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Potent and selective adenosine A_{2A} receptor antagonists: [1,2,4]-triazolo[4,3-*c*]pyrimidin-3-ones

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ABSTRACT

Antagonism of the adenosine A_{2A} receptor affords a possible treatment of Parkinson's disease. In the course of investigating pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine A_{2A} antagonists, we prepared [1,2,4]-triazolo[4,3-*c*]pyrimidin-3-ones with potent and selective (vs A_1) A_{2A} antagonist activity. Structure–activity relationships are described for this series.

Parkinson's disease (PD) is a severe neurological disorder marked by degeneration of dopaminergic neurons.[1] The use of L-dopa as dopamine replacement therapy is the central focus of treatment for Parkinson's disease, however chronic administration leads to motor skills complications and loss of drug efficacy and dyskinesia.[2] Other methods of treatment fail to achieve long-term control of motor skills, speech, and other functions.[3]

Adenosine is an important neuromodulator in the central and peripheral nervous systems. Adenosine modulates its effects through the activation of four receptors located on cell membranes, known as A_1, A_{2A}, A_{2B}, and A_3.[4] Adenosine A_{2A} antagonists have demonstrated the ability to restore the deficits caused by degeneration of the striatonigral dopamine system, which is compromised by the loss of striatal neurons in this disease.[5] A_{2A} antagonism offers a novel target for the possible treatment of PD.[6] Generally, A_{2A} antagonists have been classified as xanthine and non-xanthine derivatives, such as KW-6002 (istradefylline)[7] and SCH 420814 (preladenant)[8] currently in clinical trials (Fig. 1). Adenosine A_{2A} receptor antagonists of several structural types have been described.[9,10]

In our previous Reports, we disclosed the development of the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (preladenant) as a potential treatment for PD[8] and the activity of a set of 7-aryl-1,2,4-triazolo[1,5-*c*]pyrimidines of type **1** was also described.[11] As a part of the investigation of these series, we also explored 1,2,4-triazolo[4,3-*c*]pyrimidin-3-one **2** and pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-one **3** derivatives (Fig. 2). We focused our study on identifying a suitable replacement for the optimal

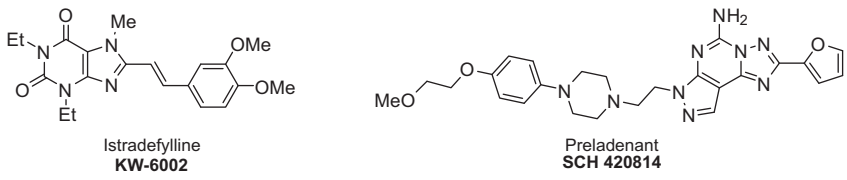

Figure 1. A_{2A} antagonists KW-6002 and preladenant.

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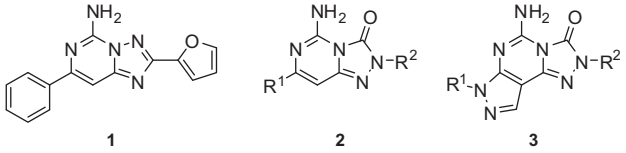


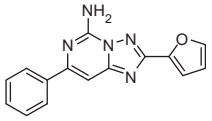
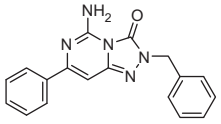
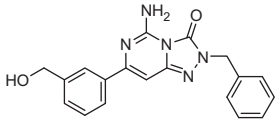
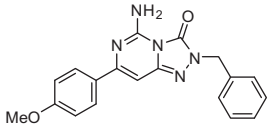
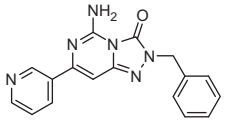
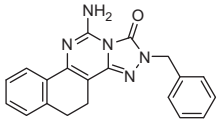
Figure 2. 1,2,4-Triazolo[4,3-*c*]pyrimidin-3-one **2** and pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-one **3**.

furan moiety, which has the potential to undergo oxidative metabolism[12] and on improving the solubility of preladenant.[13a] In the present report, we describe the synthesis and SAR of a novel series of 1,2,4-triazolo[4,3-*c*]pyrimidin-3-ones **2**[14] and pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-ones **3**.

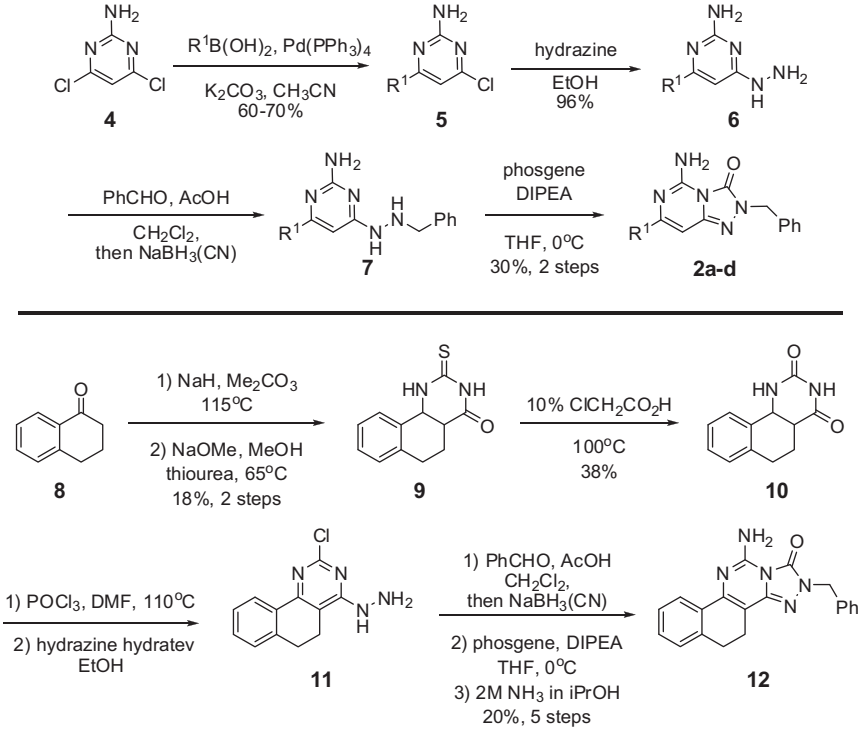
The compounds shown in Table 1 were prepared using the general procedures described in Scheme 1. The Pd-catalyzed coupling of 2-amino-4,6-dichloropyrimidine with aryl boronic acids yielded chlorides **5** which underwent displacement with hydrazine to produce **6**. Condensation of **6** with benzaldehyde, followed by reduction with $NaBH_3(CN)$ produced structures of type **7**. Subsequent cyclization of **7** with phosgene yielded compounds **2a–d**. All compounds reported herein gave satisfactory analytical results.[15] We also synthesized a fused tetracyclic analog **12** to restrict rotation of the aryl substituent. Compound **12** was prepared in 8 steps from α-tetralone. Initially, α-tetralone was treated with sodium hydride and dimethyl carbonate to provide the intermediate α-keto ester, which upon reaction with sodium methoxide and thiourea in methanol provided compound **9**. Compound **10** was prepared by reacting compound **9** with 10% chloroacetic acid at 100 °C. Subsequent treatment of compound **10** with $POCl_3$ followed by displacement with hydrazine gave compound **11**. Lastly, reductive amination of compound **11** with benzaldeyde, followed by cyclization with phosgene and displacement with ammonia provided compound **12**.

The in vitro results of the A_{2A} AR binding assays[16] are expressed as inhibition constants (K_i, nM) and A_1/A_{2A} describes the selectivity over A_1. Results in Table 1 show that compound **2a** had higher A_{2A} affinity than compound **1**, but only moderately improved selectivity over A_1. Substituted aryl- analogs **2b–c** and the pyridyl- analog

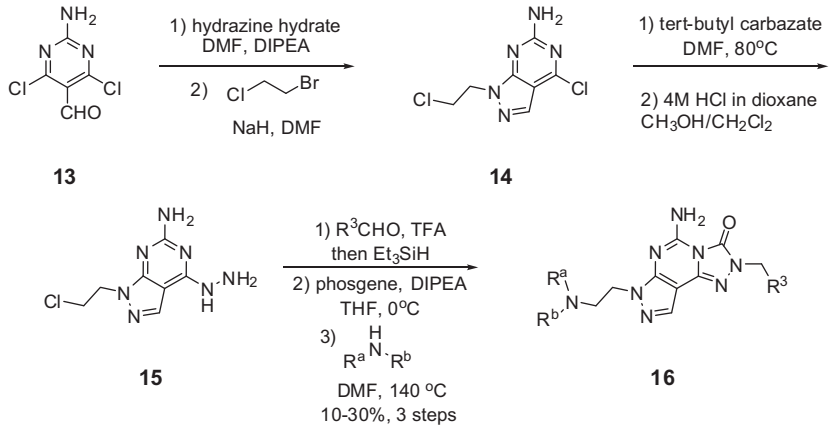
Table 1
1,2,4-Triazolo[4,3-*c*]pyrimidin-3-ones

Compound	Structure	A_{2A} K_i[a] (nM)	A_1/A_{2A}[a]	k. sol.[b] (μM)
1		7.0	3	NA
2a		1.0	26	NA
2b		1700	1	100
2c		636	2	25
2d		791	2	100
12		0.5	43	NA

[a] Average of duplicate determinations, human receptors.
[b] A single measurement of kinetic solubility at pH 7.4.[13b]

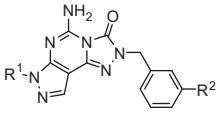


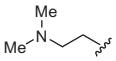
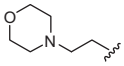
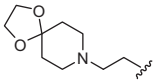
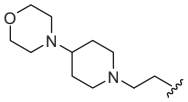
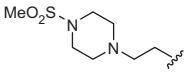
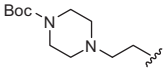
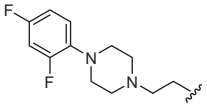
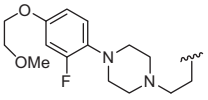
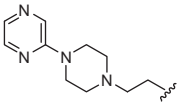
Scheme 1. Synthesis of 1,2,4-triazolo[4,3-*c*]pyrimidin-3-ones **2a–d** and **12**.



Scheme 2. Pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-ones **16**.

Table 2
Receptor affinity and solubility of pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-ones



Compound	R^1	R^2	A_{2A} K_i[a] (nM)	A_1/A_{2A}[a]	k. sol.[b] (μM)
17		H	0.3	1115	37
18		Cl	0.4	356	12
19		H	>1500	NA	>250
20		Cl	5.2	269	18
21		H	9.0	156	175
22		Cl	14.2	99	25
23		H	8.7	162	75
24		Cl	11.0	127	NA
25		H	28.4	53	NA
26		Cl	6.7	224	NA
27		H	8.9	157	75
28		Cl	10.5	133	12
29		H	>1300	NA	100
30		Cl	556	2	25
31		H	>1300	NA	NA
32		Cl	23.4	60	NA
33		H	>1300	NA	12
34		Cl	15.6	90	12
35		H	12.7	110	50
36		Cl	12.9	109	25

[a] Average of duplicate determinations, human receptors.
[b] A single measurement of kinetic solubility at pH 7.4.13.

2d had greatly decreased A_{2A} affinity and selectivity. Compound **12** demonstrated that fusing the aryl group to the pyrimidin-3-one core improved A_{2A} affinity to <1 nM, but unfortunately did not improve selectivity and was inactive in the rat catalepsy assay at an oral dose of 10 mg/kg (Table 4).[11,17]

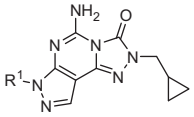
The solubility for compounds **2b–d** was greatly improved over preladenant, however, due to the lack of affinity for the A_{2A} receptor, we moved our attention to preparing analogs of type **3** based on the SAR of the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine series. The synthesis of these analogs originated from known compound **15**[18] and is shown in Scheme 2. Condensation of compound **15** with a suitable aldehyde in TFA followed by reduction with triethylsilane provided the appropriate hydrazide. Subsequent cyclization with phosgene and displacement with various amines yielded compounds of type **16**.

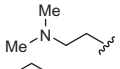
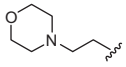
A variety of analogs (**17–39**) were prepared varying substitution at the 7-position and a benzyl, 3-chlorobenzyl, or cyclopropylmethyl group at the 2-position of the molecule (Tables 2 and 3). Compounds **17** and **18** with a vinyl group[19] installed at the 7-position afforded the most potent and selective A_{2A} receptor antagonists in this set of compounds, however, these were devoid of significant anti-cataleptic activity in the rat at an oral dose of 10 mg/kg (Table 4).[11,18] Based on previous SAR of the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine series[8,9a] incorporation of a basic nitrogen in the side chain resulted in compounds with acceptable binding affinities (5–15 nM) and selectivity (>100 fold over A_1). Introduction of a morpholine group **21** and **22** or a piperidine group **23–26** provided compounds with 7–30 nM A_{2A} K_i values and selectivity over A_1 of 50–225 fold. Several substituted piperazines **27–36** were also investigated with varying degrees of activity. Compounds of particular interest were the methyl-sulfonyl substituted piperazines **27** and **28** and pyrazinyl–piperazines **35** and **36** which displayed >100-fold selectivity over A_1. Unfortunately, compounds **31** and **33** were inactive suggesting a possible divergent structure–activity relationship compared to the preladenant series. In general, A_{2A} antagonist activity was maintained or improved by replacing the furan moiety and with a benzyl group or a 3-chlorobenzyl group, but the use of a cyclopropylmethyl group **37–39** as a benzyl isostere provided less potent compounds.

Our goal of improving the solubility relative to preladenant[8] was achieved with several compounds having solubility >25 μM at pH 7.4. Ultimately, compounds with a benzyl group in the 2-position were more soluble, while compounds with a 3-chlorobenzyl group had higher A_{2A} affinity. Unfortunalely, these compounds were inactive in the rat catalepsy assay, which was attributed to their poor rat PK, exposure in rat brain, and pharmacokinetic properties compared to preladenant.[8] The reasons for lack of activity in the catalepsy assay are not well understood but could be due to inadequate exposure of these compounds in the striatum or high non-specific binding to brain tissue (Table 4).

In summary, the exploration of SAR of 1,2,4-triazolo[4,3-*c*]pyrimidin-3-one and pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-one analogs resulted in a novel class of potent and selective A_{2A} antagonists derived from compound **1** and preladenant. Substitution with various piperazino–alkyl chains in the 7-position and furan replacements in the 2-position were shown to be tolerated and provided compounds with improved solubility compared to preladenant, however adequate in vivo activity was not achieved. Further optimization of the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine series of A_{2A} receptor antagonists will be disclosed in future publications.

Table 3
Receptor affinity and solubility of pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-ones



Compound	Structure	A_{2A} K_i[a] (nM)	A_1/A_{2A}[a]	k. sol.[b] (μM)
37		27.4	55	175
38		>1500	NA	>250
39		381	4	>250

[a] Average of duplicate determinations, human receptors.
[b] A single measurement of kinetic solubility at pH 7.4.[13b]

Table 4
In vivo activity and rat PK of selected compounds

Compound	Rat catalepsy,% inhibition 1 h/4 h @10mpk[a]	Rat plasma AUC @ 3mpk, nM•hr[b]	Exposure[c] in rat brain, ng/g	Rat clint mL/min/kg
12	0/18	0	<LOQ	14.7
17	0/8	248	<LOQ	24.4
18	13/15	NA	NA	27.7
20	13/20	398	39	37.3

[a] Average for $n = 3$. Positive control SCH 412348[8] active at 1 h and 4 h (75%, 80%). Maximum reduction attainable is 60–80%.
[b] Area under the curve.[20]
[c] At 6 h after dosing; LOQ is 10 ng/g.

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