parameters via non-compartmental analysis.

REFERENCES AND NOTES

1. Y. Yang and A. A. Sauve, *Biochim. Biophys. Acta*, 2016, **1864**, 1787.
2. L. Rajman, K. Chewalek, and D. A. Sinclari, *Cell Metab.*, 2018, **27**, 529.
3. C. Canto, K. Menzies, and J. Auwerx, *Cell Metab.*, 2015, **22**, 31.
4. E. Verdin, *Science*, 2015, **350**, 1208.
5. E. Katsuyaba and J. Auwerx, *EMBO J.*, 2017, **36**, 2670.
6. J. Yoshino, K. F. Mills, M. J. Yoon, and S. Imai, *Cell Metab.*, 2011, **14**, 528.
7. M. Akiu, T. Tsuji, K. Iida, Y. Sogawa, K. Terayama, M. Yokoyama, J. Tanaka, D. Asano, T. Honda, K. Sakurai, A. B. Pinkerton, and T. Nakamura, *Chem. Pharm. Bull.*, Accepted for publication.
8. The six-well Ames test was conducted at the following test concentrations: 1.95, 3.91, 7.81, 15.6,

31.3, 62.5, 125, 250, 500, and 1000 μg/well.

9. F. V. Vinogradova, N. H. Park, B. P. Fors, and S. C. Buchwald, *Org. Lett.*, 2013, **15**, 1394.
10. F. Gao, B. S. Kim, and P. J. Walsh, *Chem. Commun.*, 2014, **50**, 10661.
11. Y. Nishigaya, K. Umei, Y. Saito, H. Watanabe, T. Kondo, A. Kondo, N. Kawamura, K. Tatani, Y. Kohno, N. Tanaka, and S. Seto, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 4044.
12. H. Igawa, M. Takahashi, M. Ikoma, H. Kaku, K. Kakegawa, A. Kina, J. Aida, S. Okuda, Y. Kawata, T. Noguchi, N. Hotta, Y. Syunsuke, M. Nakayama, Y. Nagisa, S. Kasai, and T. Maekawa, *Bioorg. Med. Chem.*, 2016, **24**, 2504.
13. F. Ma, X. Xie, L. Zhang, Z. Peng, L. Ding, L. Fu, and Z. Zhang, *J. Org. Chem.*, 2012, **77**, 5279.
14. L. C. Behr, E. G. Alley, and O. J. Levand, *J. Org. Chem.*, 1962, **27**, 65.
15. M. K. Christensen and F. Björkling, WO2010/023307.
16. A. F. Stepan, D. P. Walker, J. Bauman, D. A. Price, T. A. Baillie, A. S. Kalgutkar, and M. D. Aleo, *Chem. Res. Toxicol.*, 2011, **24**, 1345.
17. S. J. Enoch and M. T. D. Cronin, *Mutat. Res.*, 2012, **743**, 10.
18. M. Akiu, T. Tsuji, Y. Sogawa, K. Terayama, M. Yokoyama, J. Tanaka, D. Asano, K. Sakurai, E. Sergienko, E. H. Session, S. J. Gardell, A. B. Pinkerton, and T. Nakamura, *Bioorg. Med. Chem.*, 2021, **43**, 128048.
19. L. R. Ferguson and W. A. Denny, *Mutat. Res.*, 2007, **623**, 14.
20. M. S. Malamas, Y. Ni, J. Erdei, H. Stange, R. Schindler, H. J. Lankau, C. Grunwald, K. Y. Fan, K. Parris, B. Langen, U. Egerland, T. Hage, K. L. Marquis, S. Grauer, J. Brennan, R. Navarra, R. Graf, B. L. Harrison, A. Robichaud, T. Kronbach, M. N. Pangalos, N. Hoefgen, and N. J. Brandon, *J. Med. Chem.*, 2011, **54**, 7621.
21. J. M. Ellis, M. D. Altman, A. Bass, J. W. Butcher, A. J. Byford, A. Donofrio, S. Galloway, A. M. Haidle, J. Jewell, N. Kelly, E. K. Leccese, S. Lee, M. Maddess, J. R. Miller, L. Y. Moy, E. Osimboni, R. D. Otte, M. V. Reddy, K. Spencer, B. Sun, S. H. Vincent, G. J. Ward, G. H. C. Woo, C. Yang, H. Houshyar, and A. B. Northrup, *J. Med. Chem.*, 2015, **58**, 1929.
22. NAMPT enzyme assays were performed by measuring the fluorescence of a derivative that was chemically converted from the enzymatic product NMN. Emax was calculated as a percentage of the maximal activity achieved with 30 μM compound **1** (See Ref 7, 18).
23. Cell-based assays were performed by measuring the amount of NAD^+ in HEK293A cells utilizing the NAD/NADH-Glo assay kit. Emax was calculated as a percentage of the increase in NAD^+ content achieved with 30 μM compound **1** (See Ref 7, 18).
24. The standard Ames test was conducted at the following test concentrations: 9.77, 19.5, 39.1, 78.1,

156, 313, 625, 1250, 2500, and 5000 μg/plate.

25. L. Formentini, F. Moroni, and A. Chiarugi, *Biochem. Pharmacol.*, 2009, **77**, 1612.
26. T. Ogiyama, M. Yamaguchi, N. Kurikawa, S. Honzumi, K. Terayama, N. Nagaoka, Y. Yamamoto, T. Kimura, D. Sugiyama, and S. Inoue, *Bioorg. Med. Chem.*, 2017, **25**, 4817.
27. T. Arita, M. Asano, K. Kubota, Y. Domon, and N. Machinaga, *Bioorg. Med. Chem.*, 2020, **28**, 115714.
28. T. Nagata, T. Yoshino, N. Haginoya, K. Yoshikawa, M. Nagamochi, M. Kobayashi, S. Komoriya, A. Yokomizo, R. Muto, M. Yamaguchi, K. Osanai, M. Suzuki, and H. Kanno, *Bioorg. Med. Chem.*, 2009, **17**, 1193.