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8-Substituted 2-alkynyl-N^9-propargyladenines as A_{2A} adenosine receptor antagonists

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ABSTRACT

Structure–activity relationships of 2-alkynyladenine derivatives were explored by varying substituents at the 9-, 8- and 2-positions of the purine moiety in order to optimize A_{2A} adenosine receptor antagonist activity in vitro. A propargyl group at the 9-position was found to be important for A_{2A} antagonist activity, and the introduction of a halogen, aryl, or heteroaryl at the 8-position further enhanced activity. A series of 8-substituted 2-alkynyl-N^9-propargyladenine derivatives exhibited potent antagonist activity, with IC_{50} values in the low nM range. Compound **4a** from this series was found to be orally active at a dose of 3 mg/kg in a mouse catalepsy model and a 6-hydroxydopamine-lesioned rat model of Parkinson's disease.

1. Introduction

Adenosine modulates a wide range of physiological functions by interacting with specific cell surface G-protein-coupled receptors, specifically A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor (AR) subtypes.[1,2] All adenosine receptors are associated with the cAMP second messenger system. A_1 and A_3 ARs are linked to inhibition of adenylate cyclase, while A_{2A} and A_{2B} subtypes are linked to stimulation of this enzyme.[3] The A_{2A} subtype is abundantly expressed in the striatum, whereas A_1 is widely distributed throughout the brain.[4] In recent years, A_{2A} AR antagonists have emerged as an attractive target for Parkinson's disease therapy, primarily due to localized expression in the striatum and the motor enhancement function of this receptor.[5]

Parkinson's disease (PD) is a hugely debilitating neurological disorder, and current treatments fail to achieve long-term control. Chronic over use of L-DOPA as a treatment causes complications such as wearing-off and dyskinesia, and new options to replace or reduce L-DOPA are much needed. A_{2A} AR antagonists can restore the deficiencies caused by degeneration of the striatonigral dopamine system that result from loss of striatal neurons in PD, and represent a potential novel therapeutic approach.[6] A number of potent and subtype-selective A_{2A} antagonists, based on a variety of structures have been reported, including xanthine and nonxanthine compounds.[7]

The first relatively selective A_{2A} AR antagonists reported were 8-styrylxanthines such as KW6002 (istradefylline, Fig. 1), which has a high affinity for the A_{2A} AR and 50–100-fold selectivity for A_{2A} over the A_1 subtype.[8] More recently, istradefylline has been approved for the treatment of PD in Japan. The second class of antagonists characterized were the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives, and SCH5826 is the prototype for the tricyclic derivatives, possessing a low nM affinity for A_{2A} AR in vitro.[9] However, low water solubility and consequent poor bioavailability limited the pharmacological use of these compounds. Neustadt et al. recently reported a series of arylpiperazine derivatives of pyrazolo[4,3-*e*]-1,2,4 triazolo[1,5-*c*]pyrimidines with antagonist activity for the A_{2A}.[10] Among these, SCH420814 (preladenant) was demonstrated to be a potent, selective, and orally active A_{2A} AR antagonist. The third class of antagonists to appear, the 1,2,4-triazolo[4,5-*e*]-1,3,5-triazines, as typified by ZM241385, inhibit A_{2A} with sub nM affinity, but unfortunately showed some cross-reactivity.[11] Recently, a large series of derivatives bearing various substituents at the 5-position on the triazolo-triazine nucleus, and as well as the deaza analogues (triazoro-pyrimidines), were synthesized.[7] Several purine derivatives recently reported by several groups appear to be very promising, such as the 2-amino-6-(2-furanyl)purine derivatives and ST1535, which also exhibit activity in the low nM range.[12] The discovery and development of potent and selective A_{2A} AR antagonists as potential treatment for PD and other neurodegenerative disorders has therefore been an attractive field of research in the last decade.[13]

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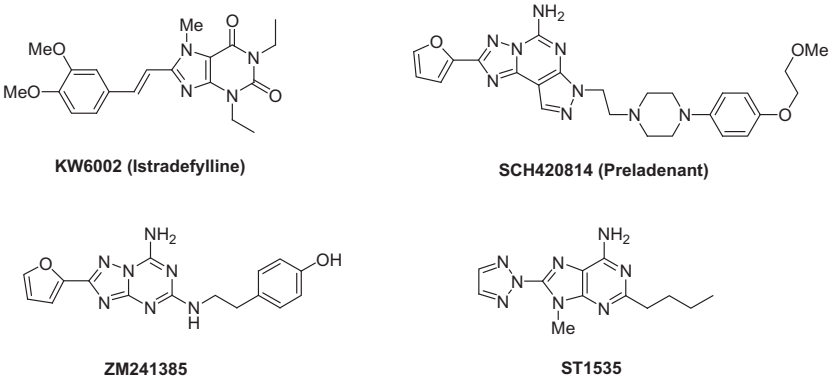


Figure 1. Representative members of selective A_{2A} AR antagonist classes.

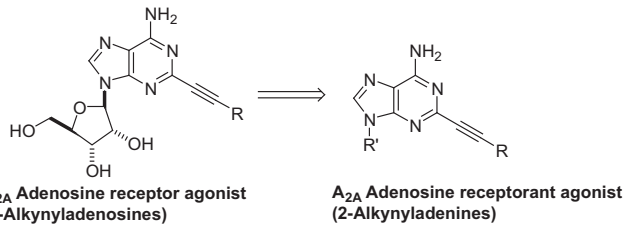
In 1993, Barrett et al. reported that N^6-cyclopentyl-N^9-methyladenine (N-0840) was a selective A_1 antagonist, in contrast to N^6-cyclopentyladenosine, which was a known A_1 agonist at the time.[14] This suggested that de-ribosylation and alkylation of the N^9-position could convert adenosine-based AR agonists into antagonists. These results, and the fact that 2-alkynyl adenosine derivatives had been found to be potent A_{2A} agonists (Scheme 1),[15] led us to design and synthesize the 2-alkynyladenine derivatives as potential A_{2A} AR antagonists. Cristalli et al. showed that 2- and 8-substituted N^9-alkyladenine derivatives possessed good affinity and selectivity for the A_{2A}.[16] On the other hand, Harada et al. reported that 2-alkynyl-8-aryl-N^9-methyladenine derivatives are candidates for antidiabetic agents through their antagonistic effects on the A_{2B} AR.[17] A three carbon linker between the adenine core and the amide moiety of the alkyl residue at the 9-position was important for A_{2B} antagonistic activity, as shown by structure–activity relationship (SAR) studies on 9-alkyl-8-(3-fluorophenyl)-2-(hydroxycyclohexyl-1-ethynyl)adenine derivatives.[18] Thus, 2-alkynyl-N^9-alkyladenine derivatives have already been evaluated as A_{2B} antagonists, but not as A_{2A} antagonists.

In this study, we describe the synthesis and SAR of novel 2-alkynyladenine derivatives, which were evaluated for A_{2A} antagonist activity by investigating their inhibitory effects on 2-octyn-1-yladenosine-induced vasodilation. We also report the discovery and characterization of potent A_{2A} antagonists. The efficacy of oral administration was tested on PD models in mice (catalepsy) and rats (turning behavior in unilateral 6-hydoxydopamine-lesioned animals).

2. Results and discussion

2.1. Synthesis and biological activities of 9-substituted 2-octyn-1-yladenines

Recently, Jaakola et al. reported the crystal structure of a human A_{2A} AR bound to an antagonist, ZM241385.[19] The key elements of the pharmacophore included H-bonds at the 15-, 17-, and 25-positions, as well as hydrophobic interactions with residues at positions 11 and 20 (Fig. 2). These key pharmacophore interactions were also present in another study.[12c] We noted that 2-alkynyladenine derivatives would also possess the appropriate functional groups (N at the 7-position, amino group at the 6-position, and hydrophobic residue at the 2-position) for potential antagonist activity (Fig. 2). We hypothesized that introduction of an aryl or heteroaryl group at the 8-position of the purine base may be potent for both affinity and selectivity of the A_{2A} AR subtype. Furthermore, we speculated that the substituents on the N at the 9-position would be involved in switching the compounds from agonists to antagonists, as mentioned above. Therefore, we first tested the effects of N^9-substituents on the 2-alkynyladenine derivatives.



Scheme 1. Design of A_{2A} AR antagonists based on A_{2A} AR agonists.

We first synthesized some N^9-alkylated 2-octyn-1-yladenine derivatives, because the octynyl group at the 2-position was expected to show a high affinity for the A_2 subtype, based on 2-octyn-1-yladenosine.[15] It was reported that de-ribosylated 2-octyn-1-yladenine (**2**) had no affinity for the A_1 and A_2 subtypes.[15b] In contrast, introduction of a methyl group on the N at the 9-position of 2-alkynyladenine derivatives was reported to increase the affinity for these receptors.[17] Therefore, the N^9-substituent seemed a logical place at which to manipulate A_{2A} agonist/antagonist activity.

2-Octyn-1-yladenosine (**1**), readily prepared from guanosine,[15c] was hydrolyzed with 0.6 M HCl–1,4-dioxane at 100 °C for 6 h, to afford 2-octyn-1-yladenine (**2**) in 92% yield.[15b] As shown in Scheme 2 and Table 1, the adenine derivative (**2**) was alkylated at the N^9-position with 2 equiv of various alkylhalides in the presence of K_2CO_3 in DMF, giving yields of 35–89%. All of these alkylated 2-octyn-1-yladenines (**3a**–**i**) were obtained as a single N^9-isomer,[20] as determined by ^1H, ^{13}C NMR and HMBC experiments on the propargyl derivative **3e** (Fig. 3). Cross-peaks from H-1′ to C-4 and H-N^6 to C-5, were observed in the HMBC spectrum of **3e**.

The A_{2A} AR antagonist activities of N^9-alkyl-2-octyn-1-yladenine derivatives (**3a**–**i**) were evaluated in vitro by measuring inhibition of 2-octyn-1-yladenosine-induced vasodilation, with comparison against istradefylline as previously reported.[21] Methyl and ethyl derivatives (**3a**, **3b**) showed moderate antagonist activity (Table 1), albeit 30-fold weaker than istradefylline. The bulkiness of the N^9-alkyl groups (*n*-propyl, allyl, *c*-pentyl and benzyl) appeared to reduce the activity. In contrast to the *n*-propyl derivative **3c**, propargyl derivative **3e** showed comparable activity to istradefylline. Interestingly, this activity disappeared upon substitution with a cyanomethyl group, which has a comparable steric bulk to the propargyl group. These results indicated that the appropriate bulkiness at the N^9-position was essential for A_{2A} AR

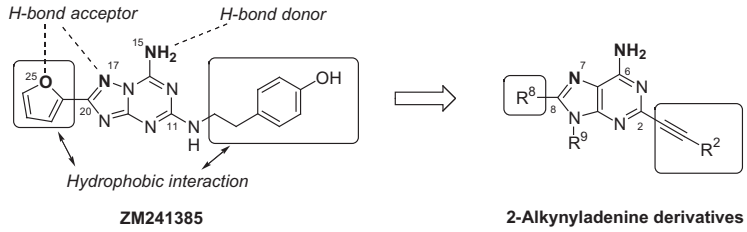
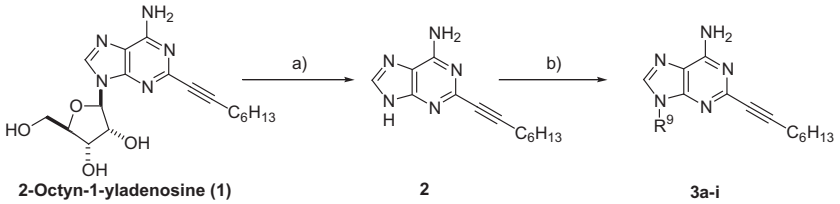


Figure 2. ZM241385 pharmacophore interactions and 2-alkynyladenine derivatives.



Scheme 2. Reagents and conditions: (a) 0.6 M HCl, 1,4-dioxane, 100 °C, (b) R–X (X = Br, I), K_2CO_3, DMF.

Table 1
Yields of N^9-alkyl-2-octynyladenine derivatives and their inhibitory effects (IC_{50} values) on 2-octyn-1-yladenosine-induced vasodilation

Compounds	R	Yield (%)	IC_{50}[a] (μM)
3a	Me	35	9.0 ± 0.16
3b	Et	79	6.7 ± 2.03
3c	*n*-Pr	46	>10
3d	Allyl	44	>10
3e	Propargyl	77	0.2 ± 0.01
3f	CH_2CN	89	>10
3g	CH_2CH_2OH	43	>10
3h	*c*-Pentyl	15	>10
3i	Bn	46	>10
Istradefylline	—	—	0.2 ± 0.02

[a] IC_{50}: compound concentration that inhibits 2-octyn-1-yladenosine-induced vasodilation by 50%.

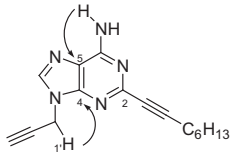


Figure 3. Assignment of the N^7-, N^9-isomer of **3e** by HMBC experiments.

antagonist activity, and polar substituents are not compatible. Based on these results, we decided to retain the propargyl substituent at the N^9-position, and explored SAR at the 8- position.

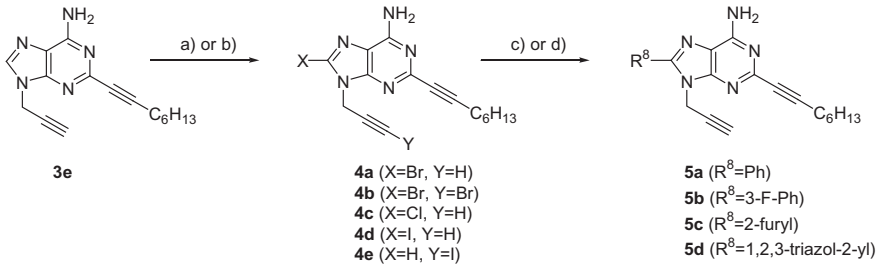
2.2. Synthesis and biological activity of 8-substituted 2-octyn-1-yl-N^9-propargyladenines

According to molecular modeling studies on 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-*c*]quinazoline (CGS15943), the presence of the furan ring at the 2-position of CGS15943, which is equivalent to the 8-position of adenine, was apparently essential for both affinity and selectivity towards the A_{2A} subtype.[22] In contrast, 2-furyl and 3-fluorophenyl groups were the preferred substituents at the 8-position of adenine for A_{2B} antagonists.[17] Recently, Lambertucci et al. introduced a bromine at the 8-position of N^9-ethyladenine derivatives, which increased the affinity for A_{2A} and resulted in compounds with nM K_i.[16b] To explore this further, we introduced halogen, aryl, and heteroaryl substituents at the 8-position.

N^9-Propargyladenine derivative **3e** was brominated with 1 equivalent of N-bromosuccinimide (NBS) in DMF to afford the 8-bromoadenine derivative **4a** in 57% yield (Scheme 3). A large excess of NBS increased the amount of 8- and 3′-dibromo derivative **4b**, which could not be separated. For example, the reaction with 4 equiv of NBS gave a 1.4:1 mixture of **4a** and **4b**. We also examined the chlorination and iodination at the 8-position using N-chlorosuccinimide (NCS) or N-iodosuccinimide (NIS). Although the reaction with NCS was slower than with NBS, 8-chloro derivative **4c** was afforded in 55% yield without generation of the dichloro byproduct. In contrast, iodination of **3e** with NIS was very rapid, but gave the undesired 3′-iodo derivative **4e** in 71% yield as a sole product. Interestingly, the diiodo derivative was not produced at all in this reaction. Halogen exchange of **4a** with iodide, and iodination of adenine derivative **2** were attempted without success.

8-Aryl and 8-heteroaryl derivatives were prepared next (Scheme 3). Phenyl, 3-fluorophenyl, 2-furyl and 1,2,3-triazol-2-yl functional groups at the 8-position were tested as these were reported to enhance the affinity for the A_{2A} AR.[12,17,19] 8-Bromoadenine derivative **4a** was reacted with phenylboronic acid in the presence of $Pd(PPh_3)_4$ to afford 8-phenyl derivative **5a** in 36% yield. Under the same conditions, 8-(3-fluoro)phenyl and 2-furyl derivatives (**5b** and **5c**) were obtained in 30% and 53% yield, respectively. The triazole derivative **5d** was prepared by a substitution reaction of the 8-bromo derivative **4a** with 1,2,3-triazole in DMF according to the literature.[12] Unexpectedly, the reaction gave a complex mixture that included the N^1- and N^2-triazolyl derivatives, with regioisomer yields of approximately were 23% (N^1) and 17% (N^2) as estimated from HPLC analysis. Following complicated purification procedures including ODS column chromatography, the desired N^2-triazolyl derivative **5d** was obtained in 10% yield.

The effects of different substituents at the 8-position of 2-octyn-1-yl-N^9-propargyladenine derivatives were evaluated in vitro (Table 2). In all cases, A_{2A} AR antagonist activity was dramatically increased compared with the lead compound **3e**. The 8-bromo, 8-chloro, 8-(2-furyl) and 8-(1,2,3-triazol-2-yl) derivatives (**4a**, **4c**, **5c** and **5d**) were particularly effective, and were 50-fold more active than **3e**.



Scheme 3. Reagents and conditions: (a) NBS, AcOK, DMF, 57% for **4a**, (b) NCS, AcOK, DMF, 55% for **4c**, (c) R^8-$B(OH)_2$, $Pd(PPh_3)_4$ (10–40 mol %), K_2CO_3, dioxane–H_2O, 80 °C, (**5a**: 36%, **5b**: 30%, **5c**: 53%), (d) 1,2,3-triazole, K_2CO_3, DMF, 100 °C, (**5d**: 10%).

Table 2
Inhibitory effects of 8-substituted 2-octyn-1-yl-N^9-propargyladenine derivatives on 2-octyn-1-yladenosine-induced vasodilation

Compounds	R^8	IC_{50}[a] (nM)
3e	H	213.0 ± 10.23
4a	Br	5.1 ± 0.32
4c	Cl	6.0 ± 0.50
5a	Ph	75.5 ± 16.95
5b	3-F-Ph	22.8 ± 3.01
5c	2-Furyl	3.6 ± 0.65
5d	1,2,3-Triazol-2-yl	1.9 ± 0.58
Istradefylline	—	228.0 ± 16.26

[a] IC_{50}: compound concentration that inhibits 2-octyn-1-yladenosine-induced vasodilation by 50%.

2.3. Synthesis and biological activity of 8-substituted 2-(1-hydroxycyclohexyl)ethyn-1-yl-N^9-propargyladenines

Finally, we examined the effects of alkynyl side chains at the 2-position. The effects of substituents at the 2-position of 2-alkynyladenosines has been investigated, and the 2-(1-hydroxycyclohexyl)ethyn-1-yl derivative showed 10-fold higher affinity towards the A_{2A} subtype than the 2-octyn-1-yl derivative.[15c] Therefore, we synthesized the 2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]adenine derivatives to test the effects on antagonist activity. 2-[2-(1-Hydroxycyclohexyl)ethyn-1-yl]adenosine (**9**) was synthesized by a Sonogashira coupling reaction of **6**, followed by amination at the 6-position (Scheme 4),[15c] using the 6-pyridinium salt as previously described.[23] 6-Chloropurine derivative **7** was treated with pyridine–H_2O (1:1) at 50 °C for 4 h to afford 6-pyridinium intermediate **8**, which was confirmed by HPLC analysis. **8** was treated with NH_4OH–1,4-dioxane (2:1) at room temperature to obtain 2-alkynyladenosine **9** in 61% yield from **7**. Furthermore, adenosine derivative **9** was converted into 2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyl adenine derivatives (**11**–**13**) using the methods described above.

As expected, the in vitro A_{2A} AR antagonist activity of the 2-[2-(1-hydroxycyclohexyl)ethyn-1-yl] series was more potent than the 2-octyn-1-yl series, with 8-(2-furyl)-2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyladenine (**13**) being the most potent compound. It is noteworthy that **13** showed 570-fold higher activity than the istradefylline positive control (Table 3).

2.4. Structure–activity relationships of 2-alkynyl-N^9-propargyladenines

SAR of 2-alkynyl-N^9-propargyladenines are summarized in Scheme 5. The lead compound **3e** showed comparable activity to istradefylline in the in vitro assay. A propargyl group at the 9-position was important for A_{2A} AR antagonist activity, as mentioned above, and substituents at the 8-position and alkynyl resides at the 2-position also markedly enhanced the affinity. The antagonist activity of **3e** increased 50–100-fold by the introduction of a halogen or 2-furyl group at the 8-position. In addition to the effects of the 8-substituents, activity was elevated 400-fold by introduction of a 2-(1-hydroxycyclohexyl) group on the 2-ethnyl residue. This result is concordant with an earlier report of the effects of 2-alkynyl resides on agonist activity.[15c] Although the most potent antagonists were **12a** and **13**, we focused on compound **4a** for biological evaluation as this was more easily prepared.

2.5. Binding affinity of compound 4a for A_1, A_{2A} and A_{2B} AR subtypes

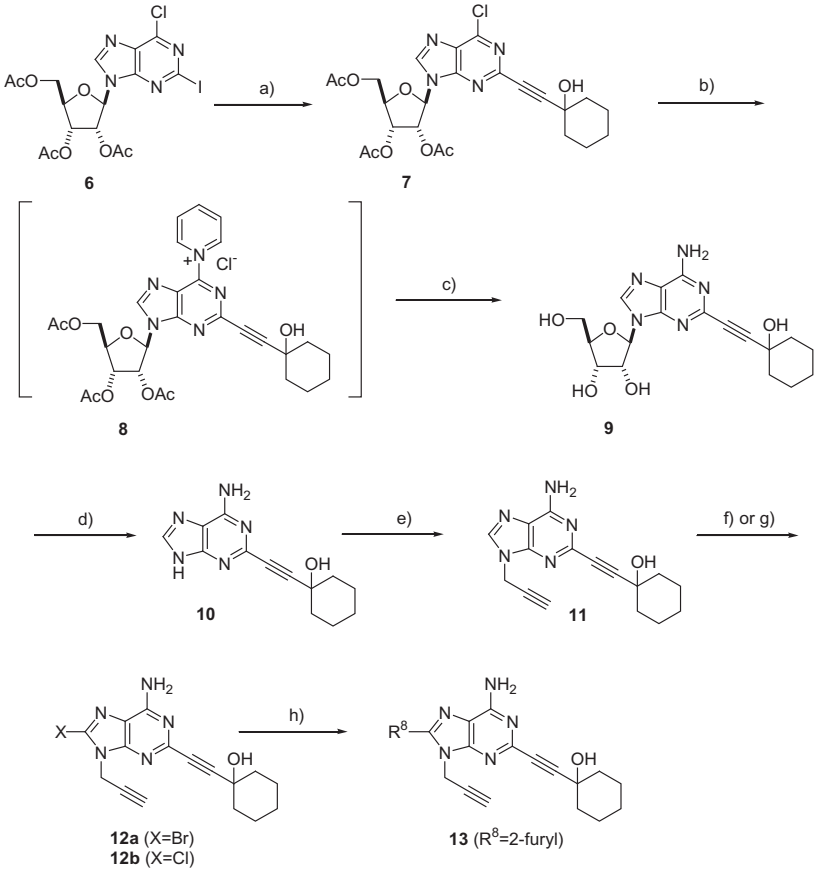
The binding affinities [K_i (nM)] of **4a** for human A_1, A_{2A} and A_{2B} subtypes (*h*-A_1, *h*-A_{2A}, and *h*-A_{2B}) expressed in CHO-K1 (A_1) and HEK-293 (A_{2A}, A_{2B}) cells were determined in order to investigate selectivity. Compound **4a** showed a higher affinity for *h*-A_{2A} (K_i = 0.56 nM) than reported for purine-type compounds.[12,16] Selectivity for A_{2B} was 1300-fold higher, while this compound was only 10-fold more selective towards the A_1 subtype, which was comparable to ST1535.

2.6. Activity as measured using the haloperidol-induced catalepsy model

To evaluate the in vivo activity of 2-alkynyl-N^9-propargyladenine derivative **4a**, the mouse catalepsy model was used. This widely used rodent model for PD involves inducing catalepsy (i.e., immobility) by subcutaneous injection with haloperidol (1 mg/kg).[24] The ability of A_{2A} AR antagonists to reduce the cataleptic responses (on a scale of 1–5) was tested. Oral administration of **4a** (and the istradefylline positive control) at a concentration of 3 mg/kg led to a significant reduction in catalepsy (Fig. 4A). Both **4a** and istradefylline achieved this in a dose-dependent manner, with a minimum effective dose (MED) of 3 mg/kg 5 h after oral administration (Fig. 4B).

2.7. Turning behavior in 6-OHDA-lesioned rats

Contralateral turning behavior in rats subjected to unilateral 6-hydroxydopamine (6-OHDA) lesions of the dopaminergic nigrostriatal pathway is another well established animal model of PD.[25] In this model, a selective A_{2A} AR antagonist reportedly potentiated the contralateral turning behavior induced by direct dopamine agonist,[26] implying that the antagonists might be beneficial for the treatment of PD. Morelli et al. reported that a selective A_{2A} AR antagonist markedly increased the number of contralateral rotations induced by a threshold dose of L-DOPA, whereas an A_{2A} AR antagonist did not induce any turning behavior when administered alone.[25] We evaluated the effects of 2-alkynyladenine derivative **4a** using this model. **4a** potentiated the turning behavior



Scheme 4. Reagents and conditions: (a) 1-ethynyl-1-cyclohexanol, $(PPh_3)_2PdCl_2$ (10 mol %), CuI, Et_3N, 1,4-dioxane, 98%, (b) pyridine–H_2O (1:1), 50 °C, (c) NH_4OH–1,4-dioxane (2:1), 61% from **7**, (d) 1 M HCl aq, 100 °C, 90%, (e) propargylbromide, K_2CO_3, DMF, 84%, (f) NBS, AcOK, DMF, 52%, (g) NCS, AcOK, DMF, 22%, (h) 2-furanboronic acid, $Pd(PPh_3)_4$ (10 mol %), K_2CO_3, 1,4-dioxane–H_2O, 100 °C, 59%.

Table 3
Inhibitory effects of 2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyladenine derivatives on 2-octyn-1-yladenosine -induced vasodilation

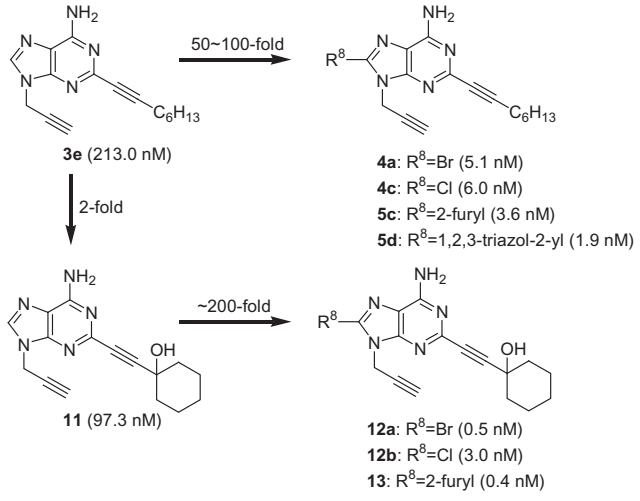
Compounds	R^8	IC_{50}[a] (nM)
11	H	97.3 ± 33.24
12a	Br	0.5 ± 0.02
12b	Cl	3.0 ± 1.67
13	2-Furyl	0.4 ± 0.12
Istradefylline	—	228.0 ± 16.26

[a] IC_{50}: compound concentration that inhibits 2-octyn-1-yladenosine-induced vasodilation by 50%.

induced by L-DOPA without inducing stereotyped behavior when administered on its own (Fig. 5). Although **4a** alone had minimal effect, turning behavior was induced significantly by administration of 100 mg/kg L-DOPA and 3 mg/kg **4a**.

3. Conclusion

In summary, we synthesized various 2-alkynyl-N^9-alkyladenine derivatives and investigated their antagonist activity against the A_{2A} AR. 8-Substituted 2-alkynyl-N^9-propargyladenine derivatives were found to antagonize the receptor with IC_{50} values in the low nM range. Furthermore, a selected member of this series (**4a**) was shown to be an orally active A_{2A} AR antagonist in the haloperidol-induced catalepsy PD model in mouse and the unilateral 6-OHDA-lesioned PD model in rat. These results suggest that novel 2-alkynyl-N^9-propargyladenine derivatives such as **4a** may be effective in the treatment of PD.



Scheme 5. Structure–activity relationships for 2-alkynyl-N^9-propargyladenines.

4. Experimental section

4.1. General

Physical data were measured as follows: Melting points were determined on a Yanagimoto MP-500D micromelting point apparatus, and were uncorrected. NMR spectra were recorded at

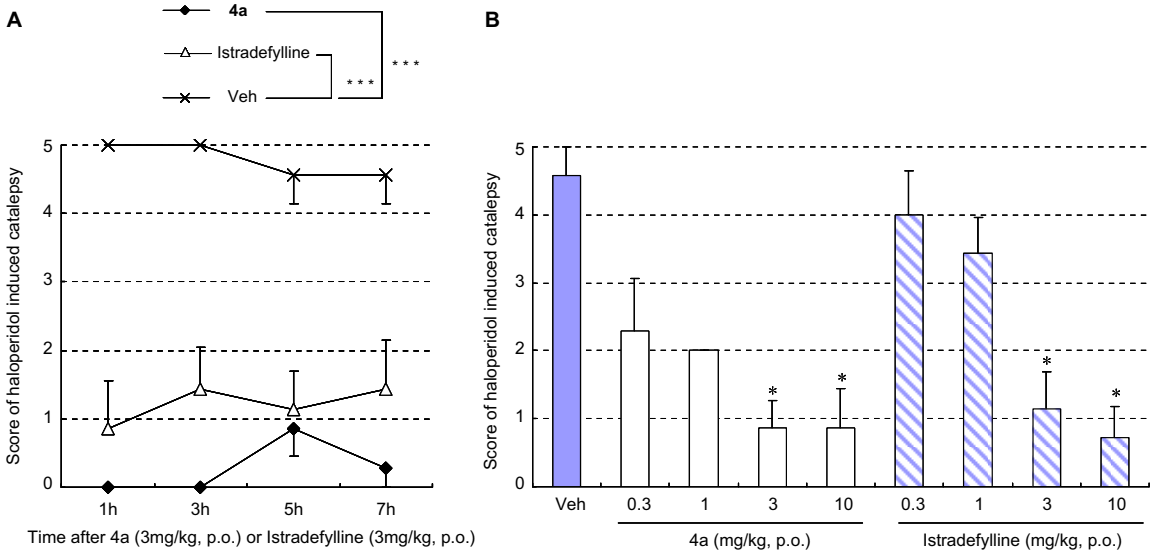


Figure 4. Anti-cataleptogenic activity of 2-alkynyl-N^9-propargyladenine derivative **4a**. (A) Effects of **4a** (3 mg/kg) and istradefylline (3 mg/kg) on catalepsy induced by haloperidol in mice. Data show the mean catalepsy score (±SEM) 1, 3, 5, and 7 h after treatment ($n = 7$ per group). $^{*}P < 0.001$ versus vehicle. Statistical significance was determined with the Repeated Two Way ANOVA test followed by the Tukey test. (B) Dose-dependence of the anti-cataleptogenic activity of **4a** (0.3–10 mg/kg) and istradefylline (0.3–10 mg/kg). Each column represents the mean catalepsy score (±SEM) 5 h after treatment ($n = 7$ per group). $^{*}P < 0.05$ versus vehicle. Statistical significance was determined with the Kruskal–Wallis test followed by the Tukey test. The cataleptic responses were scored as follows: score 0, the cataleptic posture lasted for less than 5 s for both forelimbs and hind limbs; score 1, the cataleptic posture of forelimbs lasted for 5–10 s and that of hind limbs lasted less than 5 s; score 2, the cataleptic posture of forelimbs lasted for more than 10 s and that of hind limbs lasted for less than 5 s; score 3, the cataleptic posture of forelimbs and hind limbs lasted for 5–10 s, or the cataleptic posture of forelimbs lasted for less than 5 s but that of hind limbs lasted for more than 5 s; score 4, the cataleptic posture of forelimbs lasted for more than 10 s and that of hind limbs lasted for 5–10 s, or the cataleptic posture of forelimbs lasted for 5–10 s and that of hind limbs lasted for more than 10 s; score 5, the cataleptic posture of both forelimbs and hind limbs lasted for more than 10 s.

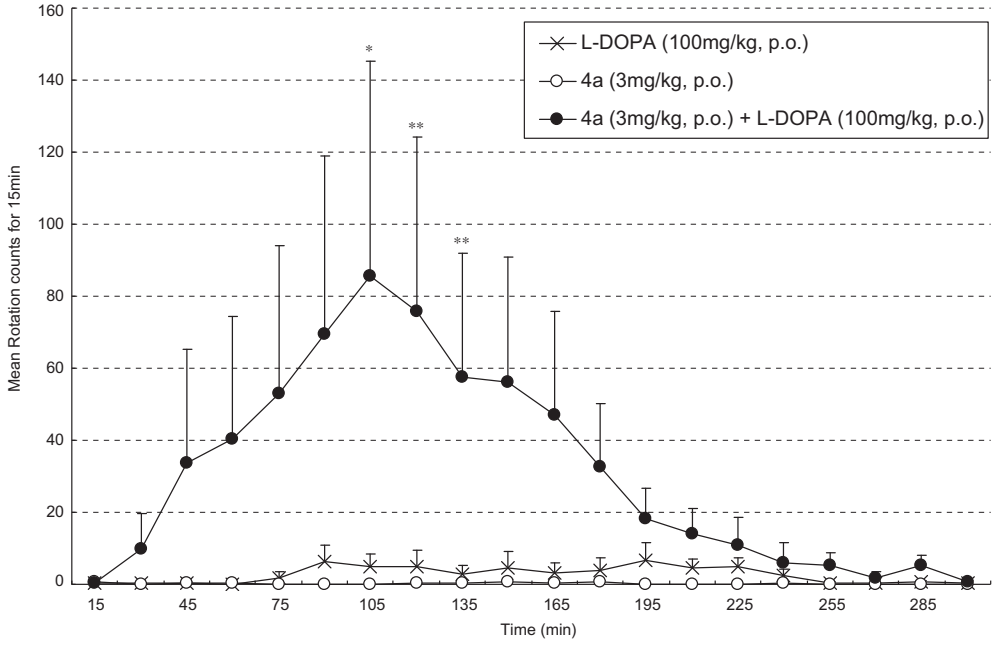


Figure 5. The effect of **4a** (3 mg/kg) on L-DOPA-induced turning behavior in 6-OHDA-lesioned, hemiparkinsonian rats. Data show mean rotation counts for a 15 min duration (±SEM). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with L-DOPA (100 mg/kg) alone. Statistical significance was determined with the Repeated Two Way ANOVA test followed by the Tukey test. Five animals were used in each group.

500 MHz (^{1}H) and at 125 MHz (^{13}C) on a Bruker AV-500 instrument in $CDCl_3$ or DMSO-d_6 as the solvent, with tetramethylsilane as the internal standards. UV spectra were recorded with a Shimadzu UV-1800 spectrophotometer. ESI mass spectra were recorded on a JEOL JMS-T100LP instrument. TLC was carried out on Merck precoated plates (Kieselgel 60F254).

4.2. Representative syntheses

4.2.1. N^9-Methyl-2-(1-octyn-1-yl)adenine (3a)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (180 mg, 1.3 mmol) in DMF (10 mL) was treated with CH_3I (100 μL, 1.6 mmol), and the mixture was stirred at room temperature for

23 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (MeOH–$CHCl_3$ = 1:16) to give **3a** (73 mg, 35%). ^{1}H NMR ($CDCl_3$) δ 7.77 (1H, s), 5.79 (2H, br s), 3.83 (3H, s), 2.45 (2H, t, J = 7.3 Hz), 1.69–1.63 (2H, m), 1.48–1.42 (2H, m), 1.35–1.27 (4H, m), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 155.1, 150.7, 147.0, 141.6, 118.9, 87.8, 80.3, 31.4, 30.0, 28.8, 28.3, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{14}H_{20}N_5$ 258.1719, found 258.1728 (MH^+); Anal. calcd for $C_{14}H_{19}N_5$: C, 65.34; H, 7.44; N, 27.22. found: C, 64.96; H, 7.46; N, 27.05.

4.2.2. N^9-Ethyl-2-(1-octyn-1-yl)adenine (3b)

A mixture of **2** (300 mg, 1.2 mmol) and K_2CO_3 (340 mg, 2.5 mmol) in DMF (4 mL) was treated with EtBr (180 μL, 2.5 mmol), and the mixture was stirred at room temperature for 16 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (EtOAc/MeOH = 15:1) to give **3b** (263 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 7.82 (1H, s), 6.00 (2H, br s), 4.26 (2H, q, J = 7.3 Hz), 2.45 (2H, t, J = 7.4 Hz), 1.69–1.63 (2H, m), 1.52 (3H, t, J = 7.3 Hz), 1.48–1.42 (2H, m), 1.34–1.28 (4H, m), 0.89 (3H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 155.2, 150.2, 146.8, 140.4, 119.0, 87.6, 80.4, 38.8, 31.4, 28.9, 28.3, 22.5, 19.4, 15.6, 14.0; ESIMS-HR calcd for $C_{15}H_{22}N_5$ 272.1875, found 272.1895 (MH^+); Anal. calcd for $C_{15}H_{21}N_5{\cdot}H_2O$: C, 62.26; H, 8.01; N, 24.20. found: C, 62.15; H, 7.95; N, 24.23.

4.2.3. 2-(1-Octyn-1-yl)-N^9-*n*-propyladenine (3c)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (180 mg, 1.3 mmol) in DMF (10 mL) was treated with *n*-PrBr (150 μL, 1.6 mmol), and the mixture was stirred at 50 °C for 7 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 1:7) to give **3c** (107 mg, 46%). ^{1}H NMR ($CDCl_3$) δ 7.79 (1H, s), 5.75 (2H, br s), 4.16 (2H, q, J = 7.3 Hz), 2.45 (2H, t, J = 7.4 Hz), 1.92 (2H, tq, J = 7.3, 7.4 Hz), 1.69–1.63 (2H, m),1.48–1.42 (2H, m), 1.35–1.28 (4H, m), 0.96 (3H, t, J = 7.4 Hz), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 155.1, 150.4, 146.8, 141.0, 119.0, 87.6, 80.4, 45.5, 31.4, 28.9, 28.3, 23.4, 22.5, 19.4, 14.0, 11.1; ESIMS-HR calcd for $C_{16}H_{24}N_5$ 286.2032, found 286.2040 (MH^+); Anal. calcd for $C_{16}H_{23}N_5{\cdot}H_2O$: C, 63.34; H, 8.31; N, 23.08. found: C, 63.14; H, 8.49; N, 23.01.

4.2.4. N^9-Allyl-2-(1-octyn-1-yl)adenine (3d)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (230 mg, 1.6 mmol) in DMF (5 mL) was treated with allylbromide (138 μL, 1.6 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 1:7) to give **3d** (103 mg, 44%). ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, s), 6.07–5.99 (1H, m), 5.85 (2H, br s), 5.32 (1H, dd, J = 0.8, 10.2 Hz), 5.23 (1H, dd, J = 0.8, 17.1 Hz), 4.82 (2H, ddd, J = 1.3, 1.4, 5.8 Hz), 2.45 (2H, t, J = 7.4 Hz), 1.69–1.63 (2H, m),1.48–1.42 (2H, m), 1.34–1.27 (4H, m), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 155.2, 150.2, 147.0, 140.9, 131.9, 119.2, 118.9, 87.8, 80.3, 45.7, 31.4, 28.9, 28.3, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{16}H_{22}N_5$ 284.1875, found 284.1881 (MH^+); Anal. calcd for $C_{16}H_{21}N_5$: C, 67.82; H, 7.47; N, 24.71. found: C, 67.64; H, 7.60; N, 24.53.

4.2.5. 2-(1-Octyn-1-yl)-N^9-propargyladenine (3e)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (230 mg, 1.6 mmol) in DMF (5 mL) was treated with propargylbromide (138 μL, 1.6 mmol), and the mixture was stirred at room temperature for 6.5 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 1:7) to give **3e** (177 mg, 77%). ^{1}H NMR (DMSO-d_6) δ 8.23 (1H, s), 7.39 (2H, br s), 5.02 (2H, d, J = 2.5 Hz), 3.48 (1H, t, J = 2.5 Hz), 2.41 (2H, t, J = 7.1 Hz), 1.57–1.51 (2H, m), 1.43–1.37 (2H, m), 1.32–1.26 (4H, m), 0.88 (3H, t, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6) δ 155.7, 149.1, 145.9, 140.7, 117.8, 85.4, 81.1, 78.2, 75.9, 32.2, 30.7, 28.0, 27.8, 21.9, 18.1, 13.8; ESIMS-HR calcd for $C_{16}H_{19}N_5$ 282.1719, found 282.1733 (MH^+); Anal. calcd for $C_{16}H_{19}N_5$: C, 68.30; H, 6.81; N, 24.89. found: C, 68.26; H, 6.90; N, 24.89.

4.2.6. N^9-Cyanomethyl-2-(1-octyn-1-yl)adenine (3f)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (230 mg, 1.6 mmol) in DMF (5 mL) was treated with bromoacetonitrile (110 μL, 1.6 mmol), and the mixture was stirred at room temperature for 6.5 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (MeOH–$CHCl_3$ = 1:19) to give **3f** (206 mg, 89%). ^{1}H NMR ($CDCl_3$) δ 7.76 (1H, s), 5.94 (2H, br s), 5.14 (2H, s), 2.46 (2H, t, J = 7.3 Hz), 1.70–1.64 (2H, m), 1.49–1.43 (2H, m), 1.34–1.29 (4H, m), 0.89 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 155.4, 149.8, 147.7, 139.2, 118.5, 113.2, 88.9, 79.9, 31.4, 30.9, 28.8, 28.2, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{15}H_{19}N^6$ 283.1671, found 283.1686 (MH^+); Anal. calcd for $C_{15}H_{18}N^6{\cdot}0.1H_2O$: C, 63.40; H, 6.46; N, 29.58. found: C, 63.25; H, 6.33; N, 29.46.

4.2.7. N^9-Hydroxyethyl-2-(1-octyn-1-yl)adenine (3g)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (230 mg, 1.6 mmol) in DMF (5 mL) was treated with 2-bromoethanol (113 μL, 1.6 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (MeOH–$CHCl_3$ = 1:13) to give **3g** (102 mg, 43%). ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, s), 5.92 (2H, br s), 4.34 (2H, t, J = 4.7 Hz), 4.19 (1H, br s), 4.03 (2H, t, J = 4.7 Hz), 2.43 (2H, t, J = 7.3 Hz), 1.68–1.61 (2H, m), 1.45–1.42 (2H, m), 1.32–1.28 (4H, m), 0.89 (3H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 155.2, 150.1, 146.6, 141.8, 118.9, 88.1, 80.1, 61.2, 47.3, 31.4, 28.8, 28.3, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{15}H_{22}N_5O$ 288.1746, found 288.1839 (MH^+); Anal. calcd for $C_{15}H_{21}N_5{\cdot}0.1H_2O$: C, 62.30; H, 7.39; N, 24.22. found: C, 62.19; H, 7.33; N, 24.34.

4.2.8. N^9-Cyclopentyl-2-(1-octyn-1-yl)adenine (3h)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (230 mg, 1.6 mmol) in DMF (5 mL) was treated with cyclopentylbromide (180 μL, 1.6 mmol), and the mixture was stirred at room temperature for 20 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (AcOEt) to give **3h** (203 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 7.86 (1H, s), 5.73 (2H, br s), 5.02–5.00 (1H, m), 2.45 (2H, t, J = 7.3 Hz), 2.32–2.27 (2H, m), 2.05–1.89 (4H, m), 1.82–1.78 (2H, m), 1.69–1.63 (2H, m), 1.48–1.42 (2H, m), 1.34–1.26 (4H, m), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 155.1, 150.4, 146.6, 139.0, 119.1, 87.5, 80.5, 55.5, 33.2, 31.4, 28.9, 28.3, 23.9, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{18}H_{26}N_5$ 312.2188, found 312.2190 (MH^+); Anal. calcd for $C_{18}H_{25}N_5$: C, 69.42; H, 8.09; N, 22.49. found: C, 69.07; H, 8.29; N, 22.28.

4.2.9. N^9-Benzyl-2-(1-octyn-1-yl)adenine (3i)

A mixture of **2** (200 mg, 0.82 mmol) and K_2CO_3 (180 mg, 1.3 mmol) in DMF (10 mL) was treated with BnBr (200 μL,

1.6 mmol), and the mixture was stirred at 50 °C for 7 h. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 1:10) to give **3i** (127 mg, 46%). ^{1}H NMR ($CDCl_3$) δ 7.71 (1H, s), 7.37–7.28 (5H, m), 5.79, (2H, br s), 5.37 (2H, s), 2.46 (2H, t, J = 7.3 Hz), 1.69–1.64 (2H, m), 1.47–1.44 (2H, m), 1.33–1.29 (4H, m), 0.89 (3H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 155.2, 150.5, 147.1, 141.0, 135.5, 129.1, 128.5, 128.0, 87.8, 80.4, 47.2, 31.4, 28.9, 28.3, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{20}H_{24}N_5$ 334.2032, found 334.2026 (MH^+); Anal. calcd for $C_{20}H_{25}N_5$: C, 72.04; H, 6.95; N, 21.00. found: C, 71.99; H, 6.97; N, 21.01.

4.2.10. 8-Bromo-2-(1-octyn-1-yl)-*N*^{9}-propargyladenine (4a)

A mixture of **3e** (281 mg, 1.0 mmol) and AcOK (29 mg, 0.30 mmol) in DMF (10 mL) was treated with NBS (178 mg, 1.0 mmol), and the mixture was stirred at room temperature for 2.5 h. The reaction was quenched with saturated $Na_2S_2O_3$ solution, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 3:1) to give **4a** (204 mg, 57%). Analytical samples were crystallized from AcOEt/hexane. Mp 146–147 °C; ^{1}H NMR ($CDCl_3$) δ 5.64 (2H, s), 4.98 (2H, d, J = 2.5 Hz), 2.45 (2H, t, J = 7.2 Hz), 2.36, (1H, t, J = 2.5 Hz), 1.69–1.62 (2H, m), 1.47–1.42 (2H, m), 1.35–1.25 (4H, m), 0.89 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 153.8, 151.0, 147.2, 127.4, 119.1, 88.6, 80.0, 73.8, 33.5, 31.4, 28.8, 28.2, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{16}H_{19}BrN_5$ 360.0824, found 360.0812 (MH^+); Anal. calcd for $C_{16}H_{18}BrN_5$: C, 53.34; H, 5.04; N, 19.44. Found: C, 53.22; H, 4.94; N, 19.40.

4.2.11. 8-Chloro-2-(1-octyn-1-yl)-*N*^{9}-propargyladenine (4c)

A mixture of **3e** (281 mg, 1.0 mmol) and AcOK (29 mg, 0.30 mmol) in DMF (10 mL) was treated with NCS (270 mg, 2.0 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with saturated $Na_2S_2O_3$ solution, and the mixture was extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 3:1) to give **4c** (173 mg, 55%). ^{1}H NMR ($CDCl_3$) δ 6.00 (2H, s), 4.98 (2H, d, J = 2.5 Hz), 2.45 (2H, t, J = 7.2 Hz), 2.37, (1H, t, J = 2.5 Hz), 1.69–1.65 (2H, m), 1.48–1.42 (2H, m), 1.34–1.25 (4H, m), 0.89 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 154.0, 150.5, 147.1, 138.5, 117.6, 88.6, 80.0, 73.8, 32.5, 31.4, 28.8, 28.2, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{16}H_{19}ClN_5$ 316.1329, found 316.1324 (MH^+); Anal. calcd for $C_{16}H_{18}ClN_5 \cdot 0.3H_2O$: C, 59.83; H, 5.84; N, 21.80. Found: C, 60.09; H, 5.73; N, 21.69.

4.2.12. 2-(1-Octyn-1-yl)-8-phenyl-*N*^{9}-propargyladenine (5a)

A mixture of **4a** (100 mg, 0.28 mmol), phenylboronic acid (68 mg, 0.52 mmol), $Pd(PPh_3)_4$ (64 mg, 0.055 mmol) and K_2CO_3 (76 mg, 0.55 mmol) in 1,4-dioxane (3 mL) and H_2O (2 mL) was stirred at 80 °C for 30 min. Additional $Pd(PPh_3)_4$ (32 mg, 0.028 mmol) was added, and the reaction mixture was stirred for further 1 h. Water was added, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 2:1) to give **5a** (36 mg, 36%). ^{1}H NMR ($CDCl_3$) δ 7.90 (2H, m), 7.57–7.55 (3H, m), 5.84 (2H, br s), 5.01 (2H, d, J = 2.3 Hz), 2.47 (2H, t, J = 7.4 Hz), 2.40 (1H, t, J = 2.5 Hz), 1.71–1.65 (2H, m), 1.48–1.45 (2H, m), 1.33–1.30 (4H, m), 0.90 (3H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 154.8, 151.5, 146.8, 130.5, 129.2, 129.1, 118.6, 88.0, 80.4, 73.9, 33.4, 31.4, 28.9, 28.3, 22.5, 19.5, 14.1; ESIMS-HR calcd for $C_{22}H_{24}N_5$ 358.2032, found 358.2025 (MH^+).

4.2.13. 8-(3-Fluorophenyl)-2-(1-octyn-1-yl)-*N*^{9}-propargyladenine (5b)

A mixture of **4a** (100 mg, 0.28 mmol), 3-fluorophenylboronic acid (78 mg, 0.56 mmol), $Pd(PPh_3)_4$ (64 mg, 0.055 mmol) and K_2CO_3 (76 mg, 0.55 mmol) in 1,4-dioxane (3 mL) and H_2O (2 mL) was stirred at 80 °C for 30 min. Additional $Pd(PPh_3)_4$ was added in twice, 0.13, 0.05 equiv, respectively and the reaction mixture was stirred for further 1 h. Water was added, and the mixture was extracted with AcOEt. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 2:1) to give **5b** (31 mg, 30%). ^{1}H NMR ($CDCl_3$) δ 7.73–7.66 (2H, m), 7.55–7.54 (1H, m), 7.26–7.26 (1H, m), 5.70 (2H, br s), 5.00 (2H, d, J = 2.5 Hz), 2.47 (2H, t, J = 7.4 Hz), 2.42 (1H, t, J = 2.5 Hz), 1.70–1.65 (2H, m), 1.48–1.45 (2H, m), 1.33–1.30 (4H, m), 0.90 (3H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) 163.9, 161.9, 154.9, 151.5, 150.0, 147.1, 131.2, 130.8, 130.8, 124.7, 124.7, 118.6, 117.7, 117.5, 116.3, 116.1, 88.2, 80.4, 74.2, 33.4, 31.4, 28.9, 28.3, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{22}H_{23}FN_5$ 376.1937, found 376.1935 (MH^+).

4.2.14. 8-(2-Furyl)-2-(1-octyn-1-yl)-*N*^{9}-propargyladenine (5c)

A mixture of **4a** (100 mg, 0.28 mmol), 2-furanboronic acid (62 mg, 0.56 mmol), $Pd(PPh_3)_4$ (32 mg, 0.028 mmol) and K_2CO_3 (77 mg, 0.56 mmol) in 1,4-dioxane (3 mL) and H_2O (2 mL) was stirred at 90 °C for 50 min. Water was added, and the mixture was extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 3/2 to 1:1) to give **5c** (51 mg, 53%). ^{1}H NMR ($CDCl_3$) δ 7.69 (1H, d, J = 1.5 Hz), 7.25 (1H, d, J = 3.6 Hz), 6.64 (1H, dd, J = 1.5, 3.6 Hz), 5.94 (2H, br s), 5.24 (2H, d, J = 2.4 Hz), 2.47 (2H, t, J = 6.7 Hz), 2.32 (1H, t, J = 2.5 Hz), 1.71–1.65 (2H, m), 1.47–1.44 (2H, m), 1.33–1.29 (4H, m), 0.90 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 154.7, 150.9, 146.9, 144.8, 144.1, 142.2, 118.6, 113.1, 112.2, 88.2, 80.3, 73.5, 33.2, 31.4, 28.9, 28.3, 22.5, 19.5, 14.0; ESIMS-HR calcd for $C_{20}H_{22}N_5O$ 348.1824, found 348.1823 (MH^+); Anal. calcd for $C_{20}H_{21}N_5O \cdot 1.1H_2O$: C, 65.41; H, 6.37; N, 19.07. Found: C, 65.31; H, 6.07; N, 19.04.

4.2.15. 2-(1-Octyn-1-yl)-*N*^{9}-propargyl-8-[2-(1,2,3-triazolyl)]adenine (5d)

A mixture of **4a** (100 mg, 0.28 mmol), 1,2,3-triazole (19 mg, 0.28 mmol) and K_2CO_3 (77 mg, 0.56 mmol) in DMF (1 mL) was stirred at room temperature overnight. The reaction was quenched with water, and the mixture was extracted with AcOEt. The organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/AcOEt = 4:1 to 1:1) to give isomer mixture. Then, the mixture was purified by ODS column chromatography (MeCN/H_2O = 3:7 to 2:3) to give **5d** (10 mg, 10%). ^{1}H NMR ($CDCl_3$) δ 8.03 (2H, s), 6.11 (2H, br s), 5.47 (2H, d, J = 2.4 Hz), 2.48 (2H, t, J = 7.3 Hz), 2.16 (1H, t, J = 2.4 Hz), 1.74–1.65 (2H, m), 1.50–1.44 (2H, m), 1.35–1.26 (4H, m), 0.90 (3H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 155.1, 150.4, 147.6, 141.9, 137.5, 116.3, 88.6, 80.2, 73.1, 34.2, 31.4, 29.7, 28.9, 28.3, 22.5, 19.4, 14.0; ESIMS-HR calcd for $C_{18}H_{21}N_8$ 349.1889, found 349.1895 (MH^+).

4.2.16. 9-(2,3,5-Tri-*O*-acetyl-1-β-D-ribofuranosyl)-6-chloro-2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]purine (7)[15c]

A mixture of **6** (108 mg, 0.20 mmol),ethynyl-1-cyclohexanol (30 mg, 0.24 mmol), $(PPh_3)_2PdCl_2$ (3 mg, 0.0040 mmol) and CuI (1.5 mg, 0.0080 mmol) in 1,4-dioxane (2 mL) was treated with Et_3N (56 μL, 0.40 mmol), and the mixture was stirred at room temperature for 8.5 h. Additionalethynyl-1-cyclohexanol (30 mg, 0.24 mmol) was added, and the mixture was stirred for further 15 h. The reaction was quenched with aqueous EDTA, and the mixture was extracted with $CHCl_3$. The organic layers were washed

with aqueous EDTA and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography ($CHCl_3$ to MeOH–$CHCl_3$ = 1:49) to give **7** (105 mg, quant). ^{1}H NMR ($CDCl_3$) δ 8.28 (1H, s), 6.21 (1H, d, J = 5.4 Hz), 5.90 (1H, t, J = 5.4 Hz), 5.74 (1H, dd, J = 4.1, 5.4 Hz), 4.51–4.43 (3H, m), 2.97 (1H, s), 2.18 (3H, s), 2.12 (3H, s), 2.10 (3H, s), 2.08–2.05 (2H, m), 1.78–1.56 (7H, m), 1.35–1.33 (1H, m); ^{13}C NMR ($CDCl_3$) δ 170.5, 169.5, 169.3, 151.5, 151.2, 146.0, 144.2, 131.5, 93.0, 87.1, 82.3, 80.9, 73.3, 70.8, 68.6, 63.1, 39.4, 25.2, 23.0, 20.8, 20.5, 20.4; ESIMS-HR calcd for $C_{24}H_{28}ClN_4O_8$ 535.1596, found 535.1597 (MH^+).

4.2.17. 2-[2-(1-Hydroxycyclohexyl)ethyn-1-yl]adenosine (9)[15c]

A solution of **7** (1.61 g, 3.0 mmol) in pyridine (6 mL) and H_2O (6 mL) was stirred at 50 °C for 4 h. The solvent was removed in vacuo, and the a solution of the resulting residue in NH_4OH (6 mL) and 1,4-dioxane (3 mL) was stirred at for 4 h. The solvent was removed in vacuo and the residue was purified by column chromatography (MeOH–$CHCl_3$ = 1:19 to 1:9) to give **9** (708 mg, 61%). ^{1}H NMR (DMSO-d_6) δ 8.42 (1H, s), 7.47 (2H, br s), 5.88 (1H, d, J = 6.1 Hz), 5.54 (1H, s), 5.47 (1H, t, J = 6.2 Hz), 5.20–5.17 (2H, m), 4.51 (1H, dd, J = 6.1, 6.2 Hz), 4.14–4.10 (1H, m), 3.96–3.94 (1H, m), 3.68–3.65 (1H, m), 3.59–3.56 (1H, m), 1.86–1.83 (2H, m), 1.65–1.47 (7H, m), 1.26–1.24 (1H, m); ^{13}C NMR (DMSO-d_6) δ 155.8, 149.3, 145.5, 140.0, 118.5, 89.1, 87.1, 85.6, 82.8, 73.8, 70.4, 66.6, 61.4, 24.8, 22.5, 22.3; ESIMS-HR calcd for $C_{18}H_{24}N_5O_5$ 390.1777, found 390.1766 (MH^+); Anal. calcd for $C_{18}H_{23}N_5O_5 \cdot 1.6H_2O$: C, 51.69; H, 6.31; N, 16.75. Found: C, 51.94; H, 6.16; N, 16.35.

4.2.18. 2-[2-(1-Hydroxycyclohexyl)ethyn-1-yl]adenine (10)

A solution of **9** (24.6 g, 63 mmol) in 1 M aqueous HCl (320 mL) was stirred at 100 °C for 1 h. After cooling, 1 M aqueous NaOH was added to the mixture to adjust the pH to 8.5, and the mixture was stirred at room temperature for 1.5 h. Then, the resulting precipitate was corrected to give **10** (14.6 g, 90%). ^{1}H NMR (DMSO-d_6) δ 12.86 (1H, br s), 8.13 (1H, br s), 7.24 (2H, br s), 5.50 (1H, s), 1.85–1.82 (2H, m), 1.66–1.46 (7H, m), 1.28–1.22 (1H, m); ^{13}C NMR (DMSO-d_6) δ 155.5, 150.2, 145.4, 139.7, 117.9, 88.2, 83.3, 74.0, 66.6, 34.7, 24.8, 22.5, 21.3; ESIMS-HR calcd for $C_{13}H_{16}N_5O$ 258.1355, found 258.1374 (MH^+).

4.2.19. 2-[2-(1-Hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyladenine (11)

A mixture of **10** (2.10 g, 8.2 mmol), K_2CO_3 (2.25 g, 16 mmol) in DMF (16.5 mL) was treated with propargylbromide (1.23 mL, 16 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water (80 mL), and the mixture was stirred at room temperature for 2 h. Then, the resulting precipitate was corrected to give **11** (2.04 g, 84%). ^{1}H NMR (DMSO-d_6) δ 8.24 (1H, s), 7.43 (2H, br s), 5.59 (1H, s), 5.03 (2H, d, J = 2.3 Hz), 3.55–3.43 (1H, m), 1.84–1.83 (2H, m), 1.65–1.46 (7H, m), 1.28–1.26 (1H, m); ^{13}C NMR (DMSO-d_6) δ 155.7, 149.1, 145.7, 140.9, 117.9, 89.0, 82.9, 78.1, 75.9, 66.6, 32.3, 24.7, 22.4; ESIMS-HR calcd for $C_{16}H_{18}N_5O$ 296.1511, found 296.1526 (MH^+); Anal. calcd for $C_{16}H_{17}N_5O \cdot 1.6H_2O$: C, 59.28; H, 6.28; N, 21.60. Found: C, 59.48; H, 5.95; N, 21.25.

4.2.20. 8-Bromo-2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyladenine (12a)

A mixture of **11** (620 mg, 2.1 mmol) and AcOK (62 mg, 0.63 mmol) in DMF (21 mL) was treated with NBS (374 mg, 2.1 mmol), and the mixture was stirred at room temperature for 2.5 h. Additional NBS was added in twice, 0.2, 0.1 equiv, respectively and the reaction mixture was stirred for further 2.5 h. The reaction was quenched with saturated $Na_2S_2O_3$ solution, and the mixture was extracted with $CHCl_3$. The organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (AcOEt) to give **12a** (405 mg, 52%). Analytical samples were crystallized from 1,4-dioxane/hexane. mp. 225–229 °C; ^{1}H NMR (DMSO-d_6) δ 7.63 (2H, s), 5.59 (1H, s), 4.95 (2H, d, J = 2.3 Hz), 3.49 (1H, t, J = 2.3 Hz), 1.84–1.82 (2H, m), 1.65–1.47 (7H, m), 1.27–1.23 (1H, m); ^{13}C NMR (DMSO-d_6) δ 154.6, 150.2, 146.0, 126.7, 118.2, 89.6, 82.7, 77.3, 75.7, 66.7, 66.3, 33.0, 24.8, 22.5; ESIMS-HR calcd for $C_{16}H_{17}BrN_5O$ 374.0616, found 374.0618 (MH^+); Anal. calcd for $C_{16}H_{16}BrN_5O \cdot 0.3H_2O$: C, 50.62; H, 4.41; N, 18.45. Found: C, 50.54; H, 4.16; N, 18.35.

4.2.21. 8-Chloro-2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyladenine (12b)

A mixture of **11** (200 mg, 0.67 mmol) and AcOK (20 mg, 0.20 mmol) in DMF (7 mL) was treated with NCS (180 mg, 1.3 mmol), and the mixture was stirred at room temperature for 28.5 h. The reaction was quenched with saturated $Na_2S_2O_3$ solution, and the mixture was extracted with $CHCl_3$. The organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was suspended in $CHCl_3$, and resulting precipitate was crystallized from 1,4-dioxane/H_2O to give **12b** (49 mg, 22%). mp. 225–235 °C; ^{1}H NMR (DMSO-d_6) δ 7.60 (2H, s), 5.55 (1H, s), 4.98 (2H, d, J = 2.3 Hz), 3.48 (1H, t, J = 2.3 Hz), 1.85–1.82 (2H, m), 1.66–1.48 (7H, m), 1.28–1.25 (1H, m); ^{13}C NMR (DMSO-d_6) δ 155.4, 150.5, 146.9, 137.6, 117.5, 90.4, 83.4, 77.8, 76.5, 67.4, 67.1, 32.9, 25.5, 23.2; ESIMS-HR calcd for $C_{16}H_{17}ClN_5O$ 330.1122, found 330.1134 (MH^+); Anal. calcd for $C_{16}H_{16}ClN_5O \cdot 0.3H_2O$: C, 57.33; H, 4.99; N, 20.89. Found: C, 57.39; H, 4.87; N, 20.58.

4.2.22. 8-(2-Furyl)-2-[2-(1-hydroxycyclohexyl)ethyn-1-yl]-N^9-propargyladenine (13)

A mixture of **12a** (1.00 g, 2.7 mmol), 2-furanboronic acid (598 mg, 5.3 mmol), $Pd(PPh_3)_4$ (309 mg, 0.27 mmol) and K_2CO_3 (738 mg, 5.3 mmol) in 1,4-dioxane (30 mL) and H_2O (20 mL) was stirred at 100 °C for 25 min. The reaction was quenched with water, and the mixture was extracted with $CHCl_3$. The organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography ($CHCl_3$ to MeOH–$CHCl_3$ = 1:32) to give **13** (567 mg, 59%). Analytical samples were crystallized from 1,4-dioxane. mp. 220–229 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 8.03 (1H, d, J = 1.7 Hz), 7.60 (2H, br s), 7.27 (1H, d, J = 3.4 Hz), 6.80 (1H, dd, J = 1.7 Hz, 3.4 Hz), 5.58 (1H, s), 5.21 (2H, d, J = 2.4 Hz), 3.42 (1H, t, J = 2.4 Hz), 1.87–1.84 (2H, m), 1.67–1.47 (7H, m), 1.28–1.27 (1H, m); ^{13}C NMR (DMSO-d_6) δ 155.4, 150.1, 145.8, 145.3, 143.7, 140.8, 117.8, 112.6, 112.3, 89.4, 82.9, 78.2, 75.5, 66.6, 66.3, 32.7, 24.8, 22.5; ESIMS-HR calcd for $C_{20}H_{20}N_5O_2$ 362.1617, found 362.1631 (MH^+); Anal. calcd for $C_{20}H_{19}N_5O_2 \cdot 0.2H_2O$: C, 65.81; H, 5.36; N, 19.19. Found: C, 65.76; H, 5.31; N, 19.02.

4.3. Inhibitory effects of the 2-alkynyladenine derivatives on 2-octyn-1-yladenosine-induced vasodilation

4.3.1. Vascular preparations

Male Wistar rats, 10 weeks old weighing 305–355 g, were purchased from Clea Japan (Tokyo, Japan). The rats were killed, under ether anesthesia, by bleeding from the abdominal aorta, and the femoral vein were isolated from each rat. The vessels were placed in physiological salt solution (PSS), composed of 118 mM NaCl, 4.7 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11 mM glucose, and continuously bubbled with 95% O_2–5% CO_2. The vessels were cleaned of loosely adhering connective tissue and cut into rings (5 mm in width) under a dissecting microscope. Special care was taken not to damage the endothelium. Each ring was mounted between two stainless steel

hooks and placed in 10-ml organ baths containing PSS at 37 °C. The isometric tension of the rings was measured by a force–displacement transducer (TB-611, Nihon-Kohden, Japan) and recorded on a pen recorder (FBR-252A, TOA, Japan). The rings were equilibrated for 60 min at a resting tension of 0.5 g for femoral vein ring, the optimal tension for inducing maximal contraction. During the equilibration time, the PSS in the bath was replaced every 20 min.

4.3.2. Vasodilation inhibition study

We previously reported 5-hydroxytryptamine (10^{-7} to 3×10^{-5} M) induced concentration-dependent contraction in the isolated rat femoral vein.[27] The contractile response to 10^{-5} M 5-hydroxytryptamine (5-HT) in the vein was stable during the time period required to construct the 10^{-7} M 2-octyn-1-yladenosine induced relaxing response. And the vein was completely dilated at 2-octyn-1-yladenosine concentrations as low as 10^{-7} M. Therefore following the 1 h equilibration period, the rings were precontracted with 10^{-5} M 5-HT. After the contraction induced by 10^{-5} M 5-HT had reached a plateau, 10^{-7} M 2-octyn-1-yladenosine were added to the bath for the maximum vasodilation response.[27] To examine the inhibitory efficacy of 2-alkynyladenine derivatives on 2-octyn-1-yladenosine induced vasodilation, 2-alkynyladenine derivatives were incubated for 10 min prior to the addition of 5-HT.

4.4. Adenosine receptor binding assay[28]

The A_1 AR binding assay was performed in human cloned receptor using [^{3}H]-DPCPX, an A_1 AR antagonist. DPCPX (10^{-5} M) was used to determine non-specific binding. The incubation was done for 60 min at 25 °C. The A_{2A} AR binding assay was performed in human cloned receptor using [^{3}H]-CGS-21680, a relatively selective A_{2A} AR agonist. CGS-21680 (10^{-5} M) was used to determine non-specific binding. The incubation was done for 90 min at 25 °C. The A_{2B} AR binding assay was performed in human cloned receptor using [^{3}H]-DPCPX, an A_1 AR antagonist. A nonselective AR agonist, 5′-(N-ethyl-carboxamide)adenosine (10^{-5} M) was used to determine non-specific binding. The incubation was done for 60 min at 25 °C. 8-Bromo-2-octyn-1-yl-9-propargyladenine (**4a**) was evaluated for their ability to inhibit specific binding at seven concentrations in duplicate in each experiment. The inhibition constant (K_i) was calculated by the Cheng and Prusoff equation using the dissociation constant (K_d) of labeled ligand and the inhibition value (IC_{50}), as described previously.[5,29]

4.5. Activity on haloperidol-induced catalepsy

This test was carried out using 7 animals per group of male ddY mice of 5 week age (22–24 g in body weight, Japan SLC). Haloperidol (Sigma) was suspended in 0.3% CMC and administered intraperitoneally into mice at dose of 1.0 mg/kg. Each test compound was used by suspending them in 0.3% CMC. Also, L-DOPA and benserazide HCl were used by suspending them in 0.3% CMC. One hour after the intraperitoneal injection of haloperidol, the suspension containing each of the test compounds or a suspension which does not contain the test compound (0.3% CMC) was orally administered (0.1 ml per 10 g mouse body weight) and, one hour after the administration of test compound, only forelimbs or only hind limbs of each animal were laid on a stand having a size of 4.5 cm in height and 1.0 cm in width, to measure catalepsy symptoms. All of the test compounds were administrated orally in a dose of 0.3–10 mg/kg.

4.6. Turning behavior in 6-OHDA-lesioned rats

6-OHDA-Lesioned eight-week-old male SD rats were purchased from Japan SLC, Inc. Animals ($n = 5$ for each group, all males) were placed in the center of open chambers (50 cm long × 50 cm wide × 30 cm high) located in a sound-attenuating behavioral test room. Illumination of test room was the same as the rat colony room. The animal's behavior was observed for 300 min. After each test, the field was cleaned with an water. The following behavioral categories were examined: distance of path, time the animal spent moving. Turning Behaviors were automatically analyzed with computer-based video tracking system (Muromachi kikai Co., Ltd, Japan).

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References and notes

1. Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. *Pharmacol. Rev.* **1994**, *46*, 143.
2. (a) Müller, C. E.; Scior, T. *Pharm. Acta Helv.* **1993**, *68*, 77; (b) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. *Curr. Med. Chem.* **1995**, *2*, 707.
3. Jacobson, M. A. *Expert Opin. Ther. Pat.* **2002**, *12*, 489.
4. Svenningsson, P.; Le Moine, C.; Fisone, G.; Fredholm, B. B. *Prog. Neurobiol.* **1999**, *59*, 355.
5. Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
6. (a) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407; (b) Muller, C. E. *Drugs Future* **2000**, *25*, 1043; (c) Schwarzschild, M. A.; Agnati, L.; Fuxe, K.; Chen, J-F.; Morelli, M. *Trends Neurosci.* **2006**, *29*, 647.
7. Cristalli, G.; Cacciari, B.; Ben, D. D.; Lambertucci, C.; Moro, S.; Spalluto, G.; Volpini, R. *Chem. Med. Chem.* **2007**, *2*, 260.
8. Shimada, J.; Koike, N.; Nonaka, H.; Shiozaki, S.; Yanagawa, K.; Kanda, T.; Kobayashi, H.; Ichimura, M.; Nakamura, J.; Kase, H.; Suzuki, F. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2349.
9. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Pineda, M. J.; Zocchi, C.; Dionisotti, S.; Ongini, E. *J. Med. Chem.* **1996**, *39*, 1164.
10. Neustadt, B. R.; Hao, J.; Lindo, N.; Greenlee, W. J.; Stamford, A. W.; Tulshian, D.; Ongini, E.; Hunter, J.; Monopoli, A.; Bertorelli, R.; Foster, C.; Arik, L.; Lachowicz, J.; Ng, K.; Fenga, K.-I. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1376.
11. Keddie, J. R.; Poucher, S. M.; Shaw, G. R.; Brooks, R.; Collis, M. G. *Eur. J. Pharmacol.* **1996**, *301*, 107.
12. (a) Kiselgof, E.; Tulshian, D. B.; Arik, L.; Zhang, H.; Fawzi, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2119; (b) Weiss, S. M.; Benwell, K.; Cliffe, L. A.; Gillespie, R. J.; Knight, A. R.; Lerpiniere, J.; Misra, A.; Pratt, R. M.; Rivell, D.; Uptan, R.; Daurish, C. T. *Neurology* **2003**, *61*, S101; (c) Minetti, P.; Tinti, M. O.; Carminati, P.; Castorina, M.; Di Cesare, M. A.; Di Serio, S.; Gallo, G.; Ghirardi, O.; Giorgi, F.; Giorgi, L.; Piersanti, G.; Bartoccini, F.; Tarzia, G. *J. Med. Chem.* **2005**, *48*, 6887.
13. Baraldi, P. G.; Tabrizi, M. A.; Gessi, S.; Borea, P. A. *Chem. Rev.* **2008**, *108*, 238.
14. Barrett, R. J.; May, J. M.; Martin, P. L.; Miller, J. R. *J. Pharmacol. Exp. Ther.* **1993**, 227.
15. (a) Abiru, T.; Yamaguchi, T.; Watanabe, Y.; Kogi, K.; Aihara, K.; Matsuda, A. *Eur. J. Pharmacol.* **1991**, *196*, 69; (b) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. *J. Med. Chem.* **1992**, *35*, 241; (c) Abiru, T.; Miyashita, T.; Watanabe, Y.; Yamaguchi, T.; Machida, H.; Matsuda, A. *J. Med. Chem.* **1992**, *35*, 2253; (d) Homma, H.; Watanabe, Y.; Abiru, T.; Murayama, T.; Nomura, Y.; Matsuda, A. *J. Med. Chem.* **1992**, *35*, 2881; (e) Konno, T.; Murakami, A.; Uchibori, T.; Nagai, A.; Kogi, K.; Nakahata, N. *J. Pharmacol. Sci.* **2005**, *97*, 501.
16. (a) Camaioni, E.; Costanzi, S.; Vittori, S.; Volpini, R.; Klotz, K.-N.; Cristalli, G. *Bioorg. Med. Chem.* **1998**, *6*, 523; (b) Lambertucci, C.; Vittori, S.; Mishra, R. C.; Ben, D. D.; Klotz, K.-N.; Volpini, R.; Cristalli, G. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 1443.
17. Harada, H.; Asano, O.; Hoshino, Y.; Yoshikawa, S.; Matsukura, M.; Kabasawa, Y.; Niijima, J.; Kotake, Y.; Watanabe, N.; Kawata, T.; Inoue, T.; Horizoe, T.; Yasuda, N.; Minami, H.; Nagata, K.; Murakami, M.; Nagaoka, J.; Kobayashi, S.; Tanaka, I.; Abe, S. *J. Med. Chem.* **2001**, *44*, 170.
18. Harada, H.; Asano, O.; Kawata, T.; Inoue, T.; Horizoe, T.; Yasuda, N.; Nagata, K.; Murakami, M.; Nagaoka, J.; Kobayashi, S.; Tanaka, I.; Abe, S. *Bioorg. Med. Chem.* **2001**, *9*, 2709.
19. Jaakola, V.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y. T.; Lane, J. R.; Ijzerman, A. P.; Stevens, R. C. *Science* **2008**, *322*, 1211.
20. Jacobson, K. A.; Siddiqi, S. M.; Olah, M. E.; Ji, X.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G. L.; Kim, H. O. *J. Med. Chem.* **1995**, *38*, 1720.
21. Hockemeyer, J.; Burbiel, J. C.; Müller, C. E. *J. Org. Chem.* **2004**, *69*, 3308.
22. Kim, S.; Gao, Z.; Rompaey, P. V.; Gross, A. S.; Chen, A.; Calenbergh, S. V.; Jacobson, K. A. *J. Med. Chem.* **2003**, *46*, 4847.
23. Napoli, L.; Montesarchio, D.; Piccialli, G.; Santacroce, C.; Varra, M. *J. Chem. Soc., Perkin Trans. 1* **1995**, 15.

24. Kanda, T.; Shiozaki, S.; Shimada, J.; Suzuki, F.; Nakamura, J. *Eur. J. Pharmacol.* **1994**, *256*, 263.
25. Morelli, M.; Pinna, A. *Drug Dev. Res.* **2001**, *52*, 387.
26. Koga, K.; Kurokawa, M.; Ochi, M.; Nakamura, J.; Kuwana, Y. *Eur. J. Pharmacol.* **2000**, *408*, 249.
27. Abiru, T.; Endo, K.; Machida, H. *Eur. J. Pharmacol.* **1995**, *281*, 9.
28. The binding affinities of **4a** for human AR subtypes were assessed at Sekisui Medical Co., Ltd, Japan.
29. (a) Varani, K.; Gessi, S.; Dalpiaz, A.; Borea, P. A. *Br. J. Pharmacol.* **1996**, *117*, 1693; (b) Brackett, L. E.; Daly, J. W. *Biochem. Pharmacol.* **1994**, *47*, 801.