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Synthesis and SAR studies of trisubstituted purinones as potent and selective adenosine A_{2A} receptor antagonists

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ABSTRACT

A series of trisubstituted purinones was synthesized and evaluated as A_{2A} receptor antagonists. The A_{2A} structure–activity relationships at the three substituted positions were studied and selectivity against the A_1 receptor was investigated. One antagonist **12o** exhibits a K_i of 9 nM in an A_{2A} binding assay, a K_b of 18 nM in an A_{2A} cAMP functional assay, and is 220-fold selective over the A_1 receptor.

Adenosine receptors are members of the G-protein-coupled receptor family. Four subtypes of adenosine receptors have been identified and designated as A_1, A_{2A}, A_{2B} and A_3.[1] The A_{2A} receptor is enriched in the striatum[2] and is currently an active area of interest in pharmaceutical research due to its potential therapeutic application in Parkinson's disease and other neurodegenerative disorders.[3–5] The main pathology of Parkinson's disease is the progressive destruction of the dopamine pathway. Current dopamine agonist therapies are primarily focused on management of the symptoms and do not change the pace of disease progression.[6,7] A_{2A} receptor antagonists are potential neuroprotective drugs that improve locomotor activity and may stop or slow the degenerative process.[7,8]

High-throughput screening of a series of ECLiPS™ (Encoded Combinatorial Libraries on Polymeric Support)[9] libraries employing an A_{2A} radioligand-binding assay resulted in the identification of multiple sets of compounds as A_{2A} antagonists. Recently we reported the identification of substituted aminothiazoles as A_{2A} antagonists from this screen.[10] Here we report the discovery and associated SAR studies of a series of trisubstituted purinones as potent and selective A_{2A} antagonists. The specific library that elicited these A_{2A} actives contains ~50,000 compounds, which share a common structural element of a purinone core. A small set of compounds with a trisubstituted purinone scaffold was identified as A_{2A} antagonist leads as exemplified by structure **7a**. To further explore the structure–activity relationships and optimize the A_{2A} binding affinity and other pharmacological properties, additional analogs were synthesized.

The purinone analogs **6** were synthesized in five steps from commercially available 2,4-dichloro-5-nitropyrimidine (**1**) as shown in Scheme 1. The first chlorine atom was displaced by a primary amine (R^1NH_2) at −78 °C with high regioselectivity for the 4-position versus the 2-position (typically 10:1 ratio).[11] The two regioisomers were readily separated by flash chromatography. The predominant regioisomer **2** was further functionalized at the C-2 position with a second amine (R^2NH_2) to provide **3**, followed by reduction of the nitro group with sodium hydrosulfite to give **4**. Treatment of **4** with carbonyl diimidazole at room temperature produced the desired purinone **5**. Subsequent N-alkylation of **5** to provide **6** was achieved by using an alkyl halide (R^3X) and polystyrene-bound 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP)[12] as the base.

The A_{2A} binding assay was performed with recombinant human A_{2A} receptor using the same radioligand protocol as described previously.[13] Due to the potential cardiovascular side effect of A_1 receptor antagonism,[5,14] selectivity against the A_1 receptor was also investigated by measuring human A_1 binding affinity using a similar protocol.[13] A set of analogs incorporating a cyclopropylamino substituent at C-2 (purine numbering protocol) was synthesized to investigate the SAR at N-7 and N-9 (Table 1). Both initial screening results and this SAR study indicated conservative structural tolerances for A_{2A} binding affinity with regard to the N-9 position (R^1) of the purinone scaffold. A substituted phenyl group at R^1 seems

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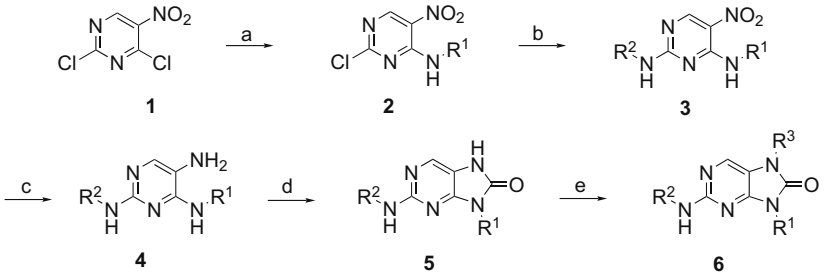
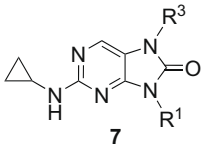


Scheme 1. Reagents and conditions: (a) R^1NH_2, DIEA, THF, −78 °C; (b) R^2NH_2, DIEA, THF, rt; (c) sodium hydrosulfite, sodium bicarbonate, THF/MeOH, rt; (d) carbonyl diimidazole, DCM, rt; (e) R^3X, polystyrene-bound BEMP, acetonitrile, rt.

Table 1
2-(Cyclopropylamino)purinone A_{2A} antagonists



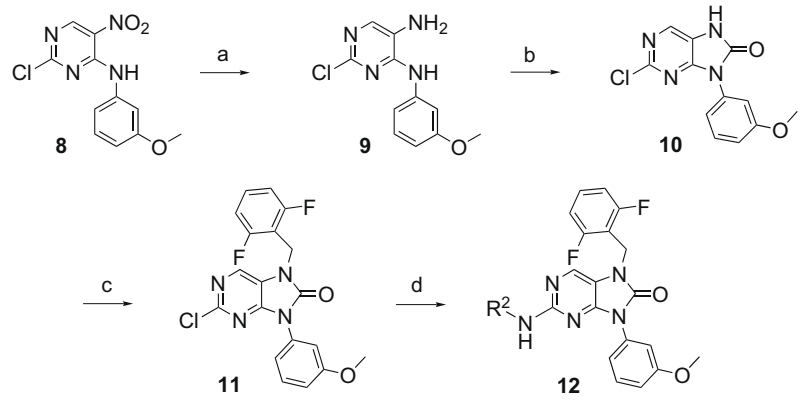
Compound	R^1	R^3	hA_{2A} K_i (nM)[a]	hA_1 K_i (nM)[b]	A_1/A_{2A} ratio
7a	3-Methoxyphenyl	2-Methoxybenzyl	24	402	17
7b	2-Methoxyphenyl	2-Methoxybenzyl	1561	N/D	N/D
7c	Phenyl	2-Methoxybenzyl	409	447	1.1
7d	3,5-Dimethoxyphenyl	2-Methoxybenzyl	76	4640	61
7e	3-Fluorophenyl	2-Methoxybenzyl	89	599	7
7f	3-Methylphenyl	2-Methoxybenzyl	316	1847	6
7g	3-Trifluoromethoxyphenyl	2-Methoxybenzyl	206	5138	25
7h	3-Cyanophenyl	2-Methoxybenzyl	2587	3237	1.3
7i	3-Methoxybenzyl	2-Methoxybenzyl	9213	>10,000	N/D
7j	3-Methoxyphenyl	Benzyl	54	564	10
7k	3-Methoxyphenyl	2-Fluorobenzyl	33	201	6
7l	3-Methoxyphenyl	2-Chlorobenzyl	86	2850	33
7m	3-Methoxyphenyl	2,6-Difluobenzyl	4	236	59
7n	3-Methoxyphenyl	2-Fluoro-6-methoxybenzyl	6	330	55
7o	3-Methoxyphenyl	(+)-α-Methylbenzyl	359	N/D	N/D
7p	3-Methoxyphenyl	Methyl	2641	N/D	N/D
7q	3-Methoxyphenyl	2-Methoxyphenyl	5122	1265	0.2

[a] K_i determined by competition binding of [3H]SCH-58261.[13]
[b] K_i determined by competition binding of [^{3}H]DPCPX.[13]

important to achieve good A_{2A} binding affinity. Among the various R^1 groups tested (**7a**–**7i**), the compound incorporating a 3-methoxyphenyl group (**7a**) shows the highest potency (K_i = 24 nM). Switching the methoxy group from the 3-position (**7a**) to the 2-position (**7b**) significantly reduces binding affinity (K_i = 1561 nM). Compound 7**b** is also 4 times weaker than the analog with the unsubstituted phenyl group (**7c**), suggesting some degree of steric restriction at the 2-position of the phenyl ring. Addition of a second methoxy substituent at the 5-position (**7d**) improves selectivity against A_1, although its A_{2A} activity is slightly decreased. Substitution at the 3-position with a fluorine atom (**7e**) also exhibits good activity (K_i = 89 nM), while substitution with a methyl group (**7f**) shows only similar activity to the unsubstituted phenyl (**7c**). This SAR might be indicative of a possible hydrogen-bond interaction at the 3-position. The weaker affinity of analogs with 3-trifluoromethoxyphenyl (**7g**) and 3-cyanophenyl (**7h**) groups demonstrate the relatively tight tolerances at this position. Interestingly, while analogs with a 3-methoxyphenyl group at R^1 display good binding, insertion of a methylene spacer between the phenyl and purinone (**7i**) completely removes binding affinity.

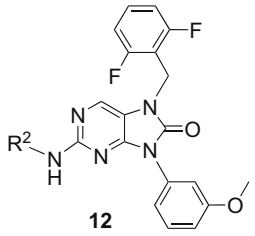
The structure–activity relationship of the R^3 group at N-7 is also detailed in Table 1. A substituted benzyl group is generally favorable at the R^3 position. While an unsubstituted benzyl group (**7j**) displays good A_{2A} activity (K_i = 54 nM), 2-methoxybenzyl (**7a**) and 2-fluorobenzyl (**7k**) further improve A_{2A} activity. The analog with a 2-chlorobenzyl group (**7l**) shows slightly decreased activity. The greatest A_{2A} activity is achieved with di-substituted benzyl analogs, for example, analogs with 2,6-difluorobenzyl (**7m**, K_i = 4 nM), and 2-fluoro-6-methoxybenzyl (**7n**, K_i = 6 nM). Interestingly, these analogs also demonstrate high selectivity against the A_1 receptor with A_1/A_{2A} K_i ratios of 55–60. α-Methylation of the benzyl group (**7o**) reduces A_{2A} activity, and a small alkyl group at the R^3 position (**7p**) is not tolerated. Also, switching from 3-methoxybenzyl (**7a**) to 3-methoxyphenyl (**7q**) at the R^3 position results in significant loss of A_{2A} activity.

In order to facilitate studies of the role of the C-2 substituent, the synthetic route was revised so that the R^2 group at C-2 was introduced in the last synthetic step (Scheme 2). Based on the SAR study results discussed above, R^1 and R^3 were fixed as 3-methoxyphenyl and 2,6-difluorobenzyl, respectively. Reduction of the nitro group of **8** provided diaminopyrimidine **9**, which was subsequently cyclized using carbonyl diimidazole to give 2-chloropurinone **10**. Subsequent N-9 alkylation to give **11** was performed with 2,6-difluorobenzyl bromide in the presence of polystyrene-bound BEMP. Final displacement of the 2-chloro group with an amine (R^2NH_2) was achieved under microwave-assisted conditions to obtain **12**. Overall, compounds with various R^2 groups display good A_{2A} affinity, with relatively wide tolerance of different struc-



Scheme 2. Reagents and conditions: (a) sodium hydrosulfite, sodium bicarbonate, THF/MeOH, rt; (b) carbonyl diimidazole, DCM, rt, 64% (from **8**); (c) 2,6-difluorobenzyl bromide, polystyrene-bound BEMP, acetonitrile, rt, 92%; (d) R^2NH_2, DMA, microwave 180 °C, 40–85%.

Table 2
7-(2,6-Difluorobenzyl)-9-(3-methoxybenzyl)purinone A_{2A} antagonists



Compound	R^2	hA_{2A} K_i (nM)[a]	hA_1 K_i (nM)[b]	A_1/A_{2A} ratio
12a	Methyl	23	1110	49
12b	Ethyl	31	1053	34
12c	Isopropyl	60	616	10
12d	Benzyl	72	2972	42
12e	Methoxyethyl	106	1473	14
12f	Cyclopentyl	213	1056	5
7m	Cyclopropyl	4	236	59
12g	Cyclopropylmethyl	499	1072	2
12h	2-Pyridylmethyl	12	1211	100
12i	4-Pyridylmethyl	50	1191	24
12j	3-Pyridylmethyl	66	1298	20
12k	2-Pyridylethyl	78	1800	23
12l	3-Pyridyl	111	1833	17
12m	4-Morpholinylethyl	259	8598	33
12n	(*N*,*N*-Dimethylamino)ethyl	261	10,000	38
12o	2-(2-Thienyl)ethyl	9	1967	220

[a] K_i determined by competition binding of [^{3}H]SCH-58261.[13]
[b] K_i determined by competition binding of [^{3}H]DPCPX.[13]

tural features (Table 2). Among the various substituents tested, small substituents are generally preferred, as indicated by the following trend of A_{2A} affinity: methyl (**12a**) > ethyl (**12b**) > isopropyl (**12c**) > benzyl (**12d**) > methoxyethyl (**12e**) > cyclopentyl (**12f**). The analog with the cyclopropylamino substituent (**7m**) demonstrates the highest A_{2A} antagonist activity (K_i = 4 nM). Insertion of a methylene spacer (**12g**) decreases activity by more than 100-fold. Pyridylmethylamino groups exhibit good activity (**12h**–**12j**), with 2-pyridylmethylamino (**12h**) being the best (K_i = 12 nM). Insertion of an additional methylene (**12k**) or removal of the methylene spacer (**12l**) results in weaker activity. Substituents containing a basic nitrogen (**12m**, **12n**) are not favorable. For A_1 receptor activity, most analogs exhibit K_i values in the micromolar range, resulting in the A_1/A_{2A} selectivity ratios of 2- to 220-fold. The highest selectivity against the A_1 receptor is achieved by the analog with a 2-(2-thienyl)ethyl group at R^2 (**12o**), which exhibits a low A_{2A} K_i of 9 nM but a high A_1 K_i of 1967 nM.

Table 3
cAMP functional assay data for A_{2A} antagonists

Compound	Rat A_{2A} cAMP K_b (nM)
7a	62
7m	88
7n	39
12a	237
12h	202
12o	18

The A_{2A} receptor is coupled to activation of adenylyl cyclase through the GTP binding-protein Gs.[2,15] Activation of the A_{2A} receptor in cells increases the intracellular accumulation of cyclic adenosine monophosphase (cAMP), a response which is blocked by A_{2A} antagonism.[15,16] Functional antagonism at the rat A_{2A} receptor was determined by measurement of cAMP levels in the presence or absence of inhibitor in CGS 21680-induced rat pheochromocytoma cells.[13] Results of the cAMP functional assay on selected purinone analogs are listed in Table 3. All the analogs tested are functional antagonists of the A_{2A} receptor with varying degrees of activity. Compound **12o** exhibits the highest functional potency with a cAMP K_b value of 18 nM.

In summary, a series of trisubstituted purinone analogs was discovered as potent A_{2A} antagonists with good selectivity against the A_1 receptor. Compound **12o** has a K_i of 9 nM in an A_{2A} binding assay and is 220-fold selective against A_1. It also exhibits high antagonistic potency in a cell-based A_{2A} cAMP functional assay. SAR studies on this series revealed that a 3-methoxyphenyl group is strongly preferred at N-9 and a substituted benzyl group is favorable at N-7. A relatively high degree of tolerance for the substituent at the C-2 position was observed, which might serve as a potential portal to investigate other pharmacological properties in future studies.

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