1,2,4-Triazolo[5,1-*i*]purine Derivatives as Highly Potent and Selective Human Adenosine A₃ Receptor Ligands

Takashi Okamura,*,† Yasuhisa Kurogi,† Hiroshi Nishikawa,† Kinji Hashimoto,† Hiroshi Fujiwara,† and Yoshimitsu Nagao‡

Nutrition Research Institute, Otsuka Pharmaceutical Factory, Inc. Tateiwa, Muya-cho, Naruto, Tokushima 772-8601 Japan, and Faculty of Pharmaceutical Sciences, The University of Tokushima, Sho-machi 1, Tokushima 770-8505 Japan

A series of triazolopurines showed structural similarity to human adenosine A₃ receptor antagonist, 9-chloro-2-(2-furanyl)-5-[(phenylacetyl)amino][1,2,4]triazolo[1,5-*c*]quinazoline (MRS 1220, **1**). In this study, we found novel 1,2,4-triazolo[5,1-*i*]purine derivatives (**2**) showing human adenosine A₃ receptor affinities. The compounds were obtained in two steps from 5-amino-4-cyanoimidazole (**33**). The affinity was determined in radioligand binding assays for the cloned human adenosine A₁, A₂A, A₂B, and A₃ receptors. After the structure−activity relationship was analyzed, we determined that there was a mild parabolic relationship between the length of alkyl groups at the 5-position and the affinities at the A₃ receptor and positive correlation between the length of the substituents on phenyl groups at the 8-position and the affinities at the A₂A receptor. These investigations led to potent and selective human adenosine A₃ receptor ligands. The most potent A₃ receptor ligand (5-*n*-butyl-8-(4-methoxyphenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine (**27**, K_i = 0.18 nM) and the most selective A₃ receptor ligand against A₁, A₂A, and A₂B receptors, (5-*n*-butyl-8-(4-*n*-propoxyphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (**29**, >19 600), were discovered.

Introduction

Adenosine is an important regulator for homeostasis of the brain, heart, kidney, and other organs.[1] Adenosine interacts with four different G-protein-coupled receptors classified as A₁, A₂A, A₂B, and A₃ receptor subtypes.[2] The A₁ and A₃ subtypes inhibit adenylate cyclase (AC) coupling to G_i protein, whereas A₂A and A₂B subtypes stimulate AC via G_s protein. Because the adenosine A₃ receptors were characterized in 1992[3] the physiological roles of the adenosine A₃ receptors have been investigated.

In 1996, Jacobson and co-workers disclosed potent and selective adenosine A₃ receptor antagonists of 1,4-dihydropyridines,[4−6] triazoloquinazolines (e.g., compound **1**),[7,8] and flavonoids.[9] Recently, triazolonaphthyridines,[10] isoquinolines,[11,12] quinazolines,[12] pyrazolotriazolopyrimidines,[13] and thiazoles[14] have been reported as new adenosine A₃ receptor antagonists. Selective adenosine A₃ receptor antagonists are considered as potential antiinflammatory,[15] antiasthmatic,[16] antiischemic,[17] and antiglaucoma agents.[18,19]

A series of 1,2,4-triazolo[5,1-*i*]purine derivatives (**2**) were synthesized as hybrid scaffolds of adenine and 5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5-*a*]pyrimidine (OT-7100, **3**). Compound **3** was synthesized in our laboratory. In experiments using rats, **3** has a unique profile with the effect of normalizing the nociceptive threshold in peripheral neuropathic pain[20] and diabetic neuropathy.[21]
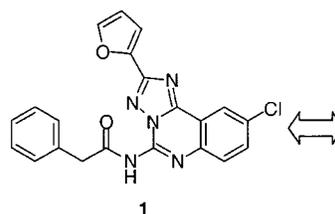
1

rA₁ = 305 nM
rA₂A = 52.0 nM
hA₃ = 0.65 nM

3

2

adenine
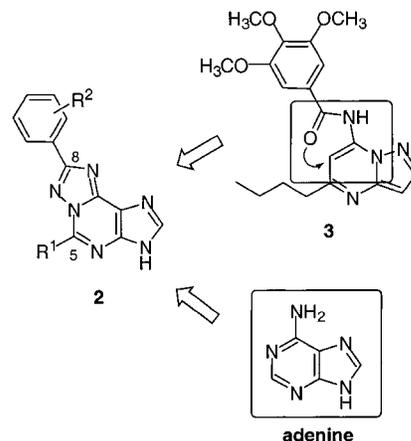
Although 1,2,4-triazolo[5,1-*i*]purines (**2**) did not show analgesic activity in vivo, the structural similarity between **1** and 1,2,4-triazolo[5,1-*i*]purines (**2**) led us to evaluate adenosine A₃ receptor affinities. In this paper, selective adenosine A₃ receptor affinities of novel 1,2,4-triazolo[5,1-*i*]purine derivatives (**2**) are described including structure−activity relationships (SAR).

* To whom correspondence should be addressed. Tel: +81-88-684-2353. Fax: +81-88-686-8176. E-mail: okamurtk@otsukakj.co.jp.
† Otsuka Pharmaceutical Factory, Inc.
‡ The University of Tokushima.

Scheme 1. Synthesis of 1,2,4-Triazolo[5,1-i]purine Derivatives[a]

Method A
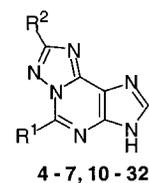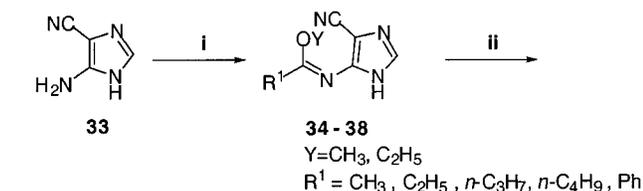
Method B
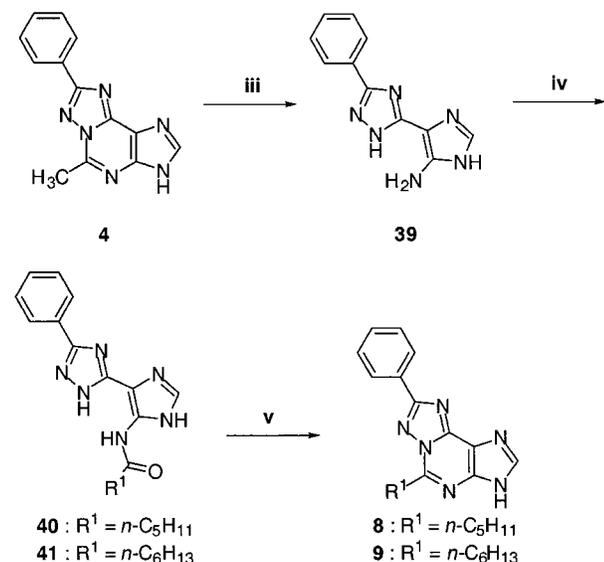
[a] Reagents: (i) $R^1C(OY)_3$, DMF, 80 °C. (ii) $R^2CONHNH_2$, DMF or diglyme, reflux. (iii) 10% HCl, reflux. (iv) R^1COCl, pyridine. (v) TMSCl, Et_3N, THF, reflux.

Chemistry

1,2,4-Triazolo[5,1-i]purines (**2**) were prepared following the synthetic strategy (Scheme 1), which was modified from that of pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidines.[22] 5-Amino-4-cyanoimidazole (**33**) was transformed into imidates **34**−**38** by treatment with commercially available ortho esters at 80 °C in dimethyl formamide (DMF). The desired compounds (**4**−**7** and **10**−**32**) were obtained by refluxing the imidates with the corresponding acylhydrazines in DMF or diglyme (method A).

On the other hand, **8** and **9** with a long alkyl chain at the R^1 position were synthesized from 5-methyl-8-phenyl-3H-[1,2,4]triazolo[5,1-i]purine (**4**) using commercially available n-hexanoyl and n-heptanoyl chloride (method B), because of the unavailability of trialkyl ortho-n-hexanoate or ortho-n-heptanoate. Compound **4** was converted into an aminoimidazole derivative (**39**) by hydrolysis with aqueous 10% HCl. The acylation of **39** with the corresponding acyl chlorides afforded the intermediates **40** and **41**. The cyclization of **40** and **41**

Table 1. Affinities of 1,2,4-Triazolo[5,1-i]purine Derivatives in Radioligand Binding Assays at Human A_{2A} and A_3

compd	R^1	R^2	IC$_{50}$ (nM) or % inhibition[c]		hA$_{2A}$/hA$_3$
			hA$_{2A}$ [a]	hA$_3$ [b]	
4	CH_3	Ph	550	1.0	550
5	C_2H_5	Ph	360	0.45	800
6	n-C_3H_7	Ph	120	0.23	520
7	n-C_4H_9	Ph	71	0.25	280
8	n-C_5H_{11}	Ph	200	0.30	670
9	n-C_6H_{13}	Ph	7800	0.61	13 000
10	Ph	Ph	23	0.41	56
11	n-C_4H_9	CH_3	46	610	0.075
12	n-C_4H_9	$PhCH_2$	47	680	0.069
13	n-C_4H_9	3-pyridyl	900	1.2	750
14	n-C_4H_9	2-furyl	210	5.9	36
15	n-C_4H_9	2-Cl-Ph	18	0.29	62
16	n-C_4H_9	3-Cl-Ph	56	1.1	51
17	n-C_4H_9	4-Cl-Ph	2600	0.41	6300
18	n-C_4H_9	4-F-Ph	510	0.25	2000
19	n-C_4H_9	4-Br-Ph	3300	1.9	1700
20	n-C_4H_9	3-CH_3-Ph	188	0.27	700
21	n-C_4H_9	4-CH_3-Ph	180	0.33	550
22	n-C_4H_9	4-t-C_4H_9-Ph	20%	1.2	>8300
23	n-C_4H_9	4-CF_3-Ph	28%	0.61	>16 000
24	n-C_4H_9	4-Biphenyl	12%	5.0	>2000
25	n-C_4H_9	4-HO-Ph	58	1.8	32
26	n-C_4H_9	3-CH_3O-Ph	67	0.22	300
27	n-C_4H_9	4-CH_3O-Ph	1600	<0.1	>16 000
28	n-C_4H_9	4-C_2H_5O-Ph	3800	0.21	18 000
29	n-C_4H_9	4-n-C_3H_7 O-Ph	9%	0.30	>33 000
30	n-C_4H_9	3,4,5-$(CH_3O)_3$-Ph	2500	1.1	2300
31	n-C_4H_9	4-CH_3S-Ph	42%	3.3	>3000
32	n-C_4H_9	4-$(CH_3)_2$N-Ph	0%	0.67	>15 000

[a] Displacement of specific [^{3}H]CGS 21680 binding at human A_{2A} receptors expressed in HEK-293 cells, in membranes, expressed as IC$_{50}$ in nanomolar ($n = 2$); CV = 4.3%. [b] Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK-293 cells, in membranes, expressed as IC$_{50}$ in nanomolar ($n = 2$); CV = 7.2%. [c] Displacement of specific binding at 10 000 nM concentration.

was performed by treatment with trimethylsilyl chloride to provide the desired compounds **8** and **9**. Yields and characterizations are shown in the Experimental Section.

Results and Discussion

The binding affinities of **4**−**32** at human adenosine A_{2A} and A_3 receptors expressed in HEK-293 cells are shown in Table 1. As initially observed among compounds **4**−**10** bearing an unsubstituted phenyl group at the R^2 position, there was a mild parabolic relationship between the length of the R^1 position and the affinities to the A_3 receptor. Among compounds (**7** and **11**−**14**) with a butyl group at the R^1 position and structurally diverse substituents at the R^2 position, **7** bearing a phenyl group at the R^2 position resulted in the highest A_3 receptor affinity.

Furthermore, compounds **15**−**32** bearing a substituted phenyl group at the R^2 position were evaluated. By analyzing SAR of these compounds, the length of substituents at the para position on phenyl group seems to correlate positively with the binding affinities (IC$_{50}$)

Table 2. Binding Affinities at Adenosine A_1, A_{2A}, A_{2B}, and A_3 Receptors of Compounds **1**, **23**, **27−29**, **32**, **42**, and **43**

| compd | K_i (nM)^a or % inhibition^b | | | | hA_1/hA_3 | hA_{2A}/hA_3 | hA_{2B}/hA_3 |
	hA_1 ^{c}	hA_{2A} ^{d}	hA_{2B} ^{e}	hA_3 ^{f}			
23	4 ± 2%	31 ± 4%	6 ± 11%	0.95 (0.72−1.24)	>10 500	>10 500	>10 500
27	398 (256−620)	892 (811−982)	1030 (772−1390)	0.18 (0.17−0.20)	2210	4960	5720
28	413 (335−509)	572 (438−749)	838 (640−1100)	0.90 (0.58−1.40)	459	636	931
29	32 ± 0%	49 ± 2%	21 ± 3%	0.51 (0.35−0.74)	>19 600	>19 600	>19 600
32	1310 (983−1730)	49 ± 3%	6660 (3580−12 400)	1.25 (0.82−1.91)	1050	>8000	5330
1^h	305 ± 51^g	52.0 ± 8.8^g		0.65 ± 0.25	470^g	80^g	
42^h	1200 (1030−1400)	140 (120−155)	2056 (1640−2580)	0.80 (0.63−1.00)	1500	175	2570
43^h	>10 000	>10 000		18	>556	>556	

^{a} Data are expressed as geometric means, with 95% confidence intervals. ^{b} A percentage of specific binding displaced at 10 000 nM concentration, mean ± SEM ($n = 2−3$). ^{c} Displacement of specific [3H]DPCPX binding at human A_1 receptors expressed in CHO cells, in membranes, expressed as K_i in nanomolar ($n = 3$). ^{d} Displacement of specific [^{3}H]CGS 21680 binding at human A_{2A} receptors expressed in HEK cells, in membranes, expressed as K_i in nanomolar ($n = 3$). ^{e} Displacement of specific [^{3}H]DPCPX binding at human A_{2B} receptors expressed in HEK cells, in membranes, expressed as K_i in nanomolar ($n = 3$). ^{f} Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK cells, in membranes, expressed as K_i in nanomolar ($n = 3$). ^{g} At rat A_1 and A_{2A} receptors. ^{h} Values taken from refs 7, 10, and 22.

at A_{2A} receptors. In fact, a series of unsubstituted (**7**), fluoro (**18**), chloro (**17**), bromo (**19**), and trifluoromethyl (**23**) derivatives and a series of hydroxy (**25**), methoxy (**27**), ethoxy (**28**), and *n*-propoxy (**29**) derivatives showed a positive correlation between the length and the A_{2A} affinity. On the other hand, no significant difference was observed regarding the affinity at A_3 receptors. Electron-donating and -withdrawing substituents on the phenyl group at the R^2 position were also unaffected by the binding affinities at A_{2A} and A_3 receptors.
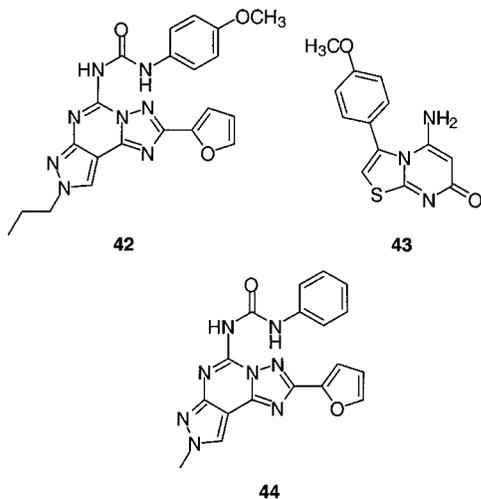
Potent and highly selective compounds (**23**, **27−29**, and **32**) were evaluated for the binding affinity as K_i values at four human adenosine receptor subtypes including A_1 and A_{2B}, as shown in Table 2.

The potent and selective affinities to human adenosine A_3 receptors against A_1, A_{2A}, and A_{2B} receptors were observed. Compound **27** was the most potent A_3 ligand ($K_i = 0.18$ nM), and compound **29** was the most selective A_3 ligand against other subtypes (>19 600) in this series. In comparison to the most selective adenosine A_3 receptor antagonists contained in the literature, compound **29** had potent and selective affinities to human adenosine A_3 receptors. For example, 5-[[(4-methoxy-

phenyl)amino]carbonyl]amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine (MRE3008F20, **42**)^{22} and 5-amino-3-(4-methoxyphenyl)thiazolo[3,2-*a*]pyrimidin-7-one (L268605, **43**)^{10} indicated $hA_3 = 0.80$ nM, $hA_1/hA_3 = 1496$, $hA_{2A}/hA_3 = 175$, $hA_{2B}/hA_3 = 2570$, and $hA_3 = 18$ nM, $hA_1/hA_3 > 556$, $hA_{2A}/hA_3 > 556$, respectively. Very recently, Baraldi et al. reported new human adenosine A_3 receptor antagonists with the good profile (e.g., compound **44**, $hA_3 = 0.16$ nM, $hA_1/hA_3 = 3713$, $hA_{2A}/hA_3 = 2381$, and $hA_{2B}/hA_3 = 1388$).^{23}

Conclusion

Novel 1,2,4-triazolo[5,1-*i*]purine derivatives (**2**) were synthesized by the modified method of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines, which showed high and selective adenosine A_3 receptor affinity. As a result of SAR analysis, the most potent and selective human adenosine A_3 receptor ligands, **27** and **29**, were found. These potent human adenosine A_3 receptor ligands might be useful as pharmacological probes and good therapeutic agents.

Experimental Section

Column chromatography was performed on silica gel 60 (Merck, particle size 63−200 mm). All melting points were determined on a Yamato micromelting point apparatus (MP-21). ^{1}H nuclear magnetic resonance (NMR) spectra were measured on a JEOL GX-270 (270 MHz) and a JEOL JNM-AL400 (400 MHz; compounds **32**, **40**, and **41**) spectrometer, and chemical shifts are indicated in δ units from tetramethylsilane (TMS) as an internal standard. Elemental analyses were performed by the analytical department of Wako Pure Chemical Industries, Ltd. or the University of Tokushima and were within ±0.4% of the calculated values.

Method A. General Procedure for Preparation of Imidates 34−38. A mixture of 5-amino-4-cyano-1*H*-imidazole (**33**, 0.14 mol) and the appropriate ortho ester derivative (0.21 mol) in 30−40 mL of DMF was heated at 90 °C for 0.5−5 h. After the solvent was evaporated, product was recrystallized from ethyl acetate (AcOEt) and hexane.

Methyl *N*-(4-Cyano-1*H*-imidazol-5-yl)acetimidate (34). Yield 93%; colorless powder. ^{1}H NMR (DMSO-d_6): δ 2.04 (3H, s), 3.78 (3H, s), 7.67 (1H, s), 12.5−13.0 (1H, brs).

Ethyl *N*-(4-Cyano-1*H*-imidazol-5-yl)propionimidate (35). Yield 66%; colorless powder. ^{1}H NMR (CDCl$_3$): δ 1.17 (3H, t,

J = 7.9 Hz), 1.34 (3H, t, J = 7.4 Hz), 2.42 (2H, q, J = 7.9 Hz), 4.26 (2H, q, J = 7.4 Hz), 7.49 (1H, s), 10.9–11.7 (1H, brs).

Methyl *N*-(4-Cyano-1*H*-imidazol-5-yl)butyrimidate (36). Yield 79%; colorless powder. ¹H NMR (CDCl₃): δ 0.89 (3H, t, J = 7.2 Hz), 1.5–1.7 (2H, m), 2.40 (2H, t, J = 7.4 Hz), 3.84 (3H, s), 7.50 (1H, s), 10.7–11.3 (1H, brs).

Methyl *N*-(4-Cyano-1*H*-imidazol-5-yl)pentanimidate (37). Yield 96%; colorless powder. ¹H NMR (CDCl₃): δ 0.86 (3H, t, J = 6.9 Hz), 1.2–1.4 (2H, m), 1.5–1.7 (2H, m), 2.43 (2H, t, J = 7.4 Hz), 3.83 (3H, s), 7.46 (1H, s), 10.1–10.5 (1H, brs).

Methyl *N*-(4-Cyano-1*H*-imidazol-5-yl)benzimidate (38). Yield 90%; colorless powder. ¹H NMR (CDCl₃): δ 4.01 (3H, s), 7.2–7.5 (6H, m), 10.2–10.7 (1H, brs).

General Procedure for the Synthesis of 1,2,4-Triazolo-[5,1-*i*]purine Derivatives 4–7 and 10–32. A mixture of the imidate **34–38** (4.85 mmol) and the corresponding acylhydrazine (5.34 mmol) in 10 mL of DMF or 2-methoxyethyl ether (diglyme) was refluxed for 3–24 h. One hundred milliliters of aqueous 50% ethanol was added to the reaction mixture at 80 °C. After it was cooled at room temperature, the precipitate was filtrated and washed with aqueous 50% ethanol. The filtrate was recrystallized from aqueous ethanol or aqueous methanol.

5-Methyl-8-phenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (4). Yield 90%; colorless powder; mp >285 °C. ¹H NMR (DMSO-d_6): δ 3.03 (3H, s), 7.5–7.7 (3H, m), 8.3–8.4 (2H, m), 8.48 (1H, s), 13.6–14.1 (1H, brs). Anal. (C₁₃H₁₀N₆) C, H, N.

5-Ethyl-8-phenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (5). Yield 87%; colorless powder; mp 253–255 °C. ¹H NMR (DMSO-d_6): δ 1.46 (3H, t, J = 7.4 Hz), 3.39 (2H, q, J = 7.4 Hz), 7.5–7.7 (3H, m), 8.2–8.3 (2H, m), 8.43 (1H, s), 13.6–14.0 (1H, brs). Anal. (C₁₄H₁₂N₆·0.75H₂O) C, H, N.

8-Phenyl-5-*n*-propyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (6). Yield 88%; colorless powder; mp 230–233 °C. ¹H NMR (DMSO-d_6): δ 1.07 (3H, t, J = 7.4 Hz), 1.9–2.1 (2H, m), 3.33 (2H, t, J = 7.7 Hz), 7.5–7.7 (3H, m), 8.2–8.3 (2H, m), 8.43 (1H, s), 13.6–14.1 (1H, brs). Anal. (C₁₅H₁₄N₆·H₂O) C, H, N.

5-*n*-Butyl-8-phenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (7). Yield 92%; colorless powder; mp 238–240 °C. ¹H NMR (DMSO-d_6): δ 1.05 (3H, t, J = 7.4 Hz), 1.5–1.6 (2H, m), 1.9–2.1 (2H, m), 3.43 (2H, t, J = 7.4 Hz), 7.6–7.7 (3H, m), 8.3–8.4 (2H, m), 8.50 (1H, s), 13.6–14.2 (1H, brs). Anal. (C₁₆H₁₆N₆·1.2H₂O) C, H, N.

5,8-Diphenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (10). Yield 46%; colorless powder; mp 268–269 °C. ¹H NMR (DMSO-d_6): d. 7.5–7.6 (3H, m), 7.6–7.7 (3H, m), 8.2–8.3 (2H, m), 8.3–8.4 (2H, m), 8.54 (1H, s), 13.7–14.2 (1H, brs). Anal. (C₁₈H₁₂N₆·0.25H₂O) C, H, N.

5-*n*-Butyl-8-methyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (11). Yield 45%; colorless powder; mp 252–254 °C. ¹H NMR (DMSO-d_6): δ 0.95 (3H, t, J = 7.2 Hz), 1.3–1.5 (2H, m), 1.8–2.0 (2H, m), 2.54 (3H, s), 3.25 (2H, t, J = 7.9 Hz), 8.38 (1H, s). Anal. (C₁₁H₁₄N₆) C, H, N.

8-Benzyl-5-*n*-butyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (12). Yield 80%; colorless prisms; mp 197–200 °C. ¹H NMR (DMSO-d_6): δ 0.95 (3H, t, J = 7.2 Hz), 1.3–1.5 (2H, m), 1.8–2.0 (2H, m), 3.28 (2H, t, J = 7.9 Hz), 4.25 (2H, s), 7.2–7.5 (5H, m), 8.38 (1H, s). Anal. (C₁₇H₁₈N₆) C, H, N.

5-*n*-Butyl-8-(3-pyridyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (13). Yield 38%; colorless powder; mp >220 °C (dec). ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.4 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 3.38 (2H, t, J = 7.4 Hz), 7.62 (1H, dd, J = 5.0, 7.9 Hz), 8.44 (1H, s), 8.59 (1H, d, J = 7.9 Hz), 8.75 (1H, d, J = 5.0 Hz), 9.42 (1H, s). Anal. (C₁₅H₁₅N₇) C, H, N; C: calcd, 61.42; found, 52.29. N: calcd, 33.43; found, 28.30.

5-*n*-Butyl-8-(2-furyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (14). Yield 64%; colorless powder; mp 255–256 °C. ¹H NMR (DMSO-d_6): δ 0.97 (3H, t, J = 7.4 Hz), 1.4–1.5 (2H, m), 1.8–2.0 (2H, m), 3.33 (2H, t, J = 7.4 Hz), 6.75 (1H, dd, J = 2.0, 3.5 Hz), 7.29 (1H, d, J = 3.5 Hz), 7.97 (1H, d, J = 2.0 Hz), 8.43 (1H, s), 13.6–14.0 (1H, brs). Anal. (C₁₄H₁₄N₆O) C, H, N.

5-*n*-Butyl-8-(2-chlorophenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (15). Yield 80%; colorless powder; mp 177–179 °C. ¹H NMR (DMSO-d_6): δ 0.96 (3H, t, J = 7.4 Hz), 1.4–1.5 (2H, m), 1.9–2.0 (2H, m), 3.36 (2H, t, J = 7.5 Hz), 7.5–7.7 (3H, m), 8.0–8.2 (1H, m), 8.47 (1H, s). Anal. (C₁₆H₁₅N₆Cl·0.5H₂O) C, H, N.

5-*n*-Butyl-8-(3-chlorophenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (16). Yield 80%; colorless powder; mp 218–221 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 3.34 (2H, t, J = 7.7 Hz), 7.6–7.7 (2H, m), 8.2–8.3 (2H, m), 8.43 (1H, s). Anal. (C₁₆H₁₅N₆Cl) C, H, N.

5-*n*-Butyl-8-(4-chlorophenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (17). Yield 88%; colorless powder; mp 273–275 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 6.9 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 3.35 (2H, t, J = 7.4 Hz), 7.64 (2H, d, J = 8.4 Hz), 8.27 (2H, d, J = 8.4 Hz), 8.43 (1H, s), 13.6–14.0 (1H, brs). Anal. (C₁₆H₁₅N₆Cl) C, H, N.

5-*n*-Butyl-8-(4-fluorophenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (18). Yield 85%; colorless powder; mp 259–260 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.4 Hz), 1.4–1.6 (2H, m), 1.9–2.0 (2H, m), 3.36 (2H, t, J = 7.7 Hz), 7.41 (2H, t, J = 8.9 Hz), 8.31 (2H, dd, J = 6.4, 8.9 Hz), 8.43 (1H, s). Anal. (C₁₆H₁₅N₆F) C, H, N.

8-(4-Bromophenyl)-5-*n*-butyl-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (19). Yield 81%; colorless needles; mp 280–282 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.4 Hz), 1.4–1.6 (2H, m), 1.9–2.0 (2H, m), 3.35 (2H, t, J = 7.4 Hz), 7.78 (2H, d, J = 8.4 Hz), 8.21 (2H, d, J = 8.4 Hz), 8.43 (1H, s). Anal. (C₁₆H₁₅N₆Br) C, H, N.

5-*n*-Butyl-8-(3-tolyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (20). Yield 70%; colorless powder; mp 201–204 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 2.43 (3H, s), 3.34 (2H, t, J = 7.7 Hz), 7.35 (1H, d, J = 7.7 Hz), 7.45 (1H, t, J = 7.7 Hz), 8.07 (1H, d, J = 7.7 Hz), 8.08 (1H, s), 8.42 (1H, s), 13.5–14.0 (1H, brs). Anal. (C₁₇H₁₈N₆·H₂O) C, H, N.

5-*n*-Butyl-8-(4-tolyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (21). Yield 74%; colorless powder; mp 254–255 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.4 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 2.40 (3H, s), 3.35 (2H, t, J = 7.4 Hz), 7.38 (2H, d, J = 7.9 Hz), 8.16 (2H, d, J = 7.9 Hz), 8.42 (1H, s), 13.5–14.0 (1H, brs). Anal. (C₁₇H₁₈N₆) C, H, N.

5-*n*-Butyl-8-(4-*t*-butylphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (22). Yield 67%; colorless powder; mp 242–244 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.35 (9H, s), 1.4–1.5 (2H, m), 1.9–2.0 (2H, m), 3.36 (2H, t, J = 7.7 Hz), 7.60 (2H, d, J = 8.2 Hz), 8.21 (2H, d, J = 8.2 Hz), 8.42 (1H, s). Anal. (C₂₀H₂₄N₆) C, H, N.

5-*n*-Butyl-8-(4-trifluoromethylphenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine (23). Yield 69%; colorless powder; mp 278–280 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 3.33 (2H, t, J = 7.7 Hz), 7.92 (2H, d, J = 7.9 Hz), 8.43 (1H, s), 8.44 (2H, d, J = 7.9 Hz). Anal. (C₁₇H₁₅N₆F₃) C, H, N.

8-(Biphenyl-4-yl)-5-*n*-butyl-3*H*-[1,2,4]triazolo[5,1-*i*]pu-rine (24). Yield 79%; colorless powder; mp 244–246 °C. ¹H NMR (DMSO-d_6): δ 0.99 (3H, t, J = 7.2 Hz), 1.4–1.6 (2H, m), 1.8–2.0 (2H, m), 3.38 (2H, t, J = 7.7 Hz), 7.3–7.6 (3H,m), 7.78 (2H, d, J = 8.2 Hz), 7.89 (2H, d, J = 7.7 Hz), 8.36 (2H, d, J = 7.7 Hz), 8.43 (1H, s). Anal. (C₂₂H₂₀N₆) C, H, N.

5-*n*-Butyl-8-(4-hydroxyphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (25). Yield 87%; colorless powder; mp >285 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.4 Hz), 1.4–1.6 (2H, m), 1.9–2.0 (2H, m), 3.34 (2H, t, J = 6.9 Hz), 6.94 (2H, d, J = 8.9 Hz), 8.11 (2H, d, J = 8.9 Hz), 8.40 (1H, s), 9.7–10.2 (1H, brs), 13.3–14.2 (1H, brs). Anal. (C₁₆H₁₆N₆O·1.8H₂O) C, H, N.

5-*n*-Butyl-8-(3-methoxyphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (26). Yield 74%; colorless prisms; mp 183–185 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.4–1.6 (2H, m), 1.9–2.0 (2H, m), 3.36 (2H, t, J = 7.9 Hz), 3.88 (3H, s), 7.12 (1H, d, J = 8.4 Hz), 7.49 (1H, dd, J = 7.7, 8.4 Hz), 7.79 (1H, s), 7.86 (1H, d, J = 7.7 Hz), 8.43 (1H, s), 13.6–14.0 (1H, brs). Anal. (C₁₇H₁₈N₆O) C, H, N.

5-*n*-Butyl-8-(4-methoxyphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (27). Yield 70%; colorless powder; mp 237–240 °C. ¹H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.4 Hz), 1.4–1.6 (2H,

m), 1.8−2.0 (2H, m), 3.34 (2H, t, J = 7.4 Hz), 3.86 (3H, s), 7.11 (2H, d, J = 8.9 Hz), 8.20 (2H, d, J = 8.9 Hz), 8.41 (1H, s). Anal. ($C_{17}H_{18}N_6O$) C, H, N.

5-*n*-Butyl-8-(4-ethoxyphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]-purine (28). Yield 71%; colorless powder; mp 241−243 °C. ^{1}H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.38 (3H, t, J = 6.9 Hz), 1.4−1.6 (2H, m), 1.9−2.0 (2H, m), 3.34 (2H, t, J = 7.4 Hz), 4.12 (2H, q, J = 6.9 Hz), 7.09 (2H, d, J = 8.7 Hz), 8.19 (2H, d, J = 8.7 Hz), 8.41 (1H, s). Anal. ($C_{18}H_{20}N_6O$) C, H, N.

5-*n*-Butyl-8-(4-*n*-propoxyphenyl)-3*H*-[1,2,4]triazolo[5,1-*i*]purine (29). Yield 87%; colorless powder; mp 233−235 °C. ^{1}H NMR (DMSO-d_6): δ 0.9−1.1 (6H, m), 1.4−1.6 (2H, m), 1.7−1.8 (2H, m), 1.9−2.0 (2H, m), 3.34 (2H, t, J = 7.7 Hz), 4.02 (2H, t, J = 6.4 Hz), 7.11 (2H, d, J = 8.4 Hz), 8.19 (2H, d, J = 8.4 Hz), 8.41 (1H, s). Anal. ($C_{19}H_{22}N_6O$) C, H, N.

5-*n*-Butyl-8-(3,4,5-trimethoxyphenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine (30). Yield 82%; colorless powder; mp 191−193 °C. ^{1}H NMR (DMSO-d_6): δ 0.99 (3H, t, J = 7.2 Hz), 1.4−1.6 (2H, m), 1.8−2.0 (2H, m), 3.33 (2H, t, J = 7.7 Hz), 3.78 (3H, s), 3.92 (6H, s), 7.52 (2H, s), 8.41 (1H, s), 13.5−14.1 (1H, brs). Anal. ($C_{19}H_{22}N_6O_3\cdot H_2O$) C, H, N.

5-*n*-Butyl-8-(4-methylthiophenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine (31). Yield 56%; colorless powder; mp 264−265 °C. ^{1}H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 1.4−1.6 (2H, m), 1.9−2.0 (2H, m), 2.56 (3H, s), 3.35 (2H, t, J = 7.4 Hz), 7.44 (2H, d, J = 8.7 Hz), 8.19 (2H, d, J = 8.7 Hz), 8.41 (1H, s). Anal. ($C_{17}H_{18}N_6S$) C, H, N.

5-*n*-Butyl-8-(4-dimethylaminophenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine (32). Yield 79%; colorless powder; mp 236−240 °C. ^{1}H NMR (DMSO-d_6): δ 0.98 (3H, t, J = 7.5 Hz), 1.4−1.5 (2H, m), 1.9−2.0 (2H, m), 3.01 (6H, s), 3.33 (2H, t, J = 7.9 Hz), 6.84 (2H, d, J = 8.7 Hz), 8.08 (2H, d, J = 8.7 Hz), 8.38 (1H, s), 13.4−14.2 (1H, brs). Anal. ($C_{18}H_{21}N_7$) C, H, N.

Method B. 5-Amino-4-(3-phenyl-1*H*-1,2,4-triazol-5-yl)-1*H*-imidazole (39). Compound **4** (1.6 g, 6.4 mmol) in 16 mL of 10% hydrochloric acid was heated at reflux temperature for 1 h. After it was cooled, the mixture was carefully basified with 25% aqueous ammonia solution. The resulting precipitate was collected by filtration and then recrystallized with aqueous ethanol. Yield 1.2 g (83%); colorless powder. ^{1}H NMR (DMSO-d_6): δ 5.4−5.8 (2H, brs), 7.2−7.6 (4H, m), 8.06 (2H, d, J = 7.9 Hz), 11.4−11.8 (1H, brs), 13.5−14.0 (1H, brs).

General Procedure for the Synthesis of 5-(*N*-Alkanoyl-amino)-4-(3-phenyl-1*H*-1,2,4-triazol-5-yl)-1*H*-imidazole 40 and 41. *n*-Hexanoyl or *n*-heptanoyl chloride (30.9 mmol) was added dropwise to a suspension of **39** (8.8 mmol) in 20 mL of pyridine at 0 °C. The reaction mixture was stirred for 0.5−1 h at 0 °C and then for 16 h at room temperature. Followed by the addition of methanol (20−40 mL), the mixture was refluxed for 1 h. After it was cooled, a precipitate was collected and washed with methanol.

5-(*N*-*n*-Hexanoylamino)-4-(3-phenyl-1*H*-1,2,4-triazol-5-yl)-1*H*-imidazole (40). Yield 66%; colorless powder. ^{1}H NMR (DMSO-d_6): δ 0.8−1.0 (3H, brs), 1.2−1.4 (4H, brs), 1.6−1.8 (2H, brs), 2.3−2.6 (2H, brs), 7.4−7.6 (4H, m), 8.0−8.2 (2H, m), 9.8−10.2 (1H, brs), 12.4−13.0 (1H, brs), 14.0−14.4 (1H, brs).

5-(*N*-*n*-Heptanoylamino)-4-(3-phenyl-1*H*-1,2,4-triazol-5-yl)-1*H*-imidazole (41). Yield 67%; colorless powder. ^{1}H NMR (DMSO-d_6): δ 0.8−1.0 (3H, brs), 1.2−1.4 (4H, brs), 1.3−1.5 (2H, brs), 1.6−1.8 (2H, brs), 2.3−2.6 (2H, brs), 7.4−7.6 (4H, m), 8.0−8.2 (2H, m), 9.8−10.3 (1H, brs), 12.4−13.0 (1H, brs), 13.9−14.5 (1H, brs).

General Procedure for the Synthesis of 5-Alkyl-8-phenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine 8 and 9. Chlorotrimethylsilane (4.9 mmol) was added dropwise to a suspension of the *N*-acylated aminoimidazole **40** or **41** (1.2 mmol) in 8 mL of tetrahydrofurane (THF) and triethylamine (9.9 mmol). The reaction mixture was refluxed for 24−72 h. After it was cooled, the reaction was quenched by adding cold water. The crude product was extracted with AcOEt and dried with sodium sulfate. The solvent was evaporated, and the residue was purified by column chromatography using CHCl$_3$/CH$_3$OH (25:1) as the eluent. Recrystallization from aqueous methanol gave the corresponding product **8** or **9** as a colorless powder.

5-*n*-Pentyl-8-phenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (8). Yield 40%; colorless powder; mp 219−220 °C. ^{1}H NMR (DMSO-d_6): δ 0.91 (3H, t, J = 6.9 Hz), 1.3−1.5 (4H, m), 1.9−2.0 (2H, m), 3.36 (2H, t, J = 7.4 Hz), 7.5−7.6 (3H, m), 8.2−8.3 (2H, m), 8.43 (1H, s). Anal. ($C_{16}H_{16}N_6\cdot 1.2H_2O$) C, H, N.

5-*n*-Hexyl-8-phenyl-3*H*-[1,2,4]triazolo[5,1-*i*]purine (9). Yield 65%; colorless powder; mp 205−209 °C. ^{1}H NMR (DMSO-d_6): δ 0.88 (3H, t, J = 7.2 Hz), 1.2−1.6 (6H, m), 1.9−2.0 (2H, m), 3.36 (2H, t, J = 7.7 Hz), 7.4−7.7 (3H, m), 8.2−8.3 (2H, m), 8.43 (1H, s), 13.6−14.1 (1H, brs). Anal. ($C_{17}H_{18}N_6$) C, H, N.

Human Cloned Adenosine A$_1$, A$_{2A}$, A$_{2B}$, and A$_3$ Receptor Binding Assay. Binding of [^{3}H]DPCPX to Chinese hamster ovary cells transfected with the human recombinant A$_1$ adenosine receptor was performed as previously described.[24] Displacement experiments were performed for 60 min at 22 °C in 0.25 mL of 50 mM Tris-HCl buffer, 5 mM MgCl$_2$, 1 mM EDTA at pH 7.4, 2 units/mL adenosine deaminase containing 1 nM [^{3}H]DPCPX, diluted membranes (20 μg of protein/assay), and eight different concentrations of examined compounds. Nonspecific binding was determined in the presence of 1 μM DPCPX.

Binding of [^{3}H]CGS 21680 to HEK-293 cells transfected with the human recombinant A$_{2A}$ adenosine receptor was performed as previously described.[25] Displacement experiments were performed for 90 min at 22 °C in 0.25 mL of 50 mM Tris-HCl buffer, 10 mM MgCl$_2$ at pH 7.4, 2 units/mL adenosine deaminase containing 6 nM [^{3}H]CGS 21680, diluted membranes (50 μg of protein/assay), and at least 3−8 different concentrations of examined compounds. Nonspecific binding was determined in the presence of 10 μM NECA.

Binding of [^{3}H]DPCPX to HEK-293 cells transfected with the human recombinant A$_{2B}$ adenosine receptor was performed as previously described.[26] Displacement experiments were performed for 120 min at 22 °C in 0.50 mL of 10 mM Hepes-Tris buffer, 1 mM MgCl$_2$, 1 mM EDTA at pH 7.4, containing 5 nM [^{3}H]DPCPX, diluted membranes (500 μg of protein/assay), and eight different concentrations of examined compounds. Nonspecific binding was determined in the presence of 100 μM NECA.

Binding of [^{125}I]AB-MECA to HEK-293 cells transfected with the human recombinant A$_3$ adenosine receptor was performed as previously described.[27] Displacement experiments were performed for 90 min at 22 °C in 0.25 mL of 50 mM Tris-HCl buffer, 5 mM MgCl$_2$, 1 mM EDTA at pH 7.4, 2 units/mL adenosine deaminase containing 0.1 nM [^{125}I]AB-MECA, diluted membranes (20 μg of protein/assay), and at least 3−8 different concentrations of examined compounds. Nonspecific binding was determined in the presence of 1 μM IB-MECA.

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