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Facile synthesis of fused 1,2,4-triazolo[1,5-*c*]pyrimidine derivatives as human adenosine A_3 receptor ligands

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Abstract—A facile synthetic method for fused triazolopyrimidine derivatives having high affinity and selectivity for human adenosine A_3 receptors is reported. The fused triazolopyrimidine derivatives were easily prepared by one-pot reaction using acylhydrazines and imidates prepared from amine derivatives bearing cyano group and orthoesters in situ. This synthetic method was useful in finding new tricyclic adenosine A_3 receptor antagonists and also in diversifying the substituents at two positions on the fused triazolopyrimidine ring.

Adenosine, an important regulator for homeostasis of the brain, heart, kidney, and other organs,[1] interacts with four different G-protein-coupled receptors classified as A_1, A_{2A}, A_{2B}, and A_3 receptor subtypes.[2] The first adenosine A_3 receptor antagonists of 1,4-dihydropyridines (**1**), flavonoids (**2**), and triazoloquinazolines (**3**), were reported by Jacobson and co-workers in 1996.[3–6] In the last 8 years, different classes of compounds with nonxanthine structures have been reported to be selective A_3 receptor antagonists.[7–13]

These nonxanthine types of A_3 receptor antagonists were classified as monocyclic, bicyclic, and tricyclic compounds, as shown in Figures 1 and 2. Recently, our group reported a series of 1,2,4-triazolo[5,1-*i*]purines, which is one of the potent and selective A_3 receptor ligands.[14] The highly potent A_3 receptor ligand, 5-*n*-butyl-8-(4-methoxyphenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine (**6**; K_i for $hA_3 = 0.18$ nM) showed excellent selectivity to hA_3 receptors against hA_1, hA_{2A}, and hA_{2B} receptors ($hA_1/hA_3 = 2210$, $hA_{2A}/hA_3 = 4960$, $hA_{2B}/hA_3 = 5720$).

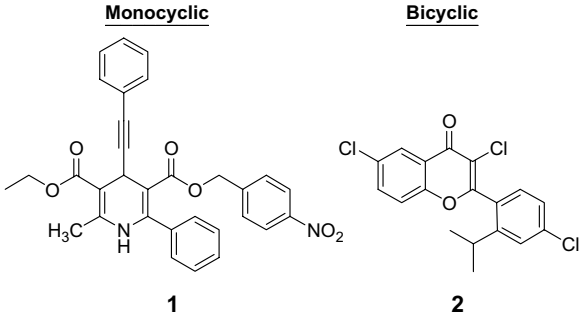

Figure 1. Chemical structures of representative adenosine A_3 receptor antagonists.

Interestingly, tricyclic antagonists have several structural similarities, such as 5–6–5 or 5–6–6 member-fused rings and two substituents at the same position of the scaffold (Fig. 2). Based on this, we hypothesized that perhaps other analogs with 5–6–5 or 5–6–6 member-fused rings also allow for binding to hA_3 receptors. Therefore, we developed a facile synthetic method and synthesized pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine and 1,2,4-triazolo[1,5-*c*]quinazoline rings bearing various substituents at the 2 and 5 positions of the ring to find novel hA_3 receptor antagonists.

The fused 1,2,4-triazolo[1,5-*c*]pyrimidine **9** was synthesized by condensation of iminoester **8** and acylhydrazine based on the synthetic strategy, as shown in Scheme 1.

Keywords: Adenosine A_3 receptor antagonist; One-pot reaction.
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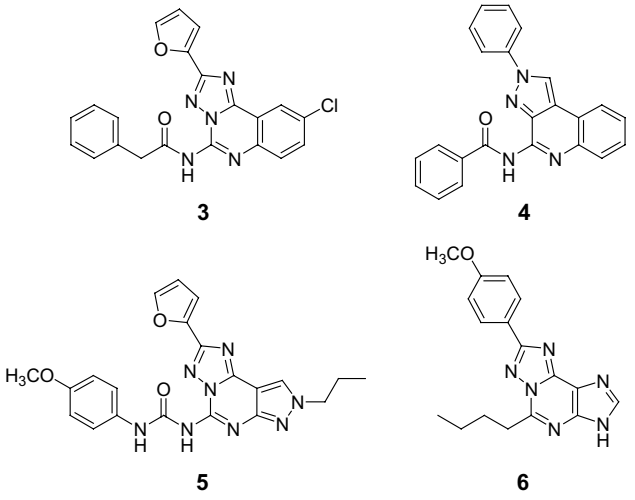
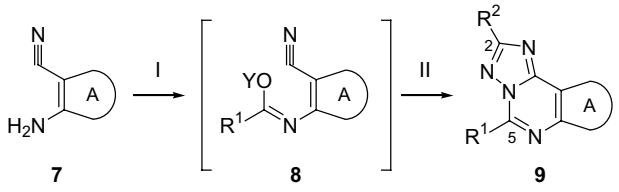


Figure 2. Chemical structures of tricyclic adenosine A_3 receptor antagonists.

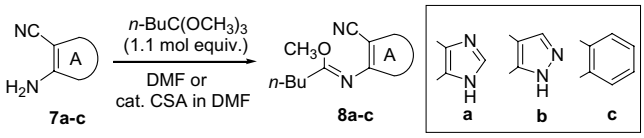


Scheme 1. Synthetic strategy for one-pot reaction of the fused 1,2,4-triazolo[1,5-*c*]pyrimidine **9**. I: orthoester, II: acylhydrazine.

Namely, the iminoester **8** was prepared by the reaction of primary amine analog **7** with a large excess of substituted orthoester.[14,15] We considered that if this reaction proceeds quantitatively with an equivalent or a small amount of orthoester to **7**, the one-pot reaction for the synthesis of the fused triazolopyrimidine derivative **9** could be accomplished by subsequently adding acylhydrazine to the same reaction vessel.

Iminoesterification of various aromatic or heterocyclic amines **7a**–**c** were accomplished by condensation of trimethyl orthovalerate under the three different conditions, as shown in Table 1. Chemical yields of the iminoesters were estimated from the ratio of amines **7** and iminoesters **8** in the reaction mixture with the integral values of ^{1}H NMR spectral data. 5-Amino-4-cyanoimidazole **7a** was converted to iminoester **8a** in good yields (62–86%) at room temperature (rt), at 90 °C, or in the presence of a catalytic amount of *dl*-camphorsulfonic acid (CSA) at rt. 3-Amino-4-cyanopyrazole **7b** was converted to **8b** quantitatively by heating at 90 °C or in the presence of a catalytic amount of CSA at rt, but there was no yield at rt without CSA. Interestingly, iminoesterification of 2-aminobenzonitrile **7c** did not afford **8c** at rt or by heating at 90 °C. However, the reaction proceeded in the presence of CSA at rt. These observations might be related to the nucleophilicity of the amino moiety of **7**. Taken together, these results suggested that using a catalytic amount of CSA enabled the primary amine analogs **7a**–**c** to be converted to iminoesters **8a**–**c** in good yields. Therefore, the reaction conditions using CSA was chosen for the one-pot synthesis of fused 1,2,4-triazolo[1,5-*c*]pyrimidine derivatives **9a**–**c**.

Table 1. Preparation conditions for iminoesters **8a**–**c**



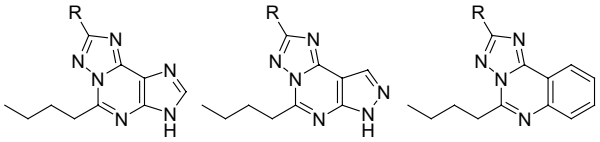
Compounds	Conditions	% Yield	Product
7a	1 h/rt	62	**8a**
7a	1 h/90 °C	86	**8a**
7a	1 h/rt/0.5 (w/v) % CSA	85	**8a**
7b	1 h/rt	0	**8b**
7b	1 h/90 °C	98	**8b**
7b	1 h/rt/0.5 (w/v) % CSA	98	**8b**
7c	1 h/rt	0	**8c**
7c	1 h/90 °C	0	**8c**
7c	1 h/rt/0.5 (w/v) % CSA	96	**8c**

Amines **7a**–**c** were transformed into the fused 1,2,4-triazolo[1,5-*c*]pyrimidine derivatives **9a**–**c** via intermediates **8a**–**c** in situ by treatment with 1.1 mol equiv of the corresponding orthoesters in the presence of 1% of CSA in DMF at rt. Subsequently, 1.2 mol equiv of the corresponding benzoylhydrazines were added in each reaction vessels and heated at reflux temperature.[16] In addition to unsubstituted phenyl compounds, 4-methoxy and 4-trifluoromethylphenyl analogs were synthesized due to the high potency and selectivity for human adenosine A_3 receptor based on our previous study.[14] The isolation yields of 1,2,4-triazolo[5,1-*i*]purines (**9a**), pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines (**9b**), and 1,2,4-triazolo[1,5-*c*] quinazolines (**9c**) were 62–82%, 60–76%, and 48–49%, respectively.

The binding affinities of the fused 1,2,4-triazolo[1,5-*c*]-pyrimidines **9a**–**c** to human adenosine A_{2A} and A_3 receptors expressed in HEK-293 cells are summarized in Table 2. All synthesized compounds showed potent and selective affinities to human adenosine A_3 receptors against A_{2A} receptors. 1,2,4-Triazolo[1,5-*c*]-quinazolines **9c** showed the weak binding affinity remarkably to human adenosine A_{2A} receptors, whereas the other two scaffolds (**9a** and **9b**) still had affinities in the range of 10–1000 nM. These results allow us to hypothesize that a hydrogen donor (NH) of imidazole (**9a**) and pyrazole (**9b**) rings is favorable for interaction with the human adenosine A_{2A} receptor subtype. In fact, Baraldi et al. reported the lower affinities to hA_{2A} receptor of unsubstituted pyrazole derivatives in comparison with N-alkylated pyrazolic series.[15] Moreover, we recently found that alkylation of the imidazole NH moiety of **9a** decreased the binding affinity to hA_{2A} receptor (data not shown).

Similar to the previous structure–activity relationship,[14] there was greater potency of hA_3 inhibition and selectivity against hA_2 receptor of 4-methoxyphenyl analogs compared to unsubstituted phenyl compounds. However, 4-trifluoromethylphenyl analogs showed poor

Table 2. Binding affinities of **9a–c** to human adenosine A_{2A} and A_3 receptors



9a **9b** **9c**

Compounds	R	IC_{50} (nM)		A_{2A}/A_3 ratio
		hA_{2A}[a]	hA_3[b]	
9a–1	Ph	71[c]	0.25[c]	280[c]
9a–2(6)	4-CH_3O-Ph	1600[c]	<0.1[c]	>16,000[c]
9a–3	4-CF_3-Ph	>10,000[c]	0.61[c]	>16,000[c]
9b–1	Ph	190	2.1	90
9b–2	4-CH_3O-Ph	2600	<10	>260
9b–3	4-CF_3-Ph	>10,000	130	>77
9c–1	Ph	>10,000	260	>38
9c–2	4-CH_3O-Ph	>10,000	160	>63
9c–3	4-CF_3-Ph	>10,000	490	>20

[a] See Refs. 17, 18; CV = 4.0%.
[b] See Refs. 19, 20; CV = 3.2%.
[c] See Ref. 14.

affinity to both receptors when compared with unsubstituted phenyl compounds. Since no significant hA_{2A} binding affinity of 4-trifluoromethyl analogs at 10 μM range would show enough selectivity to hA_3 for the animal study, further synthesis and biological evaluations might be necessary.

In conclusion, we found new scaffolds of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines (**9b**) and 1,2,4-triazolo[1,5-*c*]quinazolines (**9c**) as potent and selective hA_3 receptor ligands. This facile synthetic method for fused 1,2,4-triazolo[1,5-*c*]pyrimidine ring bearing various substituents at the 2 and 5 positions of the ring is an effective approach to finding novel adenosine A_3 receptor antagonists.

Acknowledgements

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