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The Discovery and Synthesis of Highly Potent, A_{2a} Receptor Agonists

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Abstract—A series of N6,2-disubstituted adenosine analogues have been synthesized and their functional activity measured against A_{2a} and A_1 receptors. Examples of compounds with both a lipophilic N6-substituent and amino-functionalized 2-position were highly active at the A_{2a} receptor on the human neutrophil.

Introduction

The actions of adenosine, and the family of four G-protein coupled 7-trans-membrane receptors (A_1, A_{2a}, A_{2b}, A_3) which facilitate its physiological effects, have been of great interest for a number of years.[1–3] Adenosine is used clinically for the treatment of supraventricular tachycardia,[4] but its lack of selectivity coupled with its rapid metabolism precludes its therapeutic use to any great degree. Consequently much effort has been focused on the search for metabolically stable and selective purinergic receptor agonists.[5] In this communication we will describe the discovery and synthesis of a series of highly potent A_{2a} purinergic agonists.

Results and Discussion

The structure–activity relationships of adenosine analogues using binding assays have been well-documented.[5,6] As part of our own general investigation into the structure–activity relationships of substituted adenosines using functional assays, we have investigated the effects on A_{2a} and A_1 activity of substitution at the 2 and N6 positions of the adenine ring, as shown in Table 1.

We noted that substitution at the 6-position of adenine ribosides generally conferred A_1 agonist activity whereas 2-substitution improved activity at the A_{2a} receptor, although there were many subtleties. Two pairs of compounds illustrate the marked influence of the position of substitution on activity: moving the cyclopentyl substituent from N6 in *N*-cyclopentyl-2-chloroadenosine **1** to the 2 position in 2-cyclopentylaminoadenosine **2** reduced the A_1 activity by three orders of magnitude and improved the A_{2a} activity 3-fold. The related hydroxylated compounds **3** and **4** exhibit a similar relationship: in this case, the A_{2a} activity increased by two orders of magnitude and the A_1 activity is decreased ~40-fold.

The ability to obtain potent agonists at the adenosine A_{2a} receptor which inhibited human neutrophil function[7,8] offered us the opportunity to investigate further the potential therapeutic application of A_{2a} agonists in the treatment of inflammatory diseases. Selective and potent A_{2a} agonists were required and our medicinal chemistry strategy explored three main areas: 2-alkylaminoadenosines, 2-alkylamino-NECAs and 2-alkylamino-N6-arylalkyl-NECAs.

We found that compounds with cycloalkyl substituents at the 2 position e.g. **2** and **6**, were more potent at the A_{2a} receptor than the corresponding acyclic-alkylamino compound **7**, and the incorporation of a hydroxyl group

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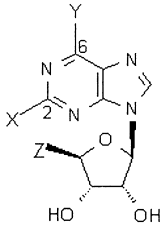
into the 2-cyclopentyl ring yielded A_{2a} agonists more potent than NECA **5**, and with significantly reduced A_1 activity. This is illustrated by the pair of compounds **8** and **9**, and notably the 4′-*N*-ethylcarboxamide substituent present in compound **9** confers several fold improvement in activity as compared to the 4′-hydroxymethyl substituent in **8**. Work investigating N6- substituted analogues in the 4′-hydroxymethyl series had resulted in the discovery of moderately active A_{2a} agonists (compounds **10**–**12**), but with comparatively lower A_1 activity than the unsubstituted compound **2**. Replacement of the 4′- hydroxymethyl group in **12** by the 4′-*N*-ethylcarboxamide substituent again increased the A_{2a} activity several fold (compound **13**), as did hydroxylation of the 2-cyclopentylamino ring (**14**). Interestingly, rat binding data was reported in the literature[12] at this time on N6- arylalkyl compounds, such as **15**. This compound has a $K_i = 10300$ nM at A_2 receptors on rat striatal membranes and $K_i = 45000$ nM at A_1 on rat whole-brain membranes, compared to its 2-H analogue which had figures of 9.4 and 24 nM respectively. The observed weakened binding affinities when a 2-cyclohexylamine substituent is present is contrasted by our functional results on compound **10** where agonist activity is retained at the A_{2a} receptor on the human neutrophil.

Table 1. Relationship between substituent position and activity[c]



	X	Y	Z	A_{2a}[a] (HN)	A_1[b] (GPI)
1	Cl		$HOCH_2$-	17	1
2		H_2N-	$HOCH_2$-	5.1	549
3	Cl		$HOCH_2$-	171	2
4		H_2N-	$HOCH_2$-	1.8	84
5	H	H_2N-	EtNH.CO-	1	1
6		H_2N-	EtNH.CO-	2.2	80
7		H_2N-	EtNH.CO-	85	No data
8		H_2N-	$HOCH_2$-	0.8	1470
9		H_2N-	EtNH.CO-	0.1	260
10		1-Naphthyl-CH_2NH-	$HOCH_2$-	25	No data
11		3-ClPhEtNH-	$HOCH_2$-	39	>1000
12		Ph_2CHCH_2NH-	$HOCH_2$-	36	>612
13		Ph_2CHCH_2NH-	EtNH.CO-	6.2	>5000
14		Ph_2CHCH_2NH-	EtNH.CO-	0.16	390
15		1-Naphthyl-CH_2NH-	$HOCH_2$-	No data	No data

[a]Agonist activities were measured in vitro using inhibition of human neutrophil activation[9,10] (HN).
[b]Inhibition of guinea pig Ileum[9,10] twitch (GPI).
[c]NECA (5′-*N*-ethylcarboxamidoadenosine) **5** was used as an internal standard[9–11] and all agonist potencies are quoted as an equieffective concentration ratio (ECR) relative to NECA. ECR values were determined by dividing the EC_{50} of the test compound by the EC_{50} of NECA.

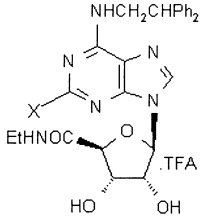
At this point, we had identified several potent compounds, but their solubilities were generally poor and potentially a hindrance to their utility as biological tools. We focused upon analogues of **13** and **14** and sought to improve solubility within the series by incorporating a basic amino group in the 2 substituent. Additionally, it had been reported[13] that NECA analogues with large 2 substituents possessed significant A_2 selectivity. The results are shown in Table 2.

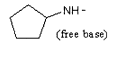
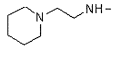
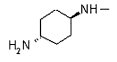
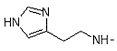
Compounds **16**–**18** are examples of analogues which showed significantly improved aqueous solubility compared to that of **13**, in addition to activity at the A_{2a} receptor on the human neutrophil at least 10 times more potent than NECA **5** itself.

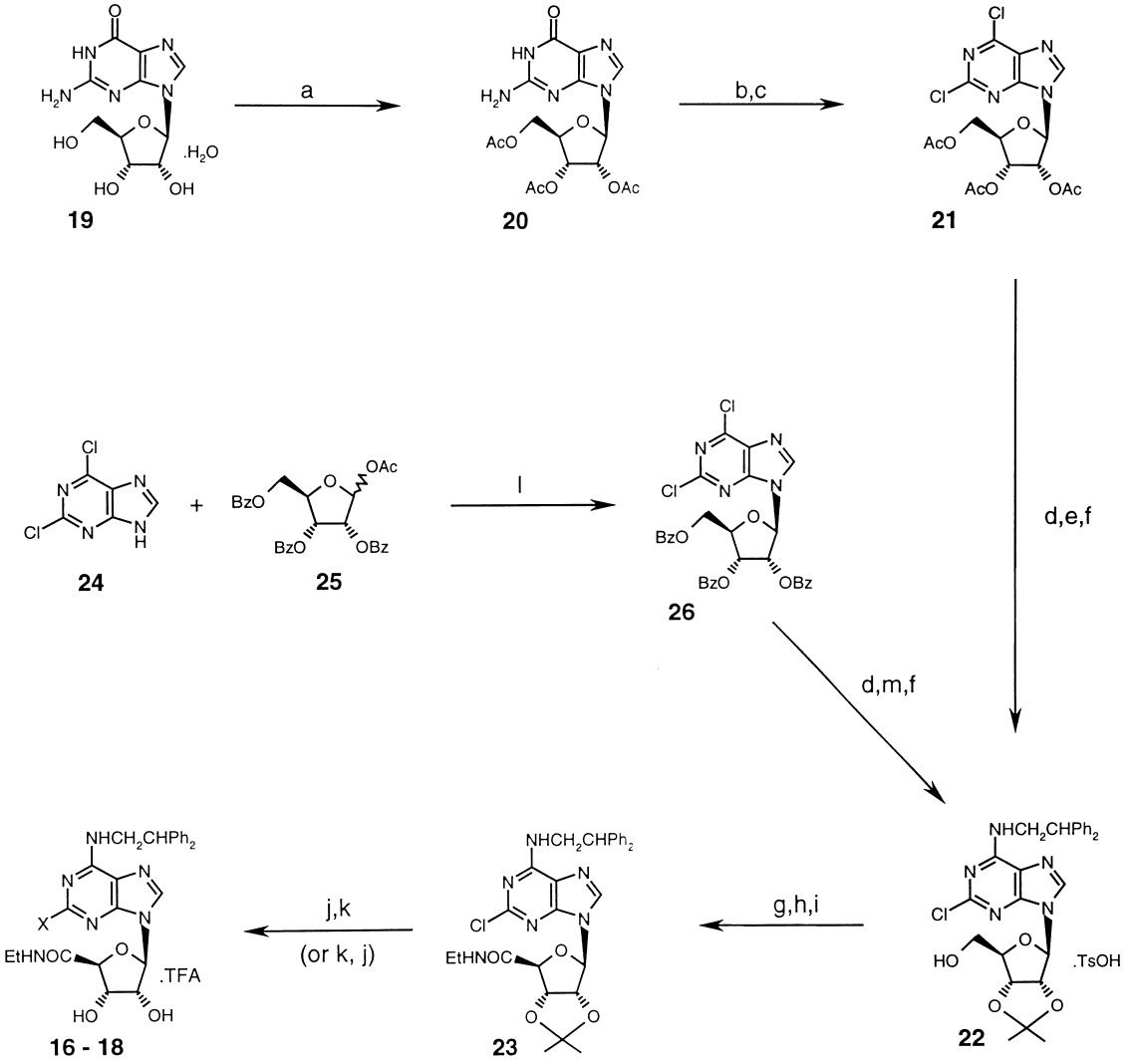
Synthesis of A2a Agonists with Improved Solubility

The analogues **16**–**18**, were prepared from the common intermediate **23**, synthesised using one of the two procedures shown in Scheme 1. Compound **22** was reached either from guanosine hydrate **19** in six steps, or in four steps from 2,6-dichloropurine **24** and a fully-protected ribofuranose sugar **25**. Tri-acetylation of **19** gave **20**,

Table 2. A_{2a} and A_1 SAR for solubilising 2-substituents



	X	A_{2a} (HN)	A_1 (GPI)	Solubility pH 2.6 (mg/ml)
13	 (free base)	6.2	>5000	0.024
16		0.08	263	>=1.87
17		0.05	369	1.57
18		0.03	672	>=1.82



Scheme 1. Synthesis of A_{2a} agonists **16**–**18**. X is defined as in Table 2. Reagents: (a) Ac_2O, Et_3N, MeCN, DMAP; (b) $POCl_3$, $PhNMe_2$, $Et_4N^+Cl^-$, MeCN, reflux; (c) *t*-BuONO, Pyridine.HCl, CuCl, DCM, 0–20 °C; (d) $Ph_2CHCH_2NH_2$, *i*-Pr_2NEt, *i*-PrOH, reflux; (e) Na_2CO_3, MeOH, H_2O; (f) $Me_2C(OMe)_2$, TsOH, Me_2CO; (g) $KMnO_4$, KOH, Dioxan; (h) $SOCl_2$, reflux; (i) $EtNH_2$, DCM; (j) XH, DMSO, 120 °C; (k) TFA, H_2O; (l) 140 °C, neat; (m) K_2CO_3, MeOH.

which was converted to **21** using a two-step procedure via the 6-chloro derivative followed by diazotization and a modified Sandmeyer reaction. Substitution of the 6-Cl by 2,2-diphenylethylamine in the presence of diisopropylethylamine in propan-2-ol was followed by removal of the acetate protecting groups and reprotection of the 2′,3′-diol as the acetonide to yield **22** as its *p*-toluenesulfonic acid salt. Alternatively, **26** was synthesised directly by heating a mixture of **24** and **25**, and yielded **22** following steps similar to the conversion of **21**. Oxidation of the free hydroxyl group yielded the intermediate acid which was elaborated to the ethyl amide **23**. Substitution of the 2-Cl with a range of amines at 120 °C in DMSO and deprotection using aqueous TFA, or *vice versa*, followed by purification using preparative HPLC yielded the series of compounds **16**–**18** as their trifluoroacetic acid salts.

Conclusions

N6,2-disubstituted adenosine analogues have been synthesized and shown to be potent A_{2a} agonists at receptors on the human neutrophil with low activity at the A_1 receptors in the system studied. The improved physical properties will allow their pharmacological effects to be further evaluated for use as potential agents in the treatment of inflammatory diseases.

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