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Structure–activity relationships of adenosine A_3 receptor ligands: new potential therapy for the treatment of glaucoma

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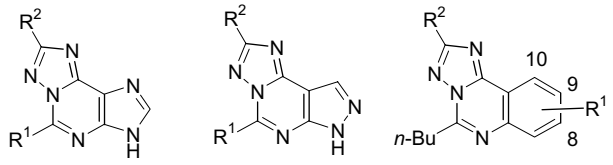
Abstract—Structure–activity relationships (SAR) of fused 1,2,4-triazolo[1,5-*c*]pyrimidine were performed. Various substituents were introduced into the heterocyclic ring to improve the potency of adenosine A_3 receptor binding affinity and A_3-selectivity against other subtypes. Potent and selective A_3 receptor antagonists were identified and were evaluated in a monkey model of intraocular pressure by eye-drop administration. As a result, compound **1c** (OT-7999) was found to significantly decrease intraocular pressure in the animal model.

Glaucoma, characterized by elevated intraocular pressure (IOP), is a leading cause of irreversible blindness in the world.[1] Patients with glaucoma may require long-term administration of IOP-lowering medications. These medications belong to several classes of molecules including beta-adrenergic blockers, cholinergic agents, alpha-adrenergic agonists, carbonic anhydrase inhibitors, and ocular hypotensive lipids. Most adverse effects associated with IOP-lowering medications are mild and ocular in nature; however, several of them are associated with systemic risks as well as serious ocular effects, especially following chronic use.[2] Civan and co-workers found that the A_3 adenosine receptors regulate Cl^- channels of nonpigmented ciliary epithelial cells.[3] In comparison of A_3 receptor knockout mice (A_3R −/−) with control mice (A_3R +/+), IOP was significantly lower in A_3R knockout mice than in normal mice.[4] In addition, the selective A_3 antagonists (MRS 1191, MRS 1097, and MRS 1523), which were identified by Jacobson and co-workers,[5] lowered IOP in the mouse.[6] These results suggest that reducing Cl^--channel activity with A_3 antagonists may provide a novel approach for treating glaucoma.[7]

We have described a discovery of lead compounds, 1,2,4-triazolo[5,1-*i*]purine derivatives **1**, as a novel series of human adenosine A_3 ligands.[8] Binding assays using human adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) of this series yielded highly potent and selective A_3 ligands versus hA_1, hA_{2A}, and hA_{2B} receptor subtypes. A facile synthetic method of fused 1,2,4-triazolo[1,5-*c*]pyrimidine was recently developed and applied to find new hA_3 ligands, such as pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine **2** and 1,2,4-triazolo[1,5-*c*]quinazoline **3** scaffolds.[9] These new compounds also showed excellent and selective affinities to hA_3 receptor.



1 **2** **3**

Based on these studies, new 1,2,4-triazolo[5,1-*i*]purines **1**, pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines **2**, and 1,2,4-triazolo[1,5-*c*]quinazolines **3** were prepared in

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order to investigate SAR of adenosine A_3 affinities. The procedure to prepare these analogs and the radioligand binding assays for human adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors have been previously described.[8,9] Binding affinities to human adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors of **1**, **2**, and **3** are summarized in Tables 1–3, respectively.

All synthesized compounds showed potent affinities to human adenosine A_3 receptors except **3s**. The alkyl chain of 1,2,4-triazolo[5,1-*i*]purines **1** was newly modified to ether and carboxylic acid derivatives (**1e**–**i**) in order to increase the water solubility. These compounds maintained potent and selective affinities to human adenosine A_3 receptors versus A_{2A} receptors (Table 1). Especially, compounds **1c**, **1d**, **1e**, **1g**, and **1h** showed excellent hA_3 selectivity versus the other adenosine receptor subtypes. These modifications were successful at increasing the water solubility. For example, the poor water solubility of **1d** (3 nM) was improved to 6 nM for **1e** and 2300 nM for **1g**.

The substitution at R^2 moiety affected both potency and selectivity of hA_3 affinity versus the other adenosine subtypes, similar to the previous SAR of 1,2,4-triazolo[5,1-*i*]purine derivatives **1**.[8] The hA_3 selectivities versus hA_2 receptors of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines **2** were better when substituted at the 4 position of the phenyl ring than at the 2 or 3 positions, or with no substitution (Table 2).

Binding affinities of 1,2,4-triazolo[1,5-*c*]quinazolines **3** changed in accordance with the substitution on the quinazoline ring (Table 3). Introduction of chlorine atom to the 8 or 9 position on 1,2,4-triazolo[1,5-*c*]-quinazoline (**3l**,**o**) enhanced the binding affinities to hA_3 receptors. However, 10-chloro-substituted compound (**3s**) reduced the potency of hA_3 binding affinity. Affinity

Table 1. Binding affinities of 1,2,4-triazolo[5,1-*i*]purines **1** in radioligand binding assays at human A_1, A_{2A}, A_{2B}, and A_3 receptors

Compd	R^1	R^2	IC_{50}; nM			
			hA_1[a]	hA_{2A}[b]	hA_{2B}[c]	hA_3[d]
1a	*n*-C_4H_9	Ph		71		0.25
1b	*n*-C_4H_9	4-Cl–Ph		2600		0.41
1c	*n*-C_4H_9	4-CF_3–Ph	>10,000	>10,000	>10,000	0.61
1d	*n*-C_4H_9	4-Biphenyl	>10,000	>10,000	>10,000	5.0
1e	$CH_3OC_2H_4$	4-Biphenyl	>10,000	>10,000	>10,000	0.9
1f	$HO_2CC_2H_4$	4-Biphenyl		>10,000		15
1g	$HO_2CC_3H_6$	4-Biphenyl	>10,000	>10,000	>10,000	8.7
1h	$HO_2CC_3H_6$	4-Cl–Ph	>10,000	>10,000	>10,000	5.4
1i	$HO_2CC_4H_8$	4-Biphenyl		>10,000		9.1

[a] Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells, in membranes, expressed as IC_{50} in nanomolar ($n = 2$).
[b] 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 ($n = 2$).
[c] Displacement of specific [^{3}H]DPCPX binding at human A_{2B} receptors expressed in HEK-293 cells, in membranes, expressed as IC_{50} in nanomolar ($n = 2$).
[d] 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 ($n = 2$).

Table 2. Binding affinities of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines **2** in radioligand binding assays at human A_1, A_{2A}, A_{2B}, and A_3 receptors

Compd	R^1	R^2	IC_{50}; nM			
			hA_1[a]	hA_{2A}[a]	hA_{2B}[a]	hA_{3a}[a]
2a	C_2H_5	Ph	27	310	<100	6.2
2b	*n*-C_3H_7	Ph	38	120	1500	4.1
2c	*n*-C_4H_9	Ph	27	190	2600	2.1
2d	*n*-C_4H_9	2-Cl–Ph		130		<10
2e	*n*-C_4H_9	3-Cl–Ph		760		<10
2f	*n*-C_4H_9	4-Cl–Ph	1200	>10,000	2700	<4.9
2g	*n*-C_4H_9	4-F–Ph	610	>10,000	9400	1.9
2h	*n*-C_4H_9	4-Br–Ph		>10,000		19
2i	*n*-C_4H_9	4-CH_3–Ph	3000	>10,000	>10,000	4.0
2j	*n*-C_4H_9	4-C_2H_5–Ph		>10,000		12
2k	*n*-C_4H_9	4-*n*-C_3H_7–Ph		>10,000		42
2l	*n*-C_4H_9	4-*t*-C_4H_9–Ph		>10,000		150
2m	*n*-C_4H_9	4-CF_3–Ph		>10,000		130
2n	*n*-C_4H_9	4-Biphenyl		>10,000		450
2o	*n*-C_4H_9	4-CH_3O–Ph		2600		<10
2p	*n*-C_4H_9	4-C_2H_5O–Ph		2700		<10

[a] See the footnote in Table 1.

Table 3. Binding affinities of 1,2,4-triazolo[1,5-*c*]quinazolines **3** in radioligand binding assays at human A_1, A_{2A}, A_{2B}, and A_3 receptors

Compd	R^1	R^2	IC_{50}; nM			
			hA_1[a]	hA_{2A}[a]	hA_{2B}[a]	hA_3[a]
3a	H	Ph	2600	>10,000	>10,000	260
3b	H	2-Furyl		650		120
3c	H	4-Cl–Ph	>10,000	>10,000	>10,000	26
3d	H	4-F–Ph		>10,000		28
3e	H	4-Br–Ph	>10,000	>10,000	>10,000	8.2
3f	H	4-CH_3–Ph		>10,000		34
3g	H	4-CF_3–Ph		>10,000		490
3h	H	4-Biphenyl		>10,000		140
3i	H	4-HO–Ph		5300		10
3j	H	4-CH_3O–Ph		>10,000		160
3k	H	4-C_2H_5O–Ph		>10,000		35
3l	8-Cl	Ph	>10,000	>10,000	>10,000	48
3m	8-Cl	4-Cl–Ph		>10,000		320
3n	8-Cl	4-CH_3O–Ph		>10,000		34
3o	9-Cl	Ph	6200	>10,000	>10,000	45
3p	9-Cl	4-Cl–Ph	>10,000	>10,000	>10,000	28
3q	9-Cl	4-Br–Ph		>10,000		22
3r	9-Cl	4-CH_3O–Ph		>10,000		33
3s	10-Cl	Ph		>10,000		>1000
3t	8-CH_3	Ph		>10,000		110

[a] See the footnote in Table 1.

of 8-methylation of 1,2,4-triazolo[1,5-*c*]-quinazoline (**3t**) to hA_3 receptors remained the same. These results allow us to hypothesize that the substitution of the electron withdrawing group (e.g., chloro) on the quinazoline ring of **3** is favorable for interaction with the human adenosine A_3 receptors, whereas the substitution at the 10 position of the quinazoline ring is unfavorable due to the hindrance of hydrogen bond interaction with the 4-imino nitrogen of the triazole ring. Further investigation for 1,2,4-triazolo[1,5-*c*]-quinazolines might be necessary to determine possible binding mechanisms.

Based on the results of the in vitro assays, we chose three hA_3 antagonists (**1c**,**g**,**h**) to evaluate in a monkey model of intraocular pressure by eye-drop administration. The pioneering study of Civan clarified that A_3 selective antagonists modulate IOP in mammalian eye, extending in vitro observations implicating A_3 receptors in tissues controlling aqueous humour physiology, and may be a novel approach for the treatment of glaucoma.[7] On the other hand, we have to be aware of major species differences of adenosine A_3 receptors (Fig. 1). MRS 1191 and MRS 1523, which showed moderate affinity to rat A_3 receptors ($K_i = 1.42$ and 0.113 μM, respectively), were used for the mouse IOP study.[6,10] However, 1,2,4-triazolo[5,1-*i*]purine **1** showed high selectivity to human A_3 versus rat A_3 receptors. For example, **1c** was >16,000-fold selective for human A_3 versus rat A_3 receptors. Therefore, we determined that the monkey model was most suitable to evaluate the IOP effects of our compounds.

The compounds (**1c**, **1g**, or **1h**) were applied topically to one eye in monkeys with normal IOP, and changes in IOP at various time points were determined without anesthesia using an Alcon applanation pneumatonograph.[11]

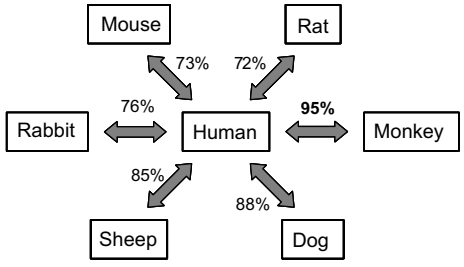

Figure 1. Homology of A_3 receptors between species. The amino acid sequences are cited from GenBank. Accession numbers are NM000677 (human), AAG35152 (monkey; partial sequence, 107AA), U54792 (sheep), O02667 (rabbit), Q61618 (mouse), and NM012896 (rat).

The obtained data were summarized and analyzed using the actual measurement values of IOP and relative values from the initial value as described in Figures 2 and 3.

The results of the actual measurements of IOP indicated that both **1c** and **1g** lowered IOP significantly whereas **1h** was ineffective (Fig. 2). Moreover, the analysis of the relative IOP values showed that **1c** reduced ΔIOP significantly whereas statistical significances of **1g** decreased. Although we expected higher efficacy of the water soluble **1g** and **1h** in the monkey, the experimental results were the exact opposite. We hypothesized that the reason may be the poor membrane permeability of carboxylic acid analogs.[12] Since it was difficult to measure the permeability of the lens and its periphery in the monkey, we could not demonstrate the validity of this hypothesis.

Finally, we found that **1c** (OT-7999) was the most potent hA_3 ($IC_{50} = 0.61$ nM) and selective hA_3 ligand versus hA_1, hA_{2A}, and A_{2B} receptors (>16,000-fold) in the

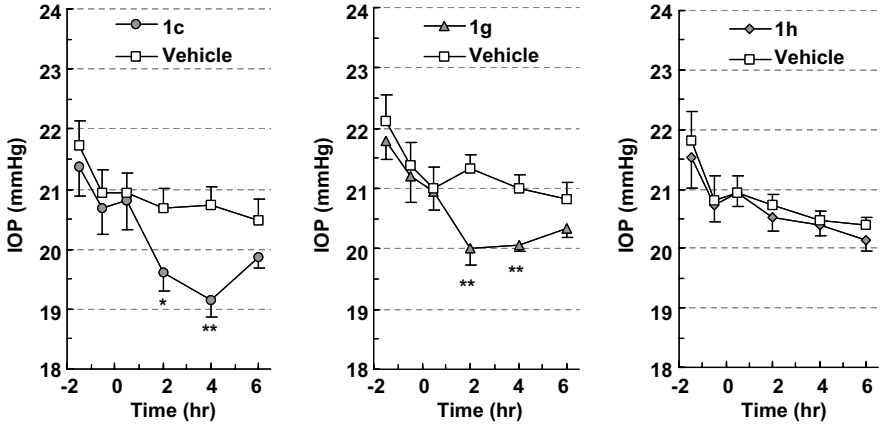

Figure 2. Effects of **1c**, **1g**, and **1h** on monkey IOP ($n = 5$). *: $P<0.05$, **: $P<0.01$, significantly different from vehicle group (Student's *t*-test).

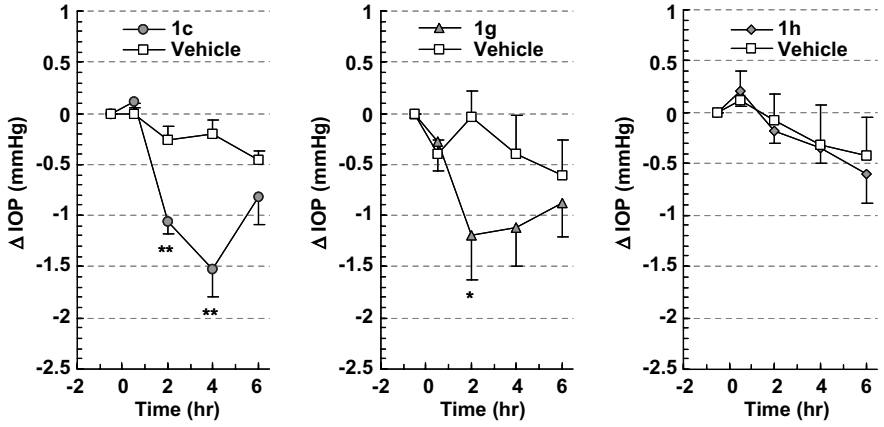

Figure 3. Changes (ΔIOP) of **1c**, **1g**, and **1h** from the initial value. *: $P<0.05$, **: $P<0.01$, significantly different from vehicle group (Student's *t*-test).

series of 1,2,4-triazolo[5,1-*i*]purines, pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidines, and 1,2,4-triazolo-[1,5-*c*]quinazolines. Compound **1c** significantly reduced IOP compared with the control eye (2 h: control = 20.7 ± 0.3 mmHg, **1c** = 19.6 ± 0.3 mmHg; 4 h: control = 20.7 ± 0.3 mmHg, **1c** = 19.1 ± 0.3 mmHg; $P<0.05$ and $P<0.01$, respectively). Moreover, no ophthalmologic side effects, such as appearance of eyelid closure, hyperemia of the external and anterior ocular segments, and abnormality of the pupil, were observed as a result of the high hA_3 selectivity. This suggests that selective human A_3 antagonists may provide a novel and safe approach for the treatment of glaucoma.

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