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The Synthesis and Biochemical Evaluation of New A_1 Selective Adenosine Receptor Agonists Containing 6-Hydrazinopurine Moieties

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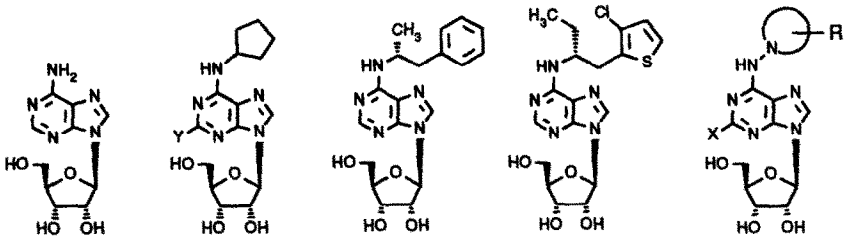
Abstract: The synthesis and SAR of a series of novel derivatives of *N*-aminoadenosine is described, along with their *in vitro* effects in biochemical assays. The rat brain A_1 adenosine receptor binding of these compounds is very dependent upon the purine 2-substituent. The novel agonist, 2-chloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine, exhibits a K_i value for A_1 receptor binding of <1 nM.

Adenosine **1** is a purine nucleoside with a wide variety of regulatory functions[1] and physiological effects[2]. In recent years, the central nervous system (CNS) effects of this purine have received greater prominence[3]. It is now clear that in the mammalian CNS, adenosine acts as a neuromodulator[4], and has been implicated both as an endogenous anticonvulsant agent[5] and a neuroprotectant[6]. Agonists at adenosine receptors have therefore been shown to possess anticonvulsant[7], antiischaemic[8] and antinociceptive[9] effects in laboratory animals, and have therefore been investigated for their therapeutic potential[10] in treatment of human CNS disorders.



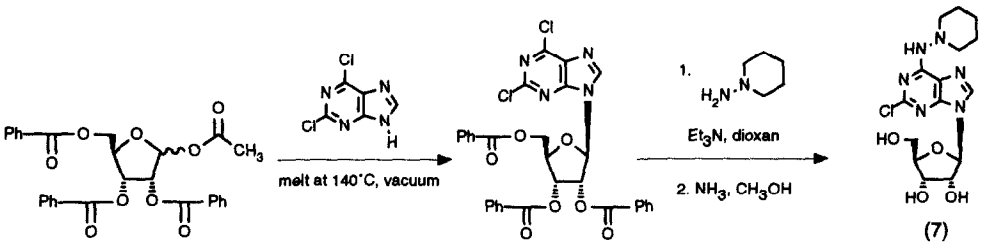
The challenge facing groups involved in designing novel A_1 receptor agonists with a view to the discovery of new drugs is to provide patentable compounds within an extensively investigated field, providing therapeutic benefit without limiting side effects. Despite over three decades of research within academia and the healthcare industry, the only adenosine receptor agonist to have been successfully registered so far as a drug for human use is adenosine itself, for treatment of arhythmias, although several other marketed drugs have purinergic components to their action.

Some potent A_1 receptor agonists currently available include *N*-cyclopentyladenosine (CPA) **2**, as well as its cyclohexyl analogue CHA[10] and (2*S*)-*N*-(2-endo-norbornyl)adenosine [*S*(-)-ENBA][11].

2-Chloro-*N*-cyclopentyladenosine (CCPA) **3** is claimed to be one of the most selective at the rat brain A_1 receptor[12]. *R*-Phenylisopropyladenosine (*R*-PIA) **4** is a classical A_1 agonist, from which a group at Rhone Poulenc Rorer have derived the 3-chloro-2-thienyl derivative RG 14718(-) **5**, which is apparently the most potent A_1 ligand described to date[13].

As part of our search for novel adenosine receptor agonists, we discovered that there are very few adenosine derivatives where an N-N bond exists at the purine 6-position[14]. In this communication we describe the synthesis, receptor binding and functional effect of a novel series of nucleosides of general structure **6**, which can be considered to be aza isosteres of reference A_1 receptor agonists such as CCPA **3**. Tables I - IV illustrate the effects of these compounds in biochemical assays comprising of adenosine A_1 and A_2 receptor binding, inhibition of cAMP accumulation and inhibition of contractile force in isolated guinea pig atria.

Scheme I: Synthesis of 2-chloro-*N*-(1-piperidinyl)adenosine.

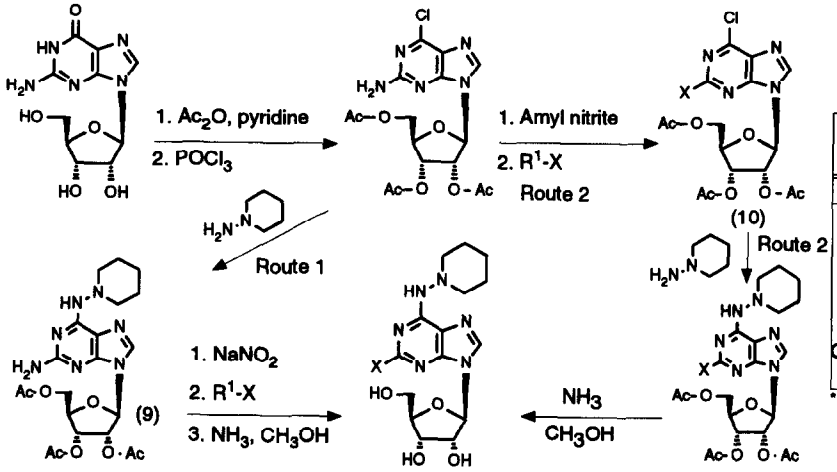


Our initial target structure, 2-chloro-*N*-(1-piperidinyl)adenosine **7**, an aza analogue of 2-chloro-*N*-cyclohexyladenosine, was prepared as shown in Scheme 1. The above route[15,16] was used to provide the 2-chloroadenosine derivatives in Tables I and III.

N-(1-piperidinyl)adenosine **8**, the deschloro analogue of **7**, was isolated by reaction of 1-amino-piperidine with 6-chloropurine riboside, and the surprising observation was made that this example showed no significant A_1 receptor binding (Table II). This was in marked contrast to **7**, which had a K_i value of 4nM for A_1 receptor binding (Table I), with high A_1/A_2 selectivity. This effect is not seen in *N*-alkyladenosine derivatives such as CCPA **3**, in which a 2-hydrogen substitution, giving CPA **2**, retains good binding affinity. The agonist **7** also showed potent effects in two functional assays indicative of A_1 agonist potency, inhibition of stimulated cAMP accumulation and inhibition of contractile force in isolated guinea pig heart (Table IV).

We therefore targeted a range of adenosine derivatives containing either a 1-piperidinyl or 1-pyrrolidinyl function at the 6-amino position, but with varying 2-substituents, in order to investigate what would be the optimal 2-substituent for A_1 agonist effect. A direct route from guanosine was used for most of these compounds[17]. Following ribosyl protection and chlorination at the purine 6-position, it was possible to substitute sequentially at either the 6-position to give **9**, or at the 2-position following diazotization of **10** (Scheme II).

Scheme II: Synthesis of 2-substituted adenosine derivatives from guanosine.



2-Substitution reagents		
X	R^1-X	Route
F	HBF_4	1
Br	$CHBr_3$	2
I*	CH_2I_2	2
CH_3S-	CH_3SSCH_3	2

* N-(1-pyrrolidinyl) derivative

The rat brain adenosine receptor binding[14,18] of this series of adenosine agonists was found to be very dependent upon the nature of the 2-substituent (Table II). Tables I and II show that whilst there is some freedom in the choice of the purine *N*-substituent, the requirements for purine 2-substituents

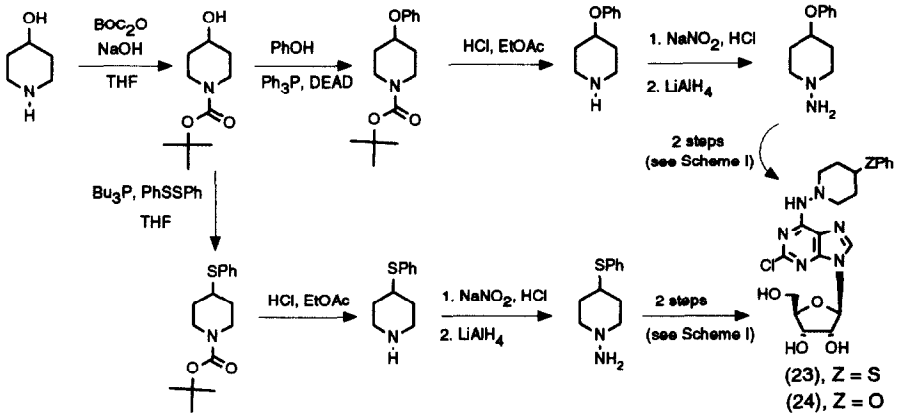
Table I: Adenosine Receptor binding and log P values[19] of 2-chloroadenosine derivatives and Table II: Other 2-substituted adenosines.

Compound number	X	Adenosine N-substituent	Receptor binding K_i, nM A1	A2	LogP
7	Cl	-N⟨ ⟩	4.2	1485	0.79
11	Cl	(S) CH_2OCH_3 -N⟨ ⟩	9.9	564	0.32
12	Cl	CH_3 -N⟨ ⟩ CH_3	4.0	33	1.38
13	Cl	(R) CH_2OCH_3 -N⟨ ⟩	10.9	459	0.32
14	Cl	-N⟨ ⟩	9.3	1130	1.42
15	Cl	-N⟨ ⟩O	13.3	1040	0.11
16	Cl	-N⟨ ⟩N CH_3	184	>30000	0.11
3	Cl	⟨ ⟩	1.5	667	2.07

Compound number	X	Adenosine N-substituent	Receptor binding K_i, nM A1	A2	LogP
17	F	-N⟨ ⟩	3.6	1050	0.35
18	Br	-N⟨ ⟩	9.3	1520	0.89
19	NH_2	-N⟨ ⟩	30	3320	-0.55
20	CH_3S	-N⟨ ⟩	390	2825	0.89
21	n-PrO	-N⟨ ⟩	400	2700	-
22	I	-N⟨ ⟩	13000	57000	1.42
8	H	-N⟨ ⟩	>3000	>10000	1.28
2	H	⟨ ⟩	1.2	321	0.72

are more restrictive. In this series with cyclic hydrazines as purine 6-substituents, we found that electronegative halogens (F, Cl, Br) at the 2-position gave the strongest A_1 receptor binding. The receptor binding and agonist activity (Table IV) of this class of adenosine agonists could generally be increased further by substituting the *N*-(1-piperidinyl) group in the purine 6-position. We found that using the synthetic route illustrated in Scheme I, but with various phenyl-, phenoxy-, phenylthio- or phenylsulphonyl-substituted 1-aminopiperidines (Scheme III), some highly potent A_1 receptor agonists were obtained (Table III). The method used involved *N*-protection of 4-piperidinol, followed by either aryl ether[20] or thioether[21] formation. The *N*-amination procedure utilized was the classical procedure involving nitrosation followed by reduction to a hydrazine[22].

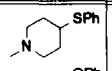
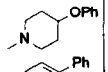
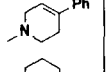
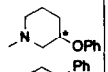
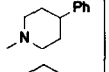
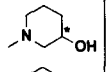
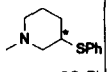
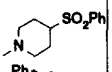
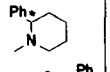
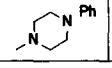
Scheme III. Synthesis of 2-chloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine and 2-chloro-*N*-[4-(phenoxy)-1-piperidinyl]adenosine.



Several conceptual steps were required to attain compound **23**, 2-chloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine, which is approximately equipotent in adenosine A_1 receptor binding to the apparently most potent commercially available A_1 agonist *S*(-)-ENBA[11]. Using **32** as a starting point, potency was increased by preparing the deaza example **27**. Further potency increase was seen when a phenyl ether oxygen was introduced to give **24**, which continued in thioether **23**. Thus, the end result, introduction of a phenyl-containing substituent in the parent structure **7** in a spatial orientation which may give some overlap with the phenyl ring in e.g. *R*-PIA **4** gave a significant increase in the *in vitro* potency of this series of compounds.

For selected compounds, the effects in biochemical assays measuring inhibition of stimulated cAMP accumulation in smooth muscle cells[23] and lowering of the force of contraction in the isolated guinea pig atria[24] were determined (Table IV). These assays are known to give a good indication of adenosine A_1 functional effect in these two tissues. All the compounds tested in the cAMP assay displayed full agonist effect when compared to the reference A_1 agonist *R*-PIA **4**. The *in vivo* CNS characterization of these examples will be described elsewhere.

Table III: Adenosine Receptor binding and log P values of substituted 2-chloro-*N*-(1-piperidinyl)adenosine derivatives and
Table IV: Effects of selected compounds in functional assays.

Compound number	X	Adenosine N-substituent	Receptor binding K_i, nM A1	A2	LogP
23	Cl		0.9	470	2.62
24	Cl		1.4	1100	2.05
25	Cl		6.1	638	2.28
26	Cl		15	1733	1.88
27	Cl		11	1200	2.36
28	Cl		11	720	-0.82
29	Cl		13	1380	2.89
30	Cl		17	220	-
31	Cl		34	17	2.25
32	Cl		55	5571	1.42

Compound number	Inhibition of stim. cAMP accum. IC_{50} (nM)	GP atria -lowering of contractile force IC_{50} (μM)
7	2.1	0.58
17	3.8	1.29
23	0.3	0.64
24	0.03	0.19
25	1.1	2.9
26	4.8	4.5
27	2.5	0.32
28	4.6	0.47
30	0.7	0.32
CPA	0.6	0.42
R-PIA	0.4	0.44
S-PIA	27	8.2

*mixture of diastereoisomers

In conclusion, we have described the synthesis, preliminary SAR and biochemical effects of a range of novel substituted *N*-aminoadenosine derivatives, some with very potent A_1 receptor binding and functional effect. These compounds are very unusual amongst adenosine receptor agonists in the apparent necessity of an electronegative halogen in the 2-position of the purine moiety for receptor binding; substitution with a hydrogen in the 2-position provided only very weakly active examples.

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