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Article

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Identification of Adenine and Benzimidazole Nucleosides as Potent Human Concentrative Nucleoside Transporter 2 Inhibitors: Potential Treatment for Hyperuricemia and Gout

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Abstract

To test the hypothesis that inhibitors of human concentrative nucleoside transporter 2 (hCNT2) suppress increases in serum urate levels derived from dietary purines, we previously identified adenosine derivative **1** as a potent hCNT2 inhibitor (IC_{50} = 0.64 μM), but further study was hampered due to its poor solubility. Here we describe the results of subsequent research to identify more soluble and more potent hCNT2 inhibitors, leading to the discovery of the

benzimidazole nucleoside **22**, which is the most potent hCNT2 inhibitor (IC_{50} = 0.062 μM) reported to date. Compound **22** significantly suppressed the increase in plasma uric acid levels after oral administration of purine nucleosides in rats. Because compound **22** was poorly absorbed orally in rats (*F* = 0.51%), its pharmacologic action was mostly limited to the gastrointestinal tract. These findings suggest that inhibition of hCNT2 in the gastrointestinal tract can be a promising approach for the treatment of hyperuricemia.

Introduction

Uric acid is the end product of purine metabolism in human beings because a gene encoding uricase has undergone mutational silencing during hominoid evolution.[1-3] Uricase is an enzyme involved in the degradation of uric acid to the more soluble allantoin, and is active in a wide range of organisms, from microorganism to mammals.[4-8] The loss of uricase activity has led to higher uric acid levels in human beings compared with most other mammals.[8,9] Uric acid is a weak acid with a p*K*a of 5.75 and is present predominantly in the form of urate at a physiologic pH of 7.4.[3,10,11] In extracellular fluid, uric acid exists mainly as a solution of monosodium urate, which is a poorly soluble excretion product unlike allantoin, and thus has the potential to cause characteristic problems in human beings.[3,10]

Hyperuricemia is a condition in which serum urate levels are abnormally high. The definition of hyperuricemia, though currently arbitrary, is a serum urate concentration in excess of the solubility limit at physiologic temperature and pH (~ 6.8 mg/dL).[11-16] Persistent hyperuricemia is widely accepted as the primary risk factor for urate deposition diseases, such as gouty arthritis, gouty tophi, and renal damage.[15,16] In addition, a number of reports suggest an association

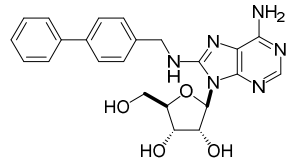
between hyperuricemia and pathologic conditions other than urate deposition disease.[17-22] For example, recent epidemiologic studies demonstrated that serum urate levels positively correlate with the frequency of metabolic syndrome[23-28] and the risk for developing hypertension.[29-35] Therefore, treatment of hyperuricemia has become increasingly important.

Serum urate levels are controlled through the balance among dietary purines, purine catabolism, and urate excretion.[36,37] Therefore, hyperuricemia can result from the excessive intake of purines, the increase in purine catabolism, the decrease in urate excretion, or combinations of them. Currently, two types of therapeutic drugs are available for the treatment of hyperuricemia.[36] Xanthine oxidase inhibitors and uricosuric agents reduce serum urate levels by blocking the biosynthesis and the tubular reabsorption of urate, respectively. However, no therapeutic drug focused on the third causative factor, dietary purine, has yet been developed. Patients with hyperuricemia are advised to refrain from habitual intake of a purine-rich diet. Hyperuricemia can be evoked in healthy subjects by ingesting yeast nucleic acid for several days.[38-40] On the other hand, recent epidemiologic studies report that high levels of meat and seafood consumption are associated with high serum urate levels[41] and an increased risk of gouty arthritis.[42] In contrast, a low purine diet lowers serum urate levels in patients with hyperuricemia.[43,44] Together, these findings suggest that orally-ingested purines increase serum urate levels and thus suppressing their gastrointestinal absorption could be a promising approach for treating hyperuricemia.

While dietary purines comprise various forms, such as DNA, RNA, nucleoproteins, oligonucleotides, purine nucleotides, purine nucleosides, and purine bases, most of these forms are unlikely to directly pass through the plasma membranes due to their high molecular weights and/or highly hydrophilic nature. Thus, carrier-mediated transport is probably involved in the

gastrointestinal absorption of purines. Nucleoside transporter proteins have been extensively studied as potential carriers.[45-50] Currently, they are roughly classified into two groups in mammals. One group comprises three concentrative nucleoside transporters, CNT1, CNT2, and CNT3 (SLC28A1, SLC28A2, and SLC28A3, respectively), which mediate unidirectional sodium- and/or proton-dependent transport.[51,52] Another group comprises four equilibrative nucleoside transporters, ENT1, ENT2, ENT3, and ENT4 (SLC29A1, SLC29A2, SLC29A3, and SLC29A4, respectively), which mediate bidirectional sodium- and proton-independent transport (facilitated diffusion).[53] In previous reports, we estimated the likelihood that human concentrative nucleoside transporter 2 (hCNT2) plays a central role in the gastrointestinal absorption of purines on the basis of the expression profile and inherent substrate selectivity of the seven transporters in the gastrointestinal tract:[54,55] In human beings, (1) CNT1, CNT2, ENT1, and ENT2 are recognized as the major players in the enterocyte.[56-58] (2) CNT1 and CNT2 are located on the apical side, while ENT1 and ENT2 are on the basolateral side.[45,48,50,56-58] (3) CNT2 is a purine nucleoside-preferring transporter, whereas CNT1 is a practically pyrimidine nucleoside-specific transporter.[50,51] Thus, we hypothesized that hCNT2 inhibitors would suppress the elevation of serum urate levels derived from dietary purines.[54,55] To test this hypothesis, we searched for potent hCNT2 inhibitors, which led to the discovery of compound **1** (Figure 1).[55] Further studies using compound **1** were hampered, however, due to its poor solubility in aqueous medium. For example, the solubility of compound **1** in the Japanese Pharmacopoeia second fluid for a dissolution test (JP2, pH 6.8)[59] at 37 °C was 0.003 mg/mL, indicating that **1** is practically insoluble. Hence, more soluble potent hCNT2 inhibitors are required. In the present paper, we describe structure-activity relationship studies leading to the discovery of potent hCNT2 inhibitors with increased solubility, and their suppressive effect on

an increase of plasma uric acid levels derived from orally administered purine nucleosides in rats.



1

hCNT2 IC_{50} = 0.64 ± 0.19 μM
Solubility (JP2, 37 °C) = 0.003 mg/mL

Figure 1. A potent hCNT2 inhibitor discovered in our previous study.

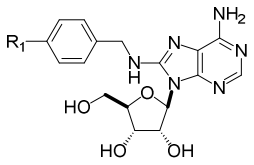
Results and Discussion

Improving solubility by manipulating the biphenyl conformation.

To enhance the solubility of compound **1**, we focused on its highly hydrophobic biphenyl moiety, and synthesized various compounds with modifications in this region. All newly synthesized final products were evaluated primarily for their inhibitory effects on sodium-dependent inosine uptake in COS-7 cells transiently expressing hCNT2 following the previously described procedure.[54,55]

First, the terminal benzene ring was replaced with a pyridine ring (Table 1). The 3-pyridyl analog **3** exhibited potent hCNT2 inhibitory activity similar to that of compound **1**, whereas the 2- and 4-pyridyl analogs (compounds **2** and **4**) resulted in somewhat reduced activity.

Table 1. Inhibitory Effects of Compounds **2**-**4** on hCNT2 and rat CNT2 (rCNT2) Activities

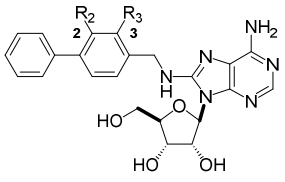


compd	R_1	IC_{50} (μM)[a]	
		hCNT2	rCNT2
1	phenyl	0.64 ± 0.19	inactive[b]
2	pyridin-2-yl	1.5 ± 0.5	inactive[b]
3	pyridin-3-yl	1.0 ± 0.2	>100[c]
4	pyridin-4-yl	2.7 ± 0.9	inactive[b]

[a]Concentration of each compound required to inhibit inosine uptake by 50%. Data are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments unless otherwise stated. [b]No inhibition was observed at the maximum concentration of 100 μM. [c]11% inhibition was observed at the maximum concentration of 100 μM.

We then introduced a polar functional group on the *endo*-benzene ring (Table 2). Among the compounds substituted at the 2-position, the 2-hydroxy derivative **5** was tolerated without a significant loss of inhibitory activity. While the inhibitory activity of the 2-methoxy derivative **6** was comparable to that of **5**, introduction of larger alkoxy groups, including ethoxy, propoxy, and isopropoxy groups, to the 2-position tended to reduce the potency depending on their bulkiness (compounds **7**-**9**). The 3-substituted derivatives **10**-**14** similarly reduced the potency depending on the bulkiness of the substituent introduced. Thus, irrespective of the substitution position, increased lipophilicity of substituents correlated with decreased activity of the compounds.

Table 2. Inhibitory Effects of Compounds **5**-**17** on hCNT2 and rCNT2 Activities



compd	R_2	R_3	IC_{50} (μM)[a]	
			hCNT2	rCNT2
1	H	H	0.64 ± 0.19	inactive[b]
5	OH	H	1.2 ± 0.6	inactive[b]
6	OMe	H	1.3 ± 0.2	inactive[b]
7	OEt	H	2.3 ± 0.6	inactive[b]
8	OPr	H	5.2 ± 1.5	inactive[b]
9	OPr-*i*	H	10.4 ± 1.0	inactive[b]
10	H	OH	1.3 ± 0.1	inactive[b]
11	H	OMe	1.1 ± 0.1	inactive[b]
12	H	OEt	5.4 ± 1.3	inactive[b]
13	H	OPr	inactive[b]	inactive[b]
14	H	OPr-*i*	9.3 ± 1.8	inactive[b]
15	OCH_2CO_2Me	H	3.3 ± 0.8	inactive[b]
16	OCH_2CONH_2	H	5.5 ± 1.4	>100[c]
17	OCH_2CH_2OH	H	3.1 ± 0.3	>100[d]

[a]Concentration of each compound required to inhibit inosine uptake by 50%. Data represent mean ± SEM of at least three independent experiments unless otherwise stated. [b]No inhibition was observed at the maximum concentration of 100 μM. [c]Less than 5% inhibition was observed at the maximum concentration of 100 μM. [d]49% inhibition was observed at the maximum concentration of 100 μM.

Although substitution with a small group at both the 2- and 3-positions of the *endo*-benzene ring did not reduce the activity, we thought that substitution at the 2-position would probably improve the solubility more effectively than substitution at the 3-position because steric repulsion between the 2-substituent and the terminal benzene ring might make the planar conformation of the biphenyl moiety unstable, and thus the biphenyl moiety would become stabilized by assuming a twisted conformation to avoid the steric repulsion. Actually, recent

studies suggested that disruption of molecular planarity is a good strategy for improving the aqueous solubility of poorly soluble compounds.[60,61] Therefore, we estimated the substituent effects on the conformation of compound **1**, especially that of the biphenyl moiety, using its 2- and 3-methyl derivatives (**A** and **B**, respectively) as model compounds by performing theoretical calculations using the density functional theory B3LYP/6-31G* method in Spartan'14. When comparing the most stable conformers (Figure 2), the biphenyl moiety of 2-methylated **A** was significantly more twisted (57.4°) than that of 3-methylated **B** (36.6°) and unmethylated **1** (37.3°).

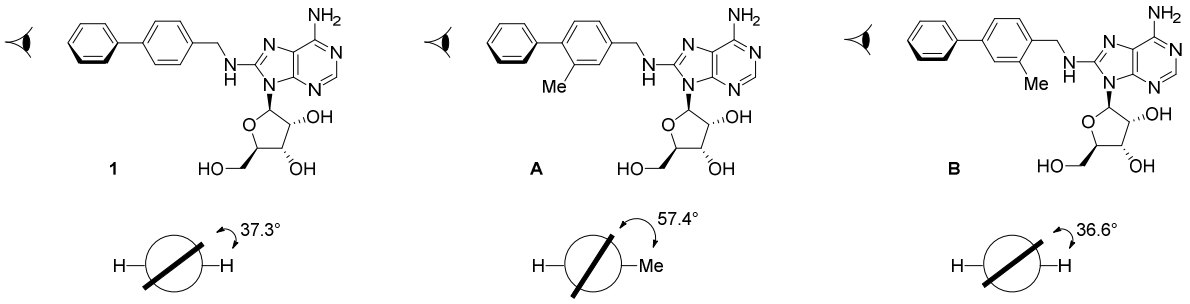


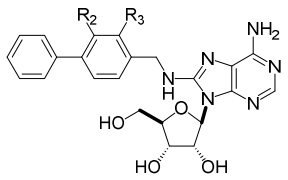
Figure 2. Simulation of substituent effects on the biphenyl structure

If the biphenyl moiety of the compound actually assumes a non-planar twisted conformation, its crystallinity would decrease compared with the planar one to improve the solubility. To test this hypothesis, we measured the solubility of compounds in JP2 at 37 °C using the 2-substituted derivatives **6**-**7** and their 3-substituted congeners **11**-**12** (Table 3). As expected, each of the 2-substituted compounds **6** and **7** had significantly improved solubility compared with the parent compound **1**. In contrast, despite introduction of the same substituents, the solubility of 3-

substituted compounds **11** and **12** were unchanged compared to the parent compound **1**. These results correlated well with the simulation of the biphenyl conformations shown in Figure 2.

On the basis of the above encouraging results, other substituents at the 2-position were investigated. Because alkoxy groups introduced at the 2-position tended to decrease hCNT2 inhibitory activity depending on their bulkiness and lipophilicity, we examined alkoxy groups substituted with a polar functional group (Table 2). Although the size of the 2-methoxy-2-oxoethoxy and 2-hydroxyethoxy groups was comparable to or greater than the propoxy group, compounds **15** and **17** exhibited more potent hCNT2 inhibitory activity than the propoxy derivative **8**, but less potent than the ethoxy derivative **7**. In contrast, the 2-amino-2-oxoethoxy derivative **16** resulted in inhibitory activity equal to that of **8**.

Table 3. Substituent Effects on the Solubility in JP2



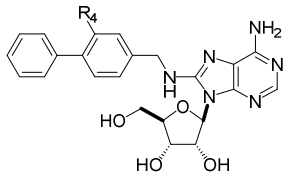
compd	R_2	R_3	solubility (mg/mL)[a]
1	H	H	0.003
6	OMe	H	0.215
7	OEt	H	0.121
11	H	OMe	0.004
12	H	OEt	0.002

[a]Solubility in JP2 (pH 6.8) after incubation at 37 °C for 1 h.

Attempt to improve inhibitory activity against rat CNT2 (rCNT2) in adenosine derivatives. We next examined the inhibitory activity of the relatively potent hCNT2 inhibitors **3**, **5**, and **6** against rCNT2.[54] Unexpectedly, both compounds **5** and **6** were inactive against rCNT2 up to the maximum concentration of 100 μM (Table 2), whereas **3** exhibited slight inhibitory activity (Table 1). These findings might be due to differences in the amino acid sequence between hCNT2 and rCNT2: hCNT2 is one amino acid shorter than rCNT2 and 83% identical to rCNT2.[57,58] Hence, we also evaluated all other compounds including parent compound **1**, and confirmed that most of these compounds were inactive, and only compounds **16** and **17** had weak activity (Tables 1 and 2).

Comparison of the 2-ethoxy derivative **7** with its hydroxylated analog **17** revealed that the terminal hydroxy group is crucial for exerting rCNT2 inhibitory activity. Therefore, we paid attention to the homologs of compound **17** and synthesized the 3-hydroxypropoxy, 4-hydroxybutoxy, and 5-hydroxypentyloxy derivatives **18**-**20**. As summarized in Table 4, elongation of the ethylene linker in compound **17** enhanced the inhibitory activity against both hCNT2 and rCNT2. Because the 4-hydroxybutoxy derivative **19** exhibited the most potent inhibitory activity against both hCNT2 and rCNT2 among the homologs, we selected this compound for further evaluation and measured its solubility in JP2 at 37 °C. As a result, compound **19** showed much higher solubility (0.190 mg/mL) than the parent compound **1** (Table 4), suggesting that the 4-hydroxybutoxy group enhanced the twisted structure of the biphenyl moiety to increase solubility, as mentioned above.

Table 4. *in vitro* Profiles of Compounds **17**-**20** and Improved Solubility of **19** in JP2



compd	R_4	IC_{50} (μM)[a]		solubility (mg/mL)[d]
		hCNT2	rCNT2	
1	H	0.64 ± 0.19	inactive[b]	0.003
17	$O(CH_2)_2OH$	3.1 ± 0.3	>100[c]	ND[e]
18	$O(CH_2)_3OH$	1.1 ± 0.1	45.1 ± 2.9	ND[e]
19	$O(CH_2)_4OH$	0.94± 0.24	38 ± 13	0.190
20	$O(CH_2)_5OH$	1.6 ± 0.1	60 ± 20	ND[e]

[a]Concentration of each compound required to inhibit inosine uptake by 50%. Data represent mean ± SEM of at least three independent experiments unless otherwise stated. [b]No inhibition was observed at the maximum concentration of 100 μM. [c]49% inhibition was observed at the maximum concentration of 100 μM. [d]Solubility in JP2 (pH 6.8) after incubation at 37 °C for 1 h. [e]ND, not determined.

Subsequently, compound **19** was evaluated by performing a purine load test in rats. Serum urate levels in rats are quite low (0.5-1 mg/mL),[8] in contrast to human beings due to the existence of uricase activity, and therefore they were pretreated with the uricase inhibitor potassium oxonate (100 mg/kg) to induce serum urate accumulation.[64] Oral administration of a purine mixture (50 mg/kg), an equal mixture of adenosine, inosine, and guanosine, significantly elevated plasma uric acid levels in the rats (Figure 3). On the other hand, the purine mixture-induced elevation of plasma uric acid levels was suppressed when compound **19** was administered simultaneously at a dose of 50 mg/kg. The suppressive ratio was calculated to be 42%, but unfortunately it was not statistically significant. This may be due to insufficient inhibitory activity against rCNT2 (IC_{50} = 38 μM).

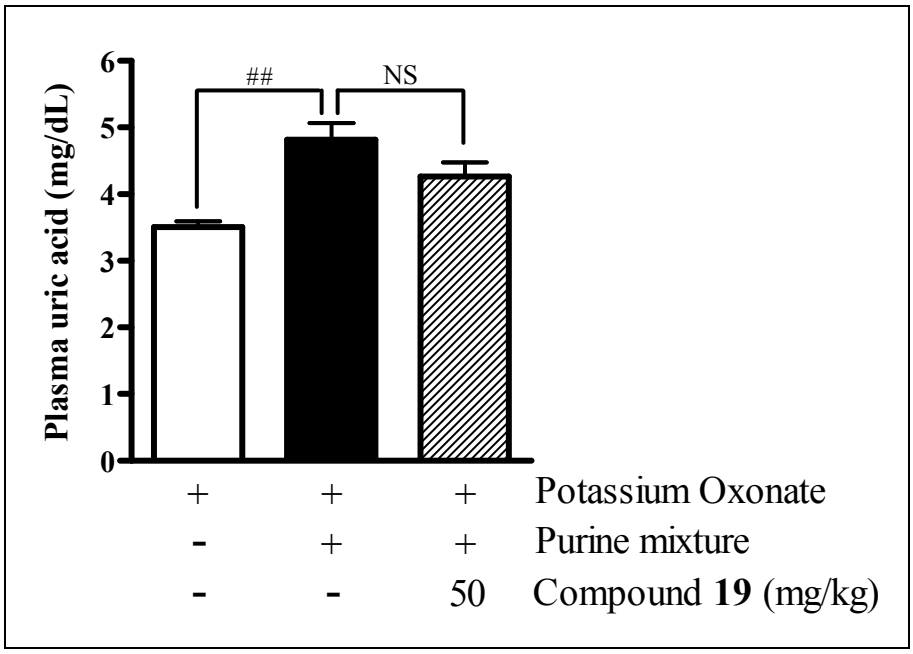


Figure 3. Effect of compound **19** on the elevation of plasma uric acid levels derived from orally administered purine mixture (an equal mixture of adenosine, guanosine, and inosine, 50 mg/kg) in rats. Data are expressed as the mean ± SEM (n = 5). ##: $p < 0.01$ versus a group treated with only potassium oxonate (100 mg/kg). NS, not significant.

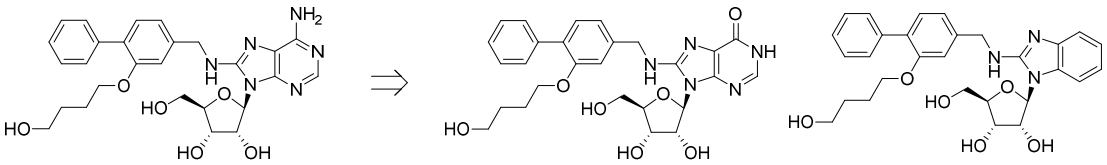
Scaffold hopping from adenine to benzimidazole.

On the basis of the above-mentioned *in vitro* profiles, it was conceivable that the different susceptibility between hCNT2 and rCNT2 observed in this study was a characteristic common to compounds having the 8-(benzylamino)adenosine substructure. Therefore, we designed compounds based on a scaffold hopping strategy to find compounds with enhanced inhibitory activity against rCNT2.

Because the sugar moiety has important interactions with CNT2,[65-67] and the substituent at the 8-position of adenosine was highly optimized through our study, we performed adenine moiety-hopping of compound **19** and designed the hypoxanthine riboside **21** and the benzimidazole

riboside **22**. As shown in Table 5, conversion to the inosine congener **21** not only reduced the inhibitory activity against both hCNT2 and rCNT2, but also increased the susceptibility difference. Replacement of adenine with the benzimidazole forming **22**, however, considerably enhanced the inhibitory activity against both hCNT2 and rCNT2. Thus, benzimidazole congener **22** was identified as the most potent hCNT2 inhibitor (IC_{50} = 0.062 μM) reported to date, which was also a potent inhibitor against rCNT2 (IC_{50} = 1.5 μM).[68] In addition, the solubility of compound **22** in JP2 at 37 °C (0.120 mg/mL) was much higher than that of parent compound **1**.

Table 5. *in vitro* Profiles and Solubility Data of Compounds **19, 21**, and **22**



compd	IC_{50} (μM)[a]		solubility (mg/mL)[c]
	hCNT2	rCNT2	
19	0.94± 0.24	38 ± 13	0.190
21	20.1 ± 2.0	>1000[b]	ND[d]
22	0.062 ± 0.017	1.5 ± 0.1	0.120

[a]Concentration of each compound required to inhibit inosine uptake by 50%. Data represent mean ± SEM of at least three independent experiments unless otherwise stated. [b]18% inhibition was observed at the maximum concentration of 1000 μM. [c]Solubility in JP2 (pH 6.8) after incubation at 37 °C for 1 h. [d]ND, not determined.

Although the issue of the susceptibility difference remained, compound **22** had more than 25-fold more potent inhibitory activity than **19** against rCNT2, and therefore was evaluated by purine load test in the same manner as described above (Figure 4). Compound **22** significantly

suppressed the increase in plasma uric acid levels derived from the purine mixture at a lower dose of 1.0 mg/kg, which was probably due to enhanced *in vitro* activity. A higher dose of compound **22** (10 mg/kg), however, resulted in little enhancement of the suppressive effect. These results suggested that rCNT2 played a central role in the absorption of purine nucleosides through the gastrointestinal tract, while pathways other than via rCNT2 might be involved in the uptake of orally-ingested purine nucleosides, including their breakdown products, in rats.

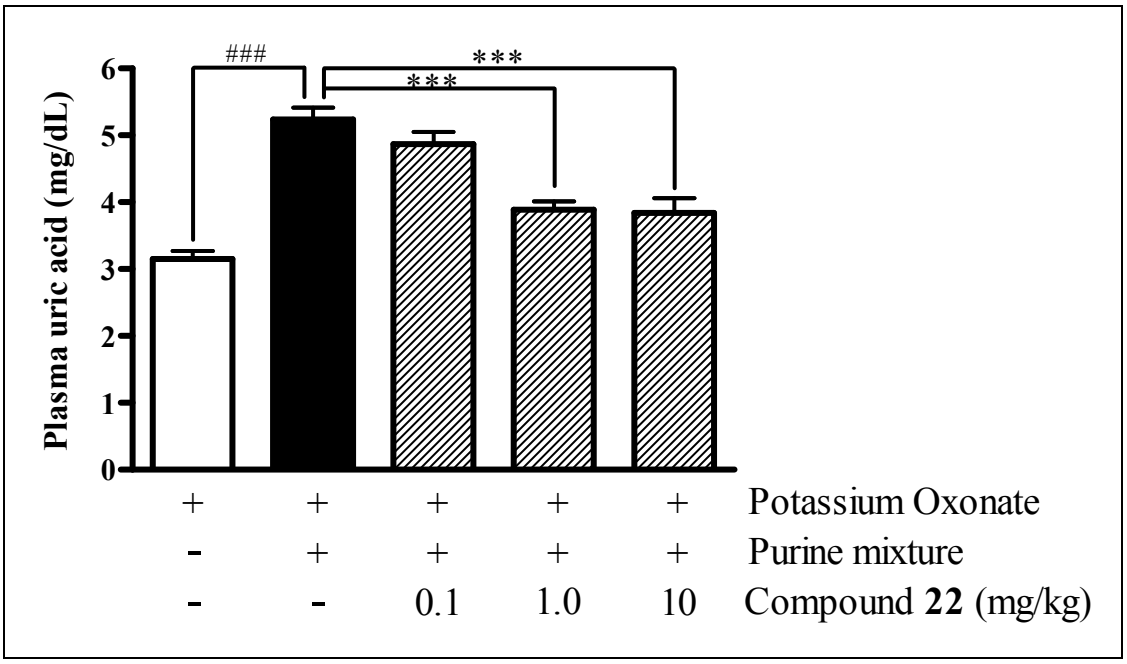


Figure 4. Effects of compound **22** on the elevation of plasma uric acid levels derived from orally administered purine mixture (50 mg/kg) in rats. Data are expressed as the mean ± SEM. (n = 5-11). ###: $p < 0.001$ versus a group treated with only potassium oxonate (100 mg/kg). ***: $p < 0.001$ versus a group treated with potassium oxonate and purine mixture.

Finally, the oral absorption properties of compound **22** were investigated in rats (Table 6). The maximum plasma concentration value (C_{max}) of compound **22** (0.11 μM) after oral administration (10 mg/kg) was below one-tenth of the IC_{50} value of **22** (1.5 μM), and the oral

bioavailability (F) was calculated to be 0.51%. These results suggested that compound **22** was poorly absorbed orally, and that its action was mostly limited to the gastrointestinal tract. Therefore, the pharmacologic effects of compound **22** in the purine load test were probably due to the uptake inhibition of purine nucleosides via rCNT2 through the gastrointestinal tract.

Table 6. Pharmacokinetic Parameters of Compound **22** in Rats after Intravenous (iv) and Oral (po) Administrations[a]

parameter	iv[b]	po[c]
CL (mL/h/kg)	394	-
V_{dss} (mL/kg)	117	-
$t_{1/2}$ (h)	1.7	-
$AUC_{0\text{-}inf}$ (μM·h)	5.0	-
C_{max} (μM)	-	0.11
t_{max} (h)	-	0.83
$AUC_{0\text{-}x}$ (μM·h)	-	0.25
F (%)	-	0.51

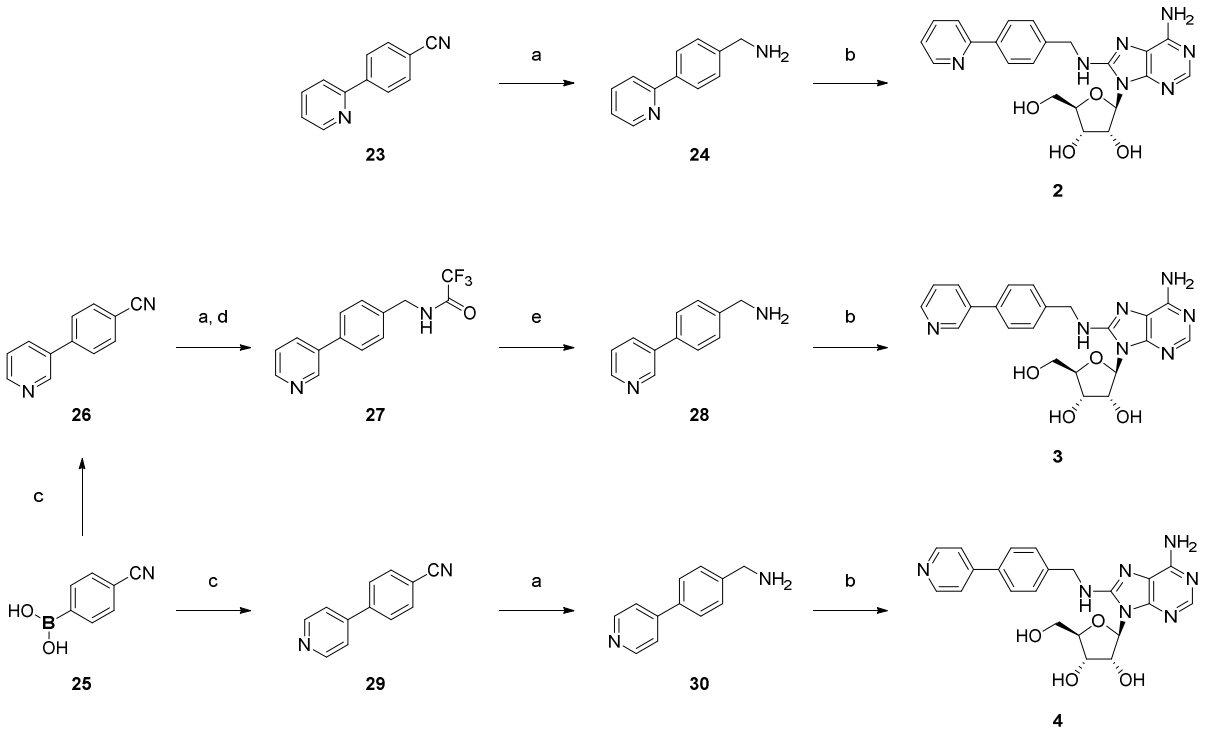
[a] Data are expressed as the mean (n = 3). [b]Compound **22** was dissolved in 50% (v/v) *N*,*N*-dimethylacetamide in 5% glucose solution and administered intravenously at a dose of 1.0 mg/kg. [c]Compound **22** was suspended in sodium carboxymethyl cellulose and administered orally at a dose of 10 mg/kg.

Chemistry

The adenosine derivatives **2-4** were synthesized by nucleophilic aromatic substitution of commercially available 8-bromoadenosine with the amines **24**, **28**, and **30**, which were prepared from commercially available starting materials (Scheme 1). The amine **24** was prepared by reducing 4-(pyridin-2-yl)benzonitrile **23** with $LiAlH_4$. Suzuki-Miyaura coupling of 4-

cyanophenylboronic acid **25** with 3-bromopyridine and subsequent treatment with $LiAlH_4$ gave 4-(pyridin-3-yl)benzylamine, which was purified after conversion to corresponding trifluoroacetamide **27**. Alkaline hydrolysis of compound **27** afforded amine **28**. In a similar fashion, amine **30** could be synthesized starting from compound **25** without the additional protection-deprotection step for purification.

Scheme 1. Synthesis of Compounds 2-4[a]

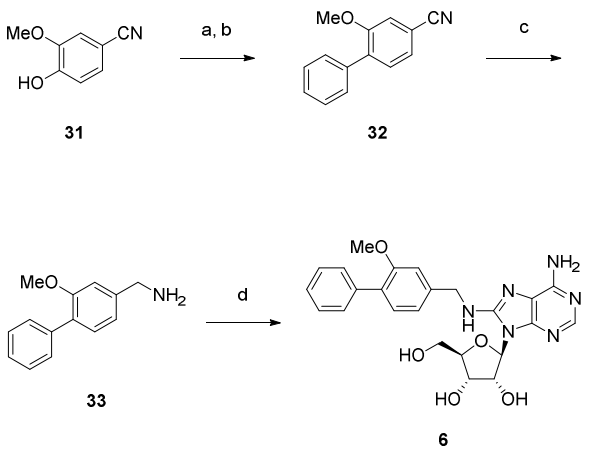


[a]Reagents and conditions: (a) $LiAlH_4$, THF, 60 °C; (b) 8-bromoadenosine, *i*-Pr_2NEt, EtOH, 120 °C, in a screw tube; (c) 3-bromopyridine or 4-bromopyridine hydrochloride, $Pd(PPh_3)_4$, 2.0 M aq Na_2CO_3, MeCN, 80 °C; (d) CF_3CO_2Et, EtOH, rt; (e) 2.0 M aq NaOH, EtOH, 60 °C.

Adenosine derivatives **5-9** and **15-20** were synthesized starting from commercially available 4-hydroxy-3-methoxybenzonitrile **31** (Schemes 2-4). Compound **31** was converted to its triflate,

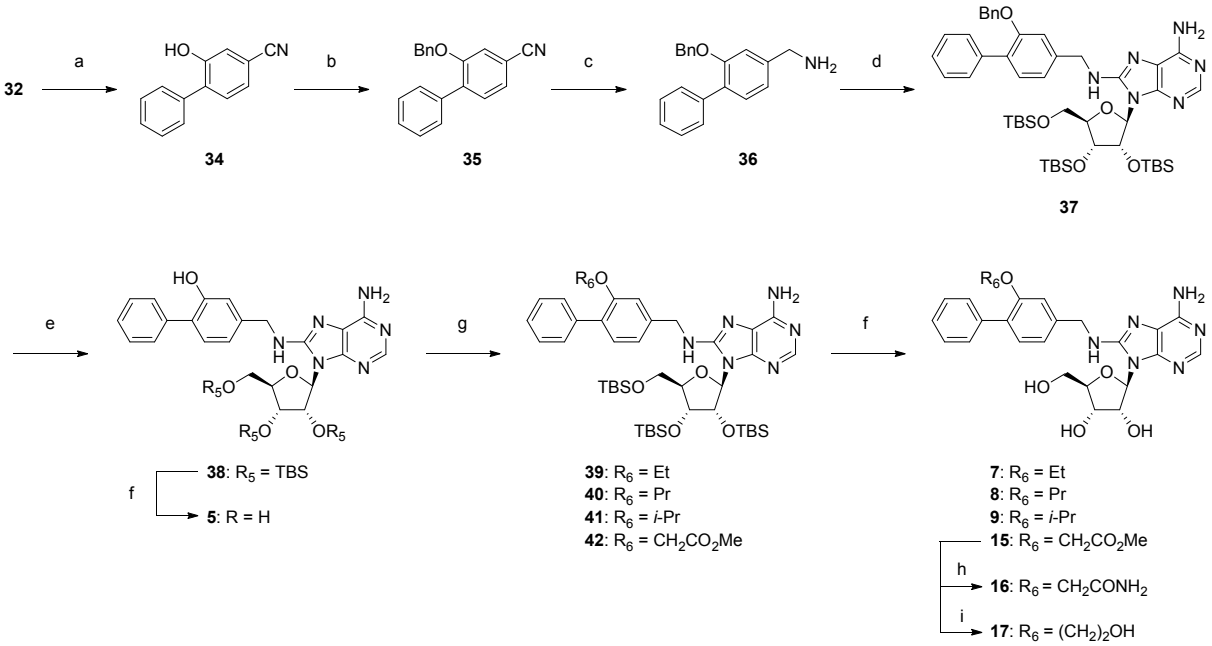
followed by Suzuki-Miyaura coupling with phenylboronic acid to give the biphenyl **32**. Reduction of compound **32** with $LiAlH_4$ and subsequent nucleophilic aromatic substitution with 8-bromoadenosine furnished **6**. In addition, methyl ether **32** was converted to the corresponding benzyl ether **35** by stepwise treatment with BBr_3 and BnBr before reduction with $LiAlH_4$ to afford amine **36**. Condensation of compound **36** with 8-bromo-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)adenosine, which was prepared according to the method described in the literature[69] with some modifications, and subsequent catalytic hydrogenation provided the common intermediate **38**. Treatment of compound **38** with NH_4F yielded **5**. On the other hand, the hydroxyl group of compound **38** was alkylated with the appropriate alkyl halide followed by treatment with NH_4F to give **7**-**9** and **15**. Reactions of the ester **15** with NH_3 and $NaBH_4$ afforded the amide **16** and alcohol **17**, respectively. Alkylation of compound **34** with the appropriate alkyl bromide and subsequent reduction with $LiAlH_4$ provided amines **46**-**48**, which were then subjected to nucleophilic aromatic substitution with 8-bromoadenosine before removal of the benzyl groups by catalytic hydrogenation to furnish **18**-**20**.

Scheme 2. Synthesis of Compound 6[a]



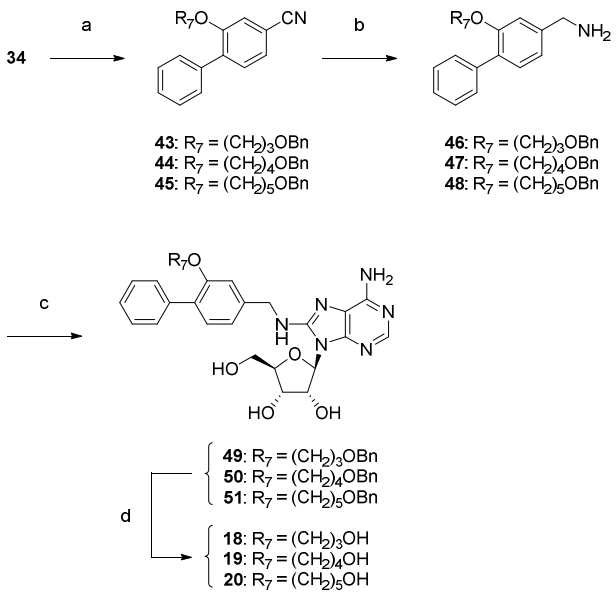
[a]Reagents and conditions: (a) Tf_2O, Et_3N, DCM, rt; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, DMF, 80 °C; (c) $LiAlH_4$, THF, 60 °C; (d) 8-bromoadenosine, *i*-Pr_2NEt, EtOH, 120 °C, in a screw tube.

Scheme 3. Synthesis of Compounds 5, 7-9, and **15-17**[a]



[a]Reagents and conditions: (a) BBr_3 (1.0 M in DCM), DCM, 30 °C; (b) BnBr, K_2CO_3, DMF, rt; (c) $LiAlH_4$, THF, 60 °C; (d) 8-bromo-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)adenosine[69], *i*-Pr_2NEt, EtOH, 120 °C, in a screw tube; (e) H_2, 10% Pd-C, AcOEt, rt; (f) NH_4F, MeOH, reflux; (g) R_6I or R_6Br, K_2CO_3, DMF, 35-50 °C; (h) NH_3 (2.0 M in MeOH), rt; (i) $NaBH_4$, EtOH, rt.

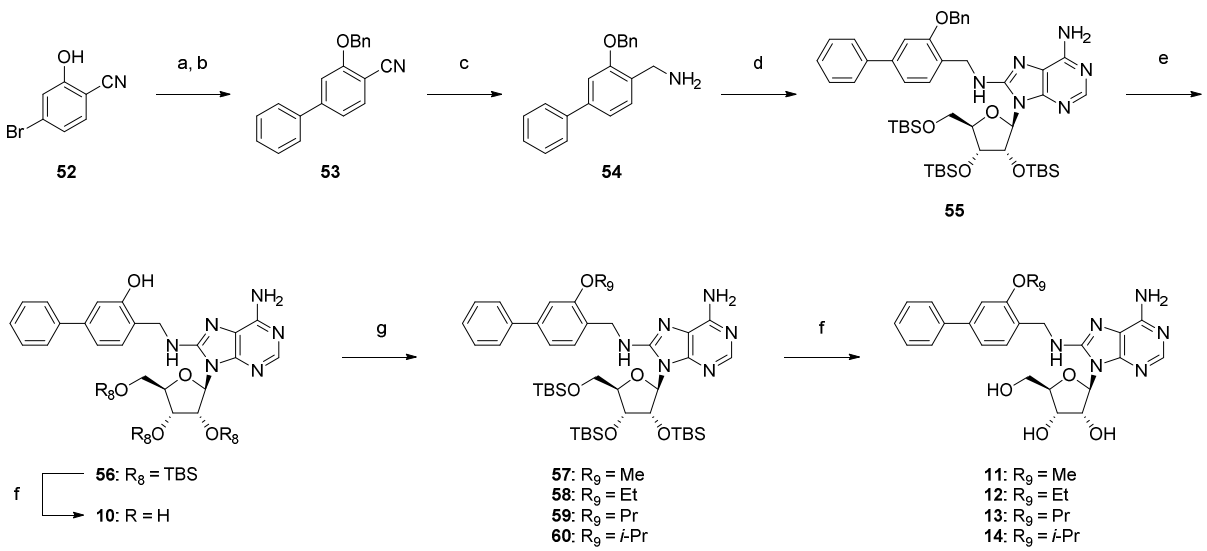
Scheme 4. Synthesis of Compounds 18-20[a]



[a]Reagents and conditions: (a) R_7Br, K_2CO_3, DMF, 50 °C; (b) $LiAlH_4$, THF, 60 °C; (c) 8-bromoadenosine, *i*-Pr_2NEt, EtOH, 120 °C, in a screw tube; (d) H_2, 10% Pd-C, MeOH, 50 °C.

The adenosine derivatives **10**-**14** were synthesized starting from commercially available 4-bromo-2-hydroxybenzonitrile **52** following a methodology similar to that described above (Scheme 5). Suzuki-Miyaura coupling with phenylboronic acid after benzylation of compound **52** and subsequent reduction with $LiAlH_4$ provided the amine **54**. Condensation of compound **54** with 8-bromo-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)adenosine[69] followed by catalytic hydrogenation yielded the common intermediate **56**. The silyl ethers of compound **56** were cleaved by treatment with NH_4F to give **10**. On the other hand, the hydroxy group of compound **56** was alkylated with the appropriate alkyl iodide before treatment with NH_4F to afford **11**-**14**.

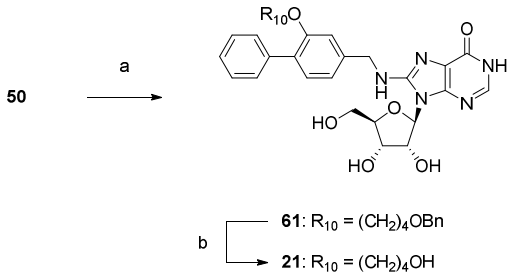
Scheme 5. Synthesis of Compounds 10-14[a]



[a]Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, DMF, 80 °C; (c) $LiAlH_4$, THF, 60 °C; (d) 8-bromo-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)adenosine[69], *i*-Pr_2NEt, EtOH, 120 °C, in a screw tube; (e) H_2, 10% Pd-C, AcOEt, rt; (f) NH_4F, MeOH, reflux; (g) R_9I, K_2CO_3, DMF, rt-50 °C.

The inosine derivative **21** was synthesized by treating the adenosine derivatives **50** with $NaNO_2$ in aqueous acetic acid followed by catalytic hydrogenation to remove the benzyl group (Scheme 6).

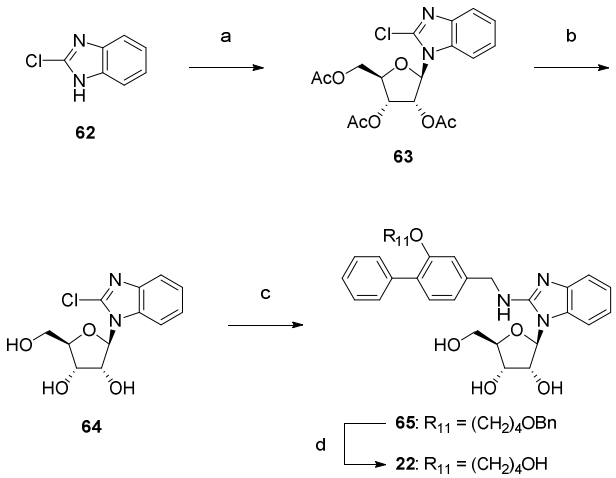
Scheme 6. Synthesis of Compound 21[a]



[a]Reagents and conditions: (a) $NaNO_2$, AcOH, H_2O, rt; (b) H_2, 10% Pd-C, MeOH, 50 °C.

The benzimidazole nucleoside **22** was synthesized starting from commercially available 2-chloro-1*H*-benzimidazole **62** (Scheme 7). Compound **62** was silylated with *N*,*O*-bis(trimethylsilyl)acetamide, followed by glycosidation with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of TMSOTf to give **63**. Treatment of compound **63** with NaOMe provided **64**, which was then subjected to a nucleophilic aromatic substitution reaction with **47** followed by catalytic hydrogenation to furnish **22**.

Scheme 7. Synthesis of Compound 22[a]



[a]Reagents and conditions: (a) *N*,*O*-bis(trimethylsilyl)acetamide, MeCN, 80 °C then TMSOTf, 1,2,3,4-tetra-*O*-acetyl-β-D-ribofuranose, rt; (b) *t*-BuOK, MeOH, rt; (c) **47**, *i*-Pr_2NEt, EtOH, 120 °C, in a screw tube; (d) H_2, 10% Pd-C, MeOH, 50 °C.

Conclusion

We searched for potent hCNT2 inhibitors with increased solubility starting from compound **1**, a potent but poorly soluble inhibitor that we reported previously. Substituents at the 2-position of the biphenyl-4-yl substructure in compound **1** effectively ameliorated the solubility in JP2 at 37 °C. Introduction of terminally-hydroxylated alkoxy groups was crucial for acquisition of the

rCNT2 inhibitory activity, which led to the discovery of compound **19**. Subsequent scaffold hopping of compound **19** by adenine replacement yielded the benzimidazole nucleoside **22** as the most potent hCNT2 inhibitor reported to date. Compound **22** exhibited potent inhibitory activity against rCNT2 and significantly suppressed increases in plasma urate levels in a purine load test using rats. Compound **22** had a poorly absorbable nature after oral administration in rats, and thus its pharmacologic action is likely limited to the gastrointestinal tract. These findings suggest that inhibition of hCNT2 in the gastrointestinal tract can be a promising approach for the treatment of hyperuricemia.

Experimental Section

General Methods. Reagents and solvents were purchased from commercial sources and used without further purification. All moisture and air sensitive reactions were carried out in an oven-dried flask under an argon atmosphere. Reaction conditions and yields were not optimized. All melting points were determined on a Yanaco micro melting point apparatus (MP-J3) and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F_{254} plates or Fuji Silysia Chemical Ltd. Chromatorex NH-TLC plates. Flash column chromatography was performed on Biotage prepacked columns or Yamazen Hi-Flash columns using an automated flash chromatography systems Biotage Isolera One (Biotage AB, Uppsala, Sweden) or W-prep 2XY (Yamazen Corporation, Osaka, Japan). ^{1}H NMR spectra were recorded on a Bruker AV400M spectrometer at 400.1 MHz. Chemical shifts (δ) are reported in parts per million (ppm) units using tetramethylsilane as an internal standard. Data are presented as follows; chemical shift, multiplicity (s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; br, broad; br s, broad singlet), coupling constant and integration. ^{13}C NMR

spectra were recorded on a Bruker AV400M spectrometer at 100.6 MHz with complete proton decoupling. Chemical shifts (δ) are reported in parts per million (ppm) with the solvent as the internal reference (δ 77.16 in $CDCl_3$ or δ 39.52 in DMSO-d_6).[70] High resolution mass spectra (HRMS) were recorded on an Agilent Technologies 6520 Accurate-Mass Q-TOF instrument using electrospray ionization (ESI, positive or negative ion mode). The purity of all tested compounds was determined to be ≥95% by HPLC-UV analysis, which was performed on a Shimadzu LC-VP series instrument (Shimadzu Corporation, Kyoto, Japan) with the following parameters: analytical column, Inertsil ODS-3, 4.6 x 250 mm, 5 μm (GL Sciences Inc., Tokyo, Japan); mobile phase, linear gradient of 10-90% MeOH in 0.1% (v/v) aq HCO_2H for 45 min with flow rate of 1.0 mL/min; column temperature, 40 °C; injection volume, 10 μL; compound concentration, 2 mM in DMSO; detection wavelength, 260 nm.

8-[4-(Pyridin-2-yl)benzylamino]adenosine (2). A mixture of **24** (343 mg, 1.86 mmol), 8-bromoadenosine (215 mg, 0.621 mmol), and *i*-Pr_2NEt (482 mg, 3.73 mmol) in EtOH (5.18 mL) was heated in a screw tube at 120 °C with stirring for 90 h. The reaction mixture was allowed to cool to room temperature and then concentrated under reduced pressure. The residue was purified by flash column chromatography on NH silica gel (gradient; 8-15% MeOH in DCM) followed by flash column chromatography on silica gel (gradient; 10-20% MeOH in DCM) to give **2** (235 mg, 84%) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 3.53-3.73 (m, 2H), 3.96-4.05 (m, 1H), 4.09-4.17 (m, 1H), 4.55-4.81 (m, 3H), 5.18 (d, J = 4.0 Hz, 1H), 5.32 (d, J = 6.8 Hz, 1H), 5.92 (dd, J = 4.0, 6.3 Hz, 1H), 5.96 (d, J = 7.5 Hz, 1H), 6.54 (br s, 2H), 7.28-7.37 (m, 1H), 7.43-7.53 (m, 2H), 7.55-7.67 (m, 1H), 7.80-7.97 (m, 3H), 7.99-8.10 (m, 2H), 8.60-8.69 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 45.1, 61.8, 71.0, 71.2, 85.9, 86.6, 117.1, 120.1, 122.5, 126.5, 127.5,

137.3 (2C), 140.9, 148.7, 149.6, 149.9, 151.4, 152.5, 156.0. HRMS calcd for $C_{22}H_{24}N_7O_4$ $(M+H)^+$ 450.1884, found 450.1886.

8-[4-(Pyridin-3-yl)benzylamino]adenosine (3). The title compound was synthesized from **28** (160 mg, 0.867 mmol) and 8-bromoadenosine (100 mg, 0.289 mmol) according to the procedure described for **2**. The crude product was purified by flash column chromatography on NH silica gel (gradient; 8-15% MeOH in DCM) followed by flash column chromatography on silica gel (gradient; 10-24% MeOH in DCM) to afford **3** (99.9 mg, 77%) as a beige amorphous solid. ^{1}H NMR (DMSO-d_6) δ 3.55-3.74 (m, 2H), 3.96-4.06 (m, 1H), 4.10-4.20 (m, 1H), 4.54-4.82 (m, 3H), 5.16 (d, J = 4.0 Hz, 1H), 5.30 (d, J = 6.8 Hz, 1H), 5.85-6.00 (m, 2H), 6.53 (br s, 2H), 7.44-7.55 (m, 3H), 7.61 (t, J = 6.0 Hz, 1H), 7.64-7.73(m, 2H), 7.90 (s, 1H), 8.02-8.10 (m, 1H), 8.55 (dd, J = 1.8, 4.8 Hz, 1H), 8.84-8.91 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 45.0, 61.8, 71.0, 71.1, 85.9, 86.6, 117.0, 123.9, 126.8, 127.9, 134.0, 135.5 (2C), 140.0, 147.6, 148.3, 148.5, 149.9, 151.4, 152.4. HRMS calcd for $C_{22}H_{24}N_7O_4$ $(M+H)^+$ 450.1884, found 450.1879.

8-[4-(Pyridin-4-yl)benzylamino]adenosine (4). A mixture of **30** (227 mg, 1.23 mmol), 8-bromoadenosine (142 mg, 0.410 mmol), and *i*-Pr_2NEt (318 mg, 2.46 mmol) in EtOH (4.10 mL) was heated in a screw tube at 120 °C with stirring for 96 h. The reaction mixture was allowed to cool to room temperature and then concentrated under reduced pressure. To the suspension of the residue in DCM (20.0 mL) was added *i*-Pr_2NEt (159 mg, 1.23 mmol), and the resulting mixture was stirred at room temperature for 20 h. The precipitate was collected by filtration, washed with DCM, air-dried, and dried under reduced pressure to give an orange brown solid (256 mg). The solid (252 mg) was suspended in EtOH (30.0 mL) and heated under reflux with stirring for 1 h before being allowed to cool to room temperature. The precipitate was collected by filtration, washed with EtOH, air-dried, and dried under reduced pressure to give **4** (93.0 mg, 50%) as a

pale brown solid. ^{1}H NMR (DMSO-d_6) δ 3.54-3.71 (m, 2H), 3.96-4.06 (m, 1H), 4.10-4.20 (m, 1H), 4.52-4.84 (m, 3H), 5.16 (d, J = 4.0 Hz, 1H), 5.30 (d, J = 6.8 Hz, 1H), 5.90 (dd, J = 4.0, 6.0 Hz, 1H), 5.95 (d, J = 7.5 Hz, 1H), 6.52 (br s, 2H), 7.48-7.59 (m, 2H), 7.62 (t, J = 6.2 Hz, 1H), 7.66-7.86 (m, 4H), 7.90 (s, 1H), 8.58-8.69 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 45.0, 61.8, 71.0, 71.1, 85.8, 86.5, 117.0, 121.1, 126.7, 127.9, 135.6, 141.3, 146.8, 148.6, 149.9, 150.2, 151.3, 152.5. HRMS calcd for $C_{22}H_{24}N_7O_4$ $(M+H)^+$ 450.1884, found 450.1884.

8-(2-Hydroxybiphenyl-4-ylmethylamino)adenosine (5). To a solution of **38** (367 mg, 0.455 mmol) in MeOH (9.09 mL) was added NH_4F (1.01 g, 27.3 mmol), and the resulting mixture was heated under reflux with stirring for 24 h. The reaction mixture was allowed to cool to room temperature, diluted with H_2O (9.09 mL), and then stirred for 1 h. The precipitate was collected by filtration, washed with MeOH/H_2O (1/1), air-dried, and then recrystallized from EtOH to give **5** (75.0 mg, 36%) as an off-white solid. mp 240-242 °C. ^{1}H NMR (DMSO-d_6) δ 3.56-3.75 (m, 2H), 3.96-4.08 (m, 1H), 4.10-4.19 (m, 1H), 4.46-4.63 (m, 2H), 4.72-4.82 (m, 1H), 5.17 (d, J = 4.3 Hz, 1H), 5.24 (d, J = 6.8 Hz, 1H), 5.85-5.93 (m, 1H), 5.95 (d, J = 7.3 Hz, 1H), 6.51 (br s, 2H), 6.84-6.91 (m, 1H), 6.92-6.99 (m, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.23-7.31 (m, 1H), 7.33-7.43 (m, 2H), 7.47-7.62 (m, 3H), 7.90 (s, 1H), 9.46 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 45.0, 61.8, 71.1, 85.8, 86.6, 114.7, 117.1, 118.2, 126.2, 126.4, 127.9, 129.0, 130.1, 138.6, 140.5, 148.5, 149.8, 151.5, 152.5, 154.2. HRMS calcd for $C_{23}H_{25}N_6O_5$ $(M+H)^+$ 465.1881, found 465.1873.

8-(2-Methoxybiphenyl-4-ylmethylamino)adenosine (6). The title compound was synthesized from **33** (370 mg, 1.73 mmol) and 8-bromoadenosine (200 mg, 0.578 mmol) according to the procedure described for **2**. The crude product was purified by flash column chromatography on NH silica gel (gradient; 8-15% MeOH in DCM) followed by flash column chromatography on silica gel (gradient; 10-17% MeOH in DCM) to afford **6** (257 mg, 93%) as a yellow solid. mp

137-139 °C. ^{1}H NMR (DMSO-d_6) δ 3.57-3.72 (m, 2H), 3.75 (s, 3H), 3.97-4.05 (m, 1H), 4.08-4.21 (m, 1H), 4.51-4.82 (m, 3H), 5.17 (d, J = 4.0 Hz, 1H), 5.30 (d, J = 6.8 Hz, 1H), 5.87-6.03 (m, 2H), 6.53 (br s, 2H), 6.98-7.08 (m, 1H), 7.13-7.51 (m, 7H), 7.52-7.64 (m, 1H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 45.3, 55.5, 61.8, 71.0, 71.1, 85.9, 86.5, 110.7, 117.1, 119.5, 126.7, 128.0, 128.2, 129.2, 130.2, 138.1, 141.0, 148.6, 149.9, 151.3, 152.5, 156.0. HRMS calcd for $C_{24}H_{27}N_6O_5$ $(M+H)^+$ 479.2037, found 479.2036.

8-(2-Ethoxybiphenyl-4-ylmethylamino)adenosine (7). To a solution of **39** (350 mg, 0.419 mmol) in MeOH (8.38 mL) was added NH_4F (931 mg, 25.1 mmol), and the resulting mixture was heated under reflux with stirring for 24 h. The reaction mixture was allowed to cool to room temperature, diluted with DCM (34.0 mL), stirred for 1 h, and then filtered through a pad of Hyflo Super-Cel. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (gradient; 10-17% MeOH in DCM) to give **7** (146 mg, 71%) as a pale yellow solid. mp 128-130 °C. ^{1}H NMR (DMSO-d_6) δ 1.24 (t, J = 6.9 Hz, 3H), 3.56-3.73 (m, 2H), 3.94-4.21 (m, 4H), 4.61 (d, J = 6.0 Hz, 2H), 4.70-4.81 (m, 1H), 5.18 (d, J = 4.0 Hz, 1H), 5.30 (d, J = 6.8 Hz, 1H), 5.90-6.04 (m, 2H), 6.54 (br s, 2H), 6.98-7.06 (m, 1H), 7.11-7.55 (m, 7H), 7.58 (t, J = 6.0 Hz, 1H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 14.6, 45.2, 61.8, 63.5, 71.0, 71.2, 85.9, 86.5, 111.8, 117.1, 119.5, 126.7, 128.0, 128.3, 129.2, 130.3, 138.2, 140.9, 148.6, 149.9, 151.3, 152.5, 155.3. HRMS calcd for $C_{25}H_{29}N_6O_5$ $(M+H)^+$ 493.2194, found 493.2199.

8-(2-Propoxybiphenyl-4-ylmethylamino)adenosine (8). The title compound was prepared from **40** (414 mg, 0.487 mmol) following the procedure described for **7**. The crude product was purified by flash column chromatography on silica gel (gradient; 10-17% MeOH in DCM) to afford **8** (236 mg, 96%) as a pale yellow foam. ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7.4 Hz, 3H),

1.56-1.71 (m, 2H), 3.57-3.72 (m, 2H), 3.92 (t, *J* = 6.4 Hz, 2H), 3.97-4.05 (m, 1H), 4.10-4.18 (m, 1H), 4.61 (d, *J* = 6.0 Hz, 2H), 4.70-4.81 (m, 1H), 5.19 (d, *J* = 4.0 Hz, 1H), 5.30 (d, *J* = 6.8 Hz, 1H), 5.89-6.01 (m, 2H), 6.54 (br s, 2H), 6.98-7.07 (m, 1H), 7.11-7.18 (m, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.25-7.33 (m, 1H), 7.33-7.64 (m, 5H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 10.6, 22.1, 45.2, 61.8, 69.3, 71.0, 71.1, 85.9, 86.5, 111.6, 117.1, 119.4, 126.7, 127.9, 128.3, 129.2, 130.2, 138.2, 140.9, 148.6, 149.9, 151.3, 152.5, 155.4. HRMS calcd for $C_{26}H_{31}N_6O_5$ $(M+H)^+$ 507.2350, found 507.2350.

8-(2-Isopropoxybiphenyl-4-ylmethylamino)adenosine (9). The title compound was prepared from **41** (310 mg, 0.365 mmol) following the procedure described for **7**. The crude product was purified by flash column chromatography on silica gel (gradient; 10-17% MeOH in DCM) to afford **9** (180 mg, 97%) as an ivory-colored foam. ^{1}H NMR (DMSO-d_6) δ 1.11-1.24 (m, 6H), 3.55-3.74 (m, 2H), 3.95-4.05 (m, 1H), 4.09-4.18 (m, 1H), 4.48-4.81 (m, 4H), 5.18 (d, *J* = 4.0 Hz, 1H), 5.29 (d, *J* = 6.8 Hz, 1H), 5.88-6.03 (m, 2H), 6.53 (br s, 2H), 6.96-7.05 (m, 1H), 7.10-7.18 (m, 1H), 7.19-7.52 (m, 6H), 7.59 (t, *J* = 6.0 Hz, 1H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 21.8 (2C), 45.2, 61.8, 69.8, 71.0, 71.1, 85.9, 86.5, 113.5, 117.1, 119.6, 126.6, 127.9, 129.1, 129.2, 130.5, 138.4, 140.8, 148.6, 149.9, 151.3, 152.5, 154.1. HRMS calcd for $C_{26}H_{31}N_6O_5$ $(M+H)^+$ 507.2350, found 507.2345.

8-(3-Hydroxybiphenyl-4-ylmethylamino)adenosine (10). To a solution of **56** (517 mg, 0.640 mmol) in MeOH (12.8 mL) was added NH_4F (1.42 g, 38.4 mmol), and the resulting mixture was heated under reflux with stirring for 24 h. The reaction mixture was allowed to cool to room temperature, diluted with DCM (40.0 mL), stirred for 1 h, and then filtered through a pad of Hyflo Super-Cel. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (gradient; 10-20% MeOH in DCM) to

give **10** (291 mg, 98%) as a yellow solid. mp 161-163 °C. ^{1}H NMR (DMSO-d_6) δ 3.53-3.74 (m, 2H), 3.96-4.05 (m, 1H), 4.09-4.19 (m, 1H), 4.46-4.64 (m, 2H), 4.68-4.84 (m, 1H), 5.18 (d, J = 4.0 Hz, 1H), 5.34 (d, J = 6.8 Hz, 1H), 5.86-6.04 (m, 2H), 6.55 (br s, 2H), 7.01-7.13 (m, 2H), 7.22-7.72 (m, 7H), 7.92 (s, 1H), 10.19 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 41.0, 61.8, 70.9, 71.2, 85.9, 86.6, 113.7, 116.6, 117.5, 125.3, 126.5, 127.3, 128.9 (2C), 140.2, 140.3, 148.8, 149.9, 151.7, 152.4, 155.4. HRMS calcd for $C_{23}H_{25}N_6O_5$ $(M+H)^+$ 465.1881, found 465.1884.

8-(3-Methoxybiphenyl-4-ylmethylamino)adenosine (11). To a solution of **57** (384 mg, 0.468 mmol) in MeOH (9.35 mL) was added NH_4F (1.04 g, 28.1 mmol), and the resulting mixture was heated under reflux with stirring for 26 h. The reaction mixture was allowed to cool to room temperature, diluted with H_2O (40.0 mL), and then stirred for 1 h. The precipitate was collected by filtration, washed with H_2O, and air-dried, and then recrystallized from EtOH to give **11** (167 mg, 75%) as a pale yellow solid. mp 236-238 °C (dec). ^{1}H NMR (DMSO-d_6) δ 3.57-3.72 (m, 2H), 3.93 (s, 3H), 3.98-4.05 (m, 1H), 4.10-4.19 (m, 1H), 4.51-4.68 (m, 2H), 4.73-4.83 (m, 1H), 5.17 (d, J = 4.0 Hz, 1H), 5.33 (d, J = 6.8 Hz, 1H), 5.86 (dd, J = 4.0, 6.0 Hz, 1H), 5.99 (d, J = 7.3 Hz, 1H), 6.51 (br s, 2H), 7.14-7.26 (m, 2H), 7.28-7.52 (m, 5H), 7.63-7.73 (m, 2H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 40.6, 55.4, 61.8, 70.9, 71.2, 85.9, 86.5, 108.8, 117.1, 118.5, 126.6, 126.8, 127.3, 127.4, 128.9, 140.1, 140.4, 148.6, 149.9, 151.4, 152.5, 157.0. HRMS calcd for $C_{24}H_{27}N_6O_5$ $(M+H)^+$ 479.2037, found 479.2046.

8-(3-Ethoxybiphenyl-4-ylmethylamino)adenosine (12). To a solution of **58** (322 mg, 0.386 mmol) in MeOH (7.71 mL) was added NH_4F (857 mg, 23.1 mmol), and the resulting mixture was heated under reflux with stirring for 24 h. The reaction mixture was allowed to cool to room temperature, diluted with DCM (24.0 mL), stirred for 1 h, and then filtered through a pad of Hyflo Super-Cel. The filtrate was concentrated under reduced pressure and the residue was

triturated in DCM. The precipitate was collected by filtration, washed with DCM, air-dried, and then recrystallized from EtOH to give **12** (120 mg, 63%) as a pale yellow solid. mp 219-221 °C. ^{1}H NMR (DMSO-d_6) δ 1.40 (t, J = 6.9 Hz, 3H), 3.56-3.71 (m, 2H), 3.97-4.06 (m, 1H), 4.10-4.27 (m, 3H), 4.52-4.69 (m, 2H), 4.73-4.84 (m, 1H), 5.17 (d, J = 4.0 Hz, 1H), 5.34 (d, J = 6.8 Hz, 1H), 5.85 (dd, J = 4.0, 6.0 Hz, 1H), 6.00 (d, J = 7.5 Hz, 1H), 6.50 (br s, 2H), 7.12-7.52 (m, 7H), 7.61-7.74 (m, 2H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 14.8, 40.6, 61.8, 63.3, 70.9, 71.2, 85.9, 86.5, 109.5, 117.1, 118.4, 126.7, 126.8, 127.2, 127.3, 128.9, 140.0, 140.4, 148.6, 149.9, 151.5, 152.5, 156.3. HRMS calcd for $C_{25}H_{29}N_6O_5$ $(M+H)^+$ 493.2194, found 493.2191.

8-(3-Propoxybiphenyl-4-ylmethylamino)adenosine (13). To a solution of **59** (378 mg, 0.445 mmol) in MeOH (8.90 mL) was added NH_4F (989 mg, 26.7 mmol), and the resulting mixture was heated under reflux with stirring for 24 h. The reaction mixture was allowed to cool to room temperature, diluted with H_2O (17.8 mL), and then stirred for 1.5 h. The precipitate was collected by filtration, washed with MeOH/H_2O (1/2), air-dried, and then recrystallized from EtOH to give **13** (121 mg, 54%) as a pale yellow solid. mp 211-213 °C. ^{1}H NMR (DMSO-d_6) δ 1.05 (t, J = 7.4 Hz, 3H), 1.71-1.90 (m, 2H), 3.55-3.72 (m, 2H), 3.97-4.21 (m, 4H), 4.53-4.69 (m, 2H), 4.74-4.83 (m, 1H), 5.19 (d, J = 4.0 Hz, 1H), 5.35 (d, J = 6.8 Hz, 1H), 5.87 (dd, J = 4.1, 5.9 Hz, 1H), 6.00 (d, J = 7.5 Hz, 1H), 6.51 (br s, 2H), 7.11-7.23 (m, 2H), 7.28 (d, J = 7.8 Hz, 1H), 7.31-7.50 (m, 4H), 7.61-7.72 (m, 2H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 10.6, 22.2, 40.6, 61.8, 69.0, 71.0, 71.2, 85.9, 86.5, 109.5, 117.1, 118.4, 126.8 (2C), 127.0, 127.3, 128.9, 140.0, 140.4, 148.6, 149.9, 151.5, 152.5, 156.4. HRMS calcd for $C_{26}H_{31}N_6O_5$ $(M+H)^+$ 507.2350, found 507.2344.

8-(3-Isopropoxybiphenyl-4-ylmethylamino)adenosine (14). The title compound was prepared from **60** (283 mg, 0.333 mmol) following the procedure described for **10**. The crude product was purified by flash column chromatography on silica gel (gradient; 10-17% MeOH in

DCM) to afford **14** (159 mg, 94%) as a pale yellow solid. mp 198-201 °C. ^{1}H NMR (DMSO-d_6) δ 1.31-1.41 (m, 6H), 3.54-3.73 (m, 2H), 3.97-4.07 (m, 1H), 4.10-4.20 (m, 1H), 4.47-4.68 (m, 2H), 4.72-4.89 (m, 2H), 5.17 (d, J = 4.0 Hz, 1H), 5.33 (d, J = 6.8 Hz, 1H), 5.86 (dd, J = 4.0, 6.0 Hz, 1H), 5.99 (d, J = 7.3 Hz, 1H), 6.49 (br s, 2H), 7.13 (dd, J = 1.5, 7.8 Hz, 1H), 7.20 (d, J = 1.5 Hz, 1H), 7.27-7.50 (m, 5H), 7.61-7.71 (m, 2H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 22.1, 40.7, 61.8, 69.6, 71.0, 71.1, 85.9, 86.5, 111.0, 117.1, 118.4, 126.8, 127.3, 127.5, 127.6, 128.9, 140.0, 140.4, 148.6, 149.9, 151.5, 152.4, 155.3. HRMS calcd for $C_{26}H_{31}N_6O_5$ $(M+H)^+$ 507.2350, found 507.2349.

8-[2-(2-Methoxy-2-oxoethoxy)biphenyl-4-ylmethylamino]adenosine (15). The title compound was prepared from **42** (335 mg, 0.381 mmol) following the procedure described for **10**. The crude product was purified by flash column chromatography on silica gel (gradient; 9-16% MeOH in DCM) to afford **15** (184 mg, 90%) as a pale brown solid. mp 121-123 °C. ^{1}H NMR (DMSO-d_6) δ 3.56-3.79 (m, 5H), 3.96-4.07 (m, 1H), 4.10-4.23 (m, 1H), 4.59 (d, J = 6.0 Hz, 2H), 4.69-4.78 (m, 1H), 4.80 (s, 2H), 5.19 (d, J = 4.0 Hz, 1H), 5.29 (d, J = 7.0 Hz, 1H), 5.88-6.07 (m, 2H), 6.55 (br s, 2H), 7.00-7.16 (m, 2H), 7.22-7.71 (m, 7H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 45.1, 51.8, 61.8, 64.5, 71.0, 71.1, 85.8, 86.5, 111.2, 117.1, 120.1, 126.8, 128.0, 128.4, 129.3, 130.5, 137.9, 140.9, 148.6, 149.8, 151.3, 152.5, 154.3, 169.3. HRMS calcd for $C_{26}H_{29}N_6O_7$ $(M+H)^+$ 537.2092, found 537.2092.

8-[2-(2-Amino-2-oxoethoxy)biphenyl-4-ylmethylamino]adenosine (16). To **15** (195 mg, 0.363 mmol) was added a 2.0 M solution of NH_3 in MeOH (14.5 mL), and the resulting mixture was stirred at room temperature for 72 h. The reaction mixture was diluted with MeOH (15 mL) and $CHCl_3$ (20 mL). The resulting suspension was heated under reflux until a clear solution was formed. The solution was allowed to cool to room temperature while stirring. The precipitate was

collected by filtration, washed with MeOH/$CHCl_3$ (6/4), air-dried, and dried at 60 °C under reduced pressure to give **16** (145 mg, 77%) as a white solid. mp 166-169 °C. ^{1}H NMR (DMSO-d_6) δ 3.56-3.72 (m, 2H), 3.97-4.03 (m, 1H), 4.10-4.18 (m, 1H), 4.43 (s, 2H), 4.52-4.64 (m, 2H), 4.70-4.80 (m, 1H), 5.16 (d, J = 4.0 Hz, 1H), 5.29 (d, J = 6.8 Hz, 1H), 5.91 (dd, J = 4.0, 6.0 Hz, 1H), 5.95 (d, J = 7.5 Hz, 1H), 6.53 (br s, 2H), 7.01-7.13 (m, 3H), 7.23-7.45 (m, 5H), 7.51-7.62 (m, 3H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 45.2, 61.8, 67.4, 71.0, 71.1, 85.9, 86.5, 112.2, 117.1, 120.3, 126.9, 128.1, 128.7, 129.3, 130.4, 138.0, 140.9, 148.6, 149.9, 151.3, 152.5, 154.6, 170.1. HRMS calcd for $C_{25}H_{28}N_7O_6$ $(M+H)^+$ 522.2096, found 522.2092.

8-[2-(2-Hydroxyethoxy)biphenyl-4-ylmethylamino]adenosine (17). To a stirred suspension of **15** (194 mg, 0.362 mmol) in EtOH (3.62 mL) was added $NaBH_4$ (68.4 mg, 1.81 mmol) in small portions, and the resulting mixture was stirred at room temperature for 25 h. The reaction mixture was quenched by addition of AcOH (434 mg, 7.23 mmol) and then concentrated under reduced pressure. To the residue was added DCM/MeOH (6/1), and the insoluble material was filtered out. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography on NH silica gel (gradient; 7-15% MeOH in DCM) to give **17** (165 mg, 90%) as a pale yellow solid. mp 143-146 °C. ^{1}H NMR (DMSO-d_6) δ 3.52-3.79 (m, 4H), 3.92-4.22 (m, 4H), 4.61 (d, J = 6.3 Hz, 2H), 4.70-4.82 (m, 2H), 5.18 (d, J = 4.0 Hz, 1H), 5.30 (d, J = 6.8 Hz, 1H), 5.90-6.04 (m, 2H), 6.54 (br s, 2H), 6.98-7.07 (m, 1H), 7.13-7.20 (m, 1H), 7.21-7.43 (m, 4H), 7.51-7.68 (m, 3H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 45.2, 59.5, 61.8, 69.9, 71.0, 71.1, 85.9, 86.5, 111.9, 117.1, 119.6, 126.6, 128.0, 128.3, 129.3, 130.3, 138.1, 140.9, 148.6, 149.9, 151.3, 152.5, 155.4. HRMS calcd for $C_{25}H_{29}N_6O_6$ $(M+H)^+$ 509.2143, found 509.2142.

8-[2-(3-Hydroxypropoxy)biphenyl-4-ylmethylamino]adenosine (18). The title compound was prepared from **49** (278 mg, 0.454 mmol) following the procedure described for **19**. The crude product was purified by flash column chromatography on NH silica gel (isocratic; 15% MeOH in DCM) to afford **18** (214 mg, 90%) as an off-white foam. ^{1}H NMR (DMSO-d_6) δ 1.71-1.88 (m, 2H), 3.42-3.55 (m, 2H), 3.57-3.73 (m, 2H), 3.96-4.18 (m, 4H), 4.45-4.52 (m, 1H), 4.61 (d, J = 6.1 Hz, 2H), 4.70-4.81 (m, 1H), 5.17 (d, J = 4.0 Hz, 1H), 5.29 (d, J = 6.8 Hz, 1H), 5.88-6.01 (m, 2H), 6.52 (br s, 2H), 6.97-7.07 (m, 1H), 7.12-7.53 (m, 7H), 7.58 (t, J = 6.1 Hz, 1H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 32.1, 45.3, 57.4, 61.8, 64.8, 71.0, 71.1, 85.9, 86.5, 111.6, 117.1, 119.4, 126.7, 127.9, 128.2, 129.2, 130.2, 138.2, 140.9, 148.6, 149.8, 151.3, 152.5, 155.4. HRMS calcd for $C_{26}H_{31}N_6O_6$ $(M+H)^+$ 523.2300, found 523.2292.

8-[2-(4-Hydroxybutoxy)biphenyl-4-ylmethylamino]adenosine (19). A mixture of **50** (354 mg, 0.565 mmol) and 10% Pd-C (51.7wt% H_2O, 220 mg) in MeOH (11.3 mL) was heated at 50 °C with stirring for 50 h under H_2 atmosphere. The reaction mixture was allowed to cool to room temperature and then filtered through a pad of Hyflo Super-Cel. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography on NH silica gel (gradient; 3-15% MeOH in DCM) to give **19** (245 mg, 81%) as a pale yellow foam. ^{1}H NMR (DMSO-d_6) δ 1.42-1.55 (m, 2H), 1.59-1.75 (m, 2H), 3.29-3.44 (m, 2H), 3.56-3.74 (m, 2H), 3.90-4.08 (m, 3H), 4.08-4.20 (m, 1H), 4.42 (t, J = 5.1 Hz, 1H), 4.61 (d, J = 6.0 Hz, 2H), 4.70-4.82 (m, 1H), 5.19 (d, J = 4.0 Hz, 1H), 5.31 (d, J = 7.0 Hz, 1H), 5.90-6.03 (m, 2H), 6.55 (br s, 2H), 6.97-7.07 (m, 1H), 7.11-7.19 (m, 1H), 7.23 (d, J = 7.8 Hz, 1H), 7.25-7.33 (m, 1H), 7.34-7.43 (m, 2H), 7.43-7.53 (m, 2H), 7.58 (t, J = 6.0 Hz, 1H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 25.5, 29.0, 45.2, 60.4, 61.8, 67.8, 71.0, 71.2, 85.9, 86.5, 111.6, 117.1, 119.4, 126.7, 127.9, 128.3,

129.2, 130.3, 138.2, 140.9, 148.6, 149.9, 151.3, 152.5, 155.4. HRMS calcd for $C_{27}H_{33}N_6O_6$ $(M+H)^+$ 537.2456, found 537.2458.

8-[2-(5-Hydroxypentyloxy)biphenyl-4-ylmethylamino]adenosine (20). The title compound was prepared from **51** (314 mg, 0.490 mmol) following the procedure described for **19**. The crude product was purified by flash column chromatography on NH silica gel (gradient; 3-10% MeOH in DCM) to afford **20** (204 mg, 76%) as an off-white foam. ^{1}H NMR (DMSO-d_6) δ 1.28-1.45 (m, 4H), 1.55-1.70 (m, 2H), 3.28-3.43 (m, 2H), 3.56-3.75 (m, 2H), 3.90-4.06 (m, 3H), 4.09-4.21 (m, 1H), 4.30-4.38 (m, 1H), 4.61 (d, J = 6.1 Hz, 2H), 4.71-4.81 (m, 1H), 5.17 (d, J = 4.0 Hz, 1H), 5.28 (d, J = 6.8 Hz, 1H), 5.89-6.03 (m, 2H), 6.52 (br s, 2H), 6.98-7.06 (m, 1H), 7.11-7.52 (m, 7H), 7.57 (t, J = 6.1 Hz, 1H), 7.90 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 22.0, 28.4, 32.0, 45.2, 60.6, 61.8, 67.7, 70.9, 71.1, 85.8, 86.5, 111.6, 117.0, 119.3, 126.6, 127.8, 128.2, 129.2, 130.2, 138.1, 140.8, 148.6, 149.8, 151.2, 152.4, 155.4. HRMS calcd for $C_{28}H_{35}N_6O_6$ $(M+H)^+$ 551.2613, found 551.2607.

8-[2-(4-Hydroxybutoxy)biphenyl-4-ylmethylamino]inosine (21). The title compound was prepared from **61** (111 mg, 0.177 mmol) following the procedure described for **19**. The crude product was purified by flash column chromatography on silica gel (gradient; 13-20% MeOH in DCM) to afford **21** (75.2 mg, 79%) as a pale yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.41-1.57 (m, 2H), 1.60-1.79 (m, 2H), 3.29-3.47 (m, 2H), 3.60-3.76 (m, 2H), 3.91-4.08 (m, 3H), 4.09-4.21 (m, 1H), 4.41 (t, J = 5.3 Hz, 1H), 4.54 (d, J = 6.0 Hz, 2H), 4.61-4.74 (m, 1H), 5.20 (d, J = 4.3 Hz, 1H), 5.38 (d, J = 6.8 Hz, 1H), 5.77 (t, J = 4.5 Hz, 1H), 5.94 (d, J = 7.5 Hz, 1H), 6.94-7.03 (m, 1H), 7.06-7.14 (m, 1H), 7.22 (d, J = 7.8 Hz, 1H), 7.24-7.63 (m, 6H), 7.83 (s, 1H), 12.11 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 25.5, 29.0, 45.1, 60.4, 61.5, 67.7, 70.7, 71.0, 85.8, 86.6, 111.4,

119.2, 121.7, 126.6, 127.9, 128.2, 129.2, 130.2, 138.2, 141.1, 142.4, 147.7, 150.5, 155.4 (2C). HRMS calcd for $C_{27}H_{32}N_5O_7$ $(M+H)^+$ 538.2296, found 538.2287.

2-[2-(4-Hydroxybutoxy)biphenyl-4-ylmethylamino]-1-(β-D-ribofuranosyl)-1*H*-benzimidazole (22). A mixture of **65** (192 mg, 0.315 mmol) and 10% Pd-C (56.5wt% H_2O, 133 mg) in MeOH (6.30 mL) was heated at 50 °C with stirring for 24 h under H_2 atmosphere. The reaction mixture was allowed to cool to room temperature and then filtered through a pad of Hyflo Super-Cel. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (gradient; 8-15% MeOH in DCM) to give **22** (144 mg, 88%) as an off-white amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.42-1.54 (m, 2H), 1.61-1.73 (m, 2H), 3.28-3.43 (m, 2H), 3.64-3.78 (m, 2H), 3.90-4.05 (m, 3H), 4.08-4.16 (m, 1H), 4.37-4.50 (m, 2H), 4.59 (d, J = 6.0 Hz, 2H), 5.23 (d, J = 4.5 Hz, 1H), 5.31 (d, J = 7.3 Hz, 1H), 5.62-5.70 (m, 1H), 5.83 (d, J = 7.5 Hz, 1H), 6.84-7.06 (m, 3H), 7.10-7.60 (m, 10H). ^{13}C NMR (DMSO-d_6) δ 25.5, 29.0, 45.5, 60.3, 61.2, 67.7, 70.4, 71.3, 85.4, 87.6, 108.7, 111.4, 115.2, 118.6, 119.3, 120.7, 126.6, 127.9, 128.1, 129.2, 130.2, 134.1, 138.2, 141.3, 142.5, 154.0, 155.4. HRMS calcd for $C_{29}H_{34}O_3N_6$ $(M+H)^+$ 520.2442, found 520.2441.

Inosine uptake inhibition assay. The cDNA encoding hCNT2 or rCNT2 was subcloned into the pCI-neo mammalian expression vector (Promega, Madison, WI). COS-7 cells (RIKEN, Japan) were then transfected with a given expression vector by using Lipofectamine 2000 (Thermo Fischer Scientific, MA, USA). Briefly, COS-7 cells were seeded at 5×10^4 cells into each well of collagen type I-coated 96-well plates containing DMEM (Thermo Fischer Scientific, MA, USA) with 10% fetal calf serum and cultured for 2 h. Then, for use in each well, 0.3 μg plasmid DNA was diluted in 25 μL of OPTI-MEM (Thermo Fischer Scientific, MA, USA). Zero point five microliter of Lipofectamine 2000 was also diluted in 25 μL of OPTI-

MEM and incubated at room temperature for 5 min. After the incubation, the diluted plasmid DNA was mixed with the diluted Lipofectamine 2000; and the mixture was incubated at room temperature for 25 min. Finally, the incubated mixture was added to each well, and the cells were cultured for 2 days, after which the inhibition assay was performed.

Transport assays were performed with a modification of the method described by Schaner et al.[71] Transport activities in COS-7 cells transiently expressing hCNT2 or rCNT2 were examined with [^{14}C]-inosine. The transfected cells were incubated with pretreatment buffer (2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM Tris, and 140 mM choline chloride; pH 7.4) at 37 °C twice for 10 min each time. Following aspiration of the pretreatment buffer, uptake buffer containing 10 μM inosine prepared with ^{14}C-labeled and unlabeled inosine was added to the cells, which were then incubated at 37 °C for 30 min. All uptake studies were carried out in the presence of 10 μM nitrobenzylthioinosine (NBMPR), which is an ENT inhibitor but not effective against CNTs. Uptake was measured in the presence of Na^+ (140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM Tris, 5 mM glucose; pH 7.4) or absence of Na^+, in which case the Na^+ was replaced by choline chloride (140 mM). The transport was stopped by aspiration of the reaction mixture, and the cells were washed twice with ice-cold Na^+-free buffer including 10 μM unlabeled inosine. Then, they were solubilized with 0.2 M sodium hydroxide. Radioactivity of the cell lysates was measured by means of a liquid scintillation counter. One-hundred percent transport was set as the difference between the uptake in the presence and the absence of Na^+, and the percentage of inosine uptake at each compound concentration was calculated. The half maximal inhibitory concentration (IC_{50} value) was calculated by nonlinear regression analysis of inosine uptake assays (at least three independent experiments) using GraphPad Prism (GraphPad Software, CA, USA).

Animals. Male Sprague-Dawley (SD) rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). Throughout the study, the rodents were housed in a constant-temperature room with a 12-h/12-h lighting cycle (lights on 8:00 a.m. to 8:00 p.m.) and allowed access to laboratory chow diet (CE-2 pellets; CLEA Japan, Inc.) and water ad libitum. All studies using rats were performed in accordance with guidelines approved by the laboratory animal committee of Kissei Pharmaceutical Co., Ltd.

Purine load test in rats. Male SD rats (5 weeks old) were fasted overnight and subcutaneously treated with potassium oxonate (Sigma-Aldrich, MO, USA; 100 mg/kg), and after 1 hour, purine mixture (adenosine:inosine:guanosine = 1:1:1, 50 mg/kg) and compound **19** (50 mg/kg) or **22** (0.1, 1.0, or 10 mg/kg) were orally administered simultaneously. A control group was treated with potassium oxonate and purine mixture, and a group treated with only potassium oxonate represented endogenous plasma uric acid level. After 1 hour, blood was collected from the abdominal aorta under anesthesia, and uric acid levels in plasma were measured by phosphotungstic acid method.

Data were presented as means ± SEM for each group. Statistical analysis was performed by using SAS System Version 9.3 (SAS Institute Inc., NC, USA). The statistical significance of the results was analyzed by the Student's t-test or the Dunnett's test.

Pharmacokinetic study of compound 22 in rats. Pharmacokinetics was investigated in male SD rats (6 weeks old, fasted for 16 h) after intravenous and oral administration. Compound **22** was dissolved in a mixture of dimethylacetamide and 5 % glucose solution (1:1v/v) for intravenous bolus injections at a dose of 1.0 mg/mL/kg. The intravenous dose was administered via tail-vein. Compound **22** was suspended in 0.5% sodium calboxymethylcellulose solution for oral administration at a dose of 10 mg/5 mL/kg. Blood samples (about 200 μL) were collected

from the jugular vein by use of heparinized syringe at 2 min, 0.25, 1, 2, 4, 6, 8, and 24 h after intravenous administration, and at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after oral administration. Plasma was prepared by centrifugation at 2280 × g for 10 min at 4 °C. The collected plasma samples were stored at -20 °C until analysis. Aliquots of the plasma (50 μL) were deproteined with 150 μL of acetonitrile and added 50 μL of internal standard (100 nmol/L warfarin). The samples were then centrifuged at 2280 × g, for 20 minutes at 4 °C. The supernatant (5 μL) was injected into LC/MS. Samples were analyzed using a LC system coupled to a Q Exactive orbitrap mass spectrometer (Thermo Fisher scientific, MA, USA). For MS detection, the heated ESI source was operated in positive ion mode. Ions monitored in the SIM mode were m/z 520.2442 for compound **22**. LC separation was performed using an Ultimate 3000 system (Thermo Fisher scientific, MA, USA) with a gradient elution from a Cadenza CD-C18 HT column (3 μm, 50 mm x 2.0 mm ID; Imtakt, Kyoto, JAPAN). The flow rate and column temperature were set at 0.4 ml/min and 50 °C, respectively. The mobile phase consisted of solvent A, 10 mM ammonium acetate aqueous solution, and solvent B, acetonitrile containing 0.1% formic acid and 20% 2-propanol. The gradient program is shown as follows: 10% B at 0-0.1 min; 90% at 0.1-0.5 min; 90% at 0.5-1.7 min; 10% at 1.7-1.8 min. The flow rate at 1.7-1.8 min was set at 1.4 ml/min for reequilibration. The standard curve was prepared in a concentration range from 1 to 5000 ng/mL for compound **22**. Pharmacokinetic calculation was performed by noncompartmental analysis using WinNonlin version 6 (Pharsight Corporation, CA, USA) to obtain the maximal plasma concentration (C_{max}), and the area under the curve (AUC). Bioavailability (F) was calculated as F = (AUC (po) / AUC (iv)) × (dose (iv) / dose (po)) × 100. The plasma total clearance (CL_{tot}), the volume of distribution (V_{dss}) and the elimination half–life ($t_{1/2}$) were calculated after intravenous administration.

ASSOCIATED CONTENT

Supporting Information.

Synthetic procedures and characterization data for all intermediates, HPLC analysis data for all tested compounds, experimental procedures for conformational analysis and determination of solubility in JP2 (PDF); Molecular formula strings (CSV). This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CNT, concentrative nucleoside transporter; ENT, equilibrative nucleoside transporter; JP2, Japanese Pharmacopoeia second fluid; SEM, standard error of the mean

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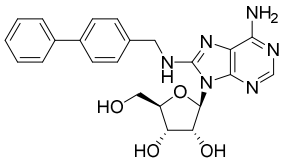
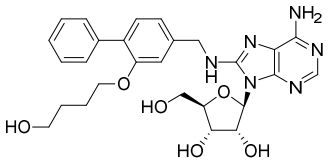
Insert Table of Contents Graphic and Synopsis Here



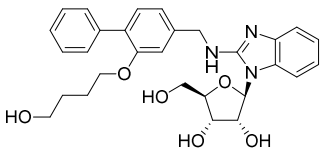
1

hCNT2 IC_{50} = 0.64 ± 0.19 μM
rCNT2 IC_{50}: inactive at 100 μM
Solubility in JP2 = 0.003 mg/mL



19

hCNT2 IC_{50} = 0.94 ± 0.24 μM
rCNT2 IC_{50} = 38 ± 13 μM
Solubility in JP2 = 0.190 mg/mL



22

hCNT2 IC_{50} = 0.062 ± 0.017 μM
rCNT2 IC_{50} = 1.5 ± 0.1 μM
Solubility in JP2 = 0.120 mg/mL