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Synthesis and evaluation of two series of 4′-aza-carbocyclic nucleosides as adenosine A_{2A} receptor agonists

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ABSTRACT

The synthesis of two series of 4′-aza-carbocyclic nucleosides are described in which the 4′-substituent is either a reversed amide, relative to the carboxamide of NECA, or an *N*-bonded heterocycle. Using established purine substitution patterns, potent and selective examples of agonists of the human adenosine A_{2A} receptor have been identified from both series. The propionamides **14**–**18** and the 4-hydroxymethylpyrazole **32** were determined to be the most potent and selective examples from the 4′-reversed amide and 4′-*N*-bonded heterocyclic series, respectively.

Adenosine plays a key role in regulating many aspects of physiology, in part, through the interaction with the four purinergic P1 receptors termed: A_1, A_{2A}, A_{2B} and A_3.[1] This family of four G protein-coupled receptors has been, and continues to be the subject of extensive investigations to identify selective agonists and antagonists as potential therapies for treating a wide range of diseases involving: smooth muscle contraction, neurotransmission, platelet aggregation, pain, wound healing, cardiac function, cardioprotection, sleep disorders and immune response.[2] One example being the activation of the adenosine A_{2A} receptor which has been demonstrated to result in broad spectrum anti-inflammatory activity, directly effecting multiple inflammatory cell types in man.[3] Our interest related to the potential for adenosine A_{2A} receptor agonism as a treatment for the chronic inflammation associated with the respiratory diseases asthma and chronic obstructive pulmonary disease (COPD).[4] In vivo studies supporting this possibility include the A_{2A} receptor agonist CGS21680 having been shown to inhibit ovalbumin and lipopolysaccharide (LPS) induced pulmonary inflammation in rat and murine models.[5] However, the hypotensive effects associated with systemic adenosine A_{2A} receptor activation are predicted to lead to the requirement for direct delivery of compounds to the lung if effective treatments for asthma and COPD are to be realised. This route of administration is anticipated to provide the potential for agents with acceptable cardiovascular side-effect profiles in the case of ligands specifically designed to maximise their local efficacy at the site of administration whilst also being associated with a controlled level of systemic exposure.[6] The challenge in identifying inhaled A_{2A} receptor agonists with such lung-targeted profiles has been highlighted recently by the clinical data reported for GW328267X and the preclinical studies leading to the identification of the clinical candidate UK432,097.[7]

To date essentially all reported agonists of the adenosine A_{2A} receptor have been based upon modifications of the endogenous ligand adenosine.[8] Changes to the 5′-hydroxy residue and the introduction of substituents into the purine base have led to the identification of potent and selective A_{2A} receptor agonists such as CGS21680 and 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (HENECA), Figure 1.[9] Both of these ligands are based upon the 5′-carboxamide modification of the potent pan adenosine receptor agonist 5′-*N*-ethylcarboxamidoadenosine (NECA).[10]

Further transformation of the 5′-carboxamide into a heterocyclic residue has also been shown to be the basis for potent adenosine A_{2A} receptor agonists, as exemplified by the 2-ethyl-5-tetrazolyl moiety of GW328267X and the analogues described in related claims.[11]

As part of our interest in identifying lung-targeted adenosine A_{2A} receptor agonists for inhaled administration we were interested in novel nucleoside templates that could offer advantages in terms of biological activity, stability and synthetic accessibility. In particular, carbocyclic nucleosides in which an amino group is introduced at

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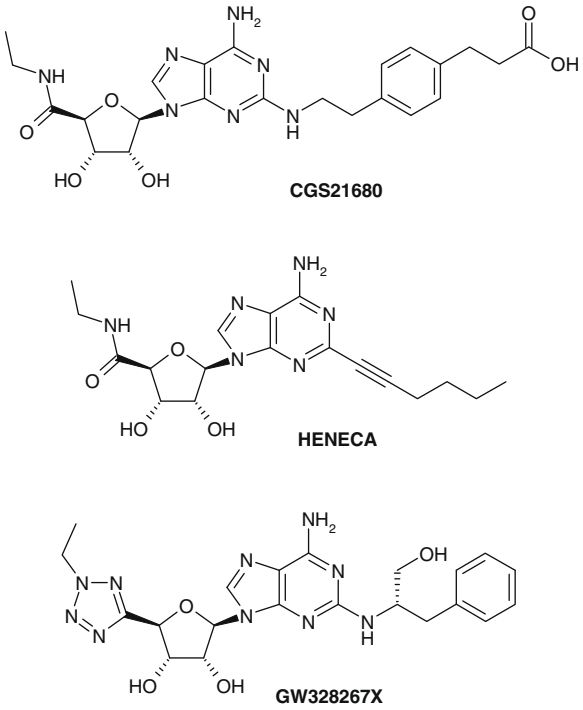


Figure 1. Structures of the adenosine A_{2A} receptor agonists CGS21680, HENECA and GW328267X.

the 4′-position provide the opportunity to generate reverse-amide variants of NECA and *N*-bonded heterocyclic variants of the *C*-bonded 4′-(2-tetrazolyl) residue present in GW328267X. The electron density comparisons shown in Figure 2 highlight the isosteric potential of these two 4′-aza-carbocyclic nucleoside series in which the 4′-ethyl carboxamide residue of NECA is compared with 4′-propionamide and 4′-methylpyrazole moieties as representative examples of the reversed amide and the *N*-bonded heterocyclic series.[12] Comparing the electron density surfaces, a high degree of spatial alignment is evident between the high electron density regions, highlighted in red, associated with the oxygen atoms of the carbonyl residue of NECA and the carbonyl residue of the reversed amide example. The carbonyl residue of NECA having been proposed to be involved in an important hydrogen bonding interaction with serine 277 on the seventh transmembrane spanning domain of the activated conformation of the adenosine A_{2A} receptor.[13]

Similarly for the *N*-bonded heterocycle series, the imino-nitrogen of the pyrrazole ring exhibits a comparable high electron density surface which also closely matches the position of the carbonyl residue of NECA. Thus, highlighting the need for an imino-nitrogen adjacent to the site of attachment to the carbocyclic ring in the 4′-heterocyclic series, if this key interaction is to be maintained. Additionally, the substitution of the pyrazole residue in the 4-position presents a methyl residue in a similar spatial orientation to the alkyl residues of both NECA and the reversed-amide series providing the opportunity for homologation along a similar vector. Interestingly, in contrast to the 4′-aza-carbocyclic nucleoside series, described above, the corresponding 4′-aza-ribose analogues would be anticipated to be of relatively low stability. The reason for the instability of such 4′-aza-ribose variants being as a consequence of their doubly-anomeric nature, and to the best of our knowledge no reports of either have been made.

The preparation of 4′-aza analogues of the carbocyclic nucleoside aristeromycin as antiviral agents, including reversed amide analogues, has been reported previously.[14] These derivatives have been prepared starting from either: (1*R*,3*S*)-4-cyclopentene-1,3-diol monoacetate in which the purine base is introduced via a palladium(0) catalysed allylic coupling reaction and the 4′-amino substituent via a Mitsunobu reaction, with azide as the nucleophile, or from the cycloaddition product 2-oxa-3-azabicyclo[2.2.1]hept-5-ene in which cleavage of the *N*-*O* bond provides the 4′-amino substituent directly as well as an allylic alcohol for the introduction of the purine base via a palladium(0) allylic coupling reaction. In both cases the 2′,3′-diol is introduced via a *cis*-dihydroxylation reaction with addition to the double bond required to occur from the sterically less hindered face. The nature of the substituents at the 1′- and 4′-positions having been shown to be important in controlling the facial selectivity of the *cis*-dihydroxylation reaction.[15] We had envisioned a similar approach starting from (1*R*,3*S*)-4-cyclopentene-1,3-diol monoacetate, but using two palladium(0) catalysed allylic coupling reactions to introduce both the purine base and the 4′-aza substituent, as outlined in Figure 3. This approach was selected because it was anticipated to provide: the opportunity for the shortest synthetic sequences, common intermediates to

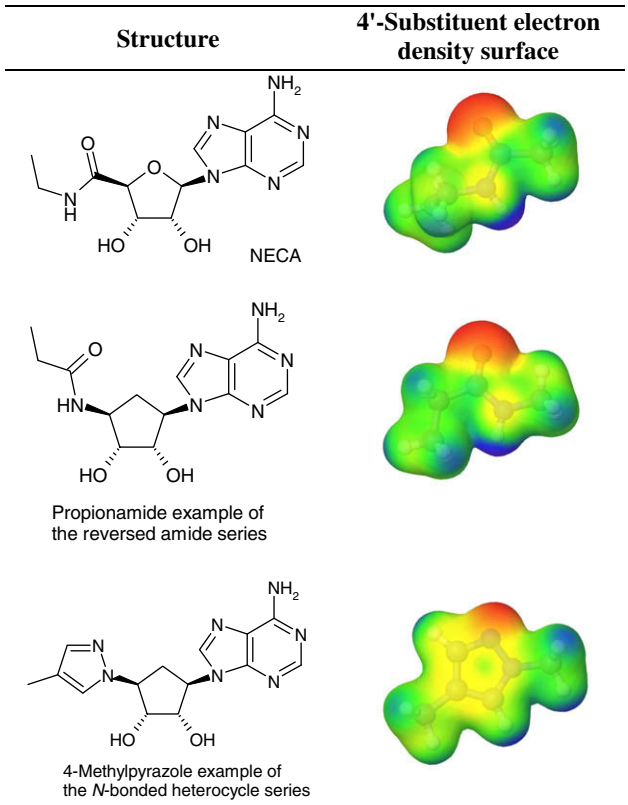


Figure 2. Electron density profiles of prototype reversed amide and *N*-bonded heterocyclic 4′-aza-carbocyclic nucleoside analogues in comparison with NECA.

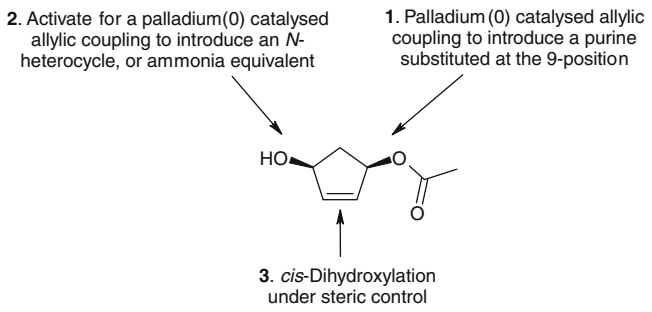
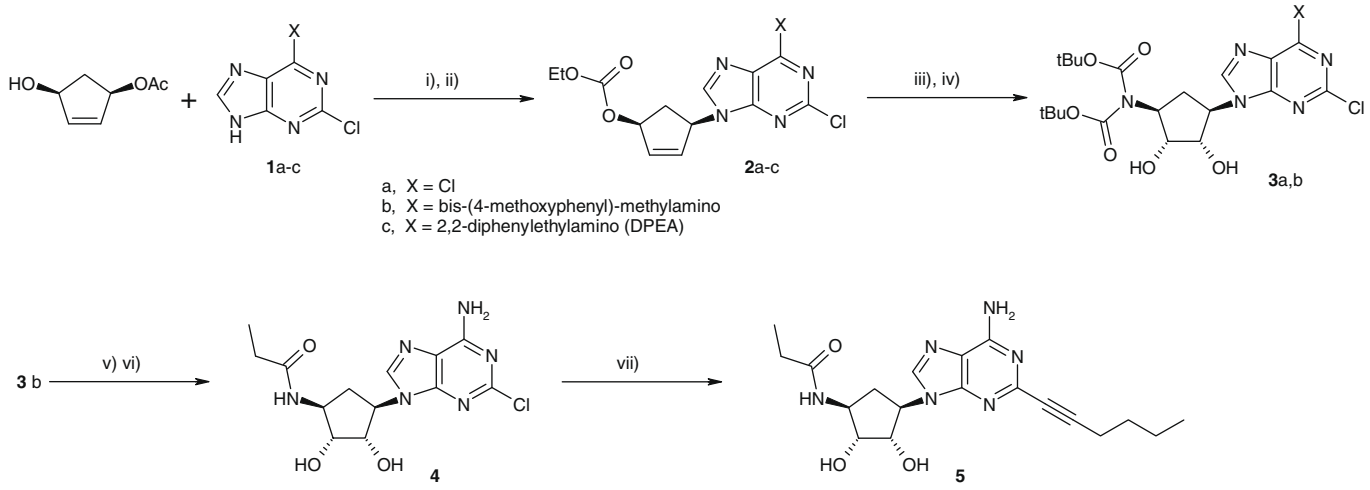


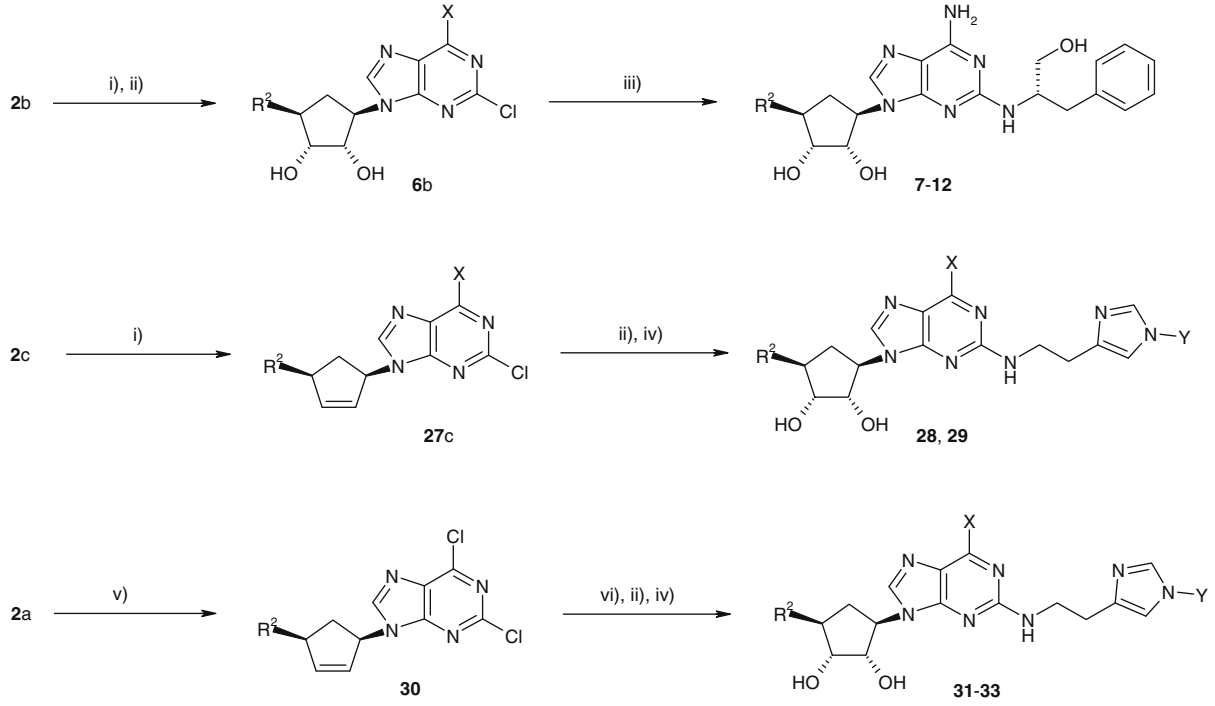
Figure 3. Key aspects of the synthetic approach to the 4′-aza-carbocyclic nucleoside series from (1*R*,3*S*)-4-cyclopentene-1,3-diol monoacetate.



Scheme 1. Synthesis of the 2-(1-hexynyl) 4′-aza-carbocyclic reversed amide nucleoside **5**. Reagents and conditions: (i) 1.0 equiv NaH, 0.05 equiv $(Ph_3P)_4Pd$, 0.15 equiv Ph_3P, 50:1 THF/DMSO, 2 h, 50 °C (a 45–62%, b,c 64–84%); (ii) to prepare **2a**: 4 equiv EtOC(O)Cl, 3 equiv pyridine, THF, 1 h 0 °C (67–75%), or to prepare **2b**,**c**: 2 equiv 3-ethoxycarbonylbenzotriazole-1-oxide, cat. DMAP, 5:1 *i*-Pr_2NEt/pyridine, 18 h, rt (65–86%); (iii) to prepare **3a**: 1.1 eqiuv $(Boc)_2NH$, 0.05 equiv $Pd_2(dba)_3$, 0.15 equiv Ph_3P, THF, 3 h, rt (23–50%), or to prepare **3b**: 1.1 eqiuv $(Boc)_2NH$, 0.05 equiv $(Ph_3P)_4Pd$, 0.15 equiv Ph_3P, THF, 3 h, rt (32%); (iv) 0.15 equiv OsO_4, 2 equiv NMMO, 10:1 THF/H_2O, 18 h, rt (58–89%); (v) 2:1 CH_2Cl_2/CF_3CO_2H, 18 h, rt (75%); (vi) 1 equiv propionyl chloride, 5 equiv *i*-Pr_2NEt, THF, 3 h, rt (>90%); (vii) 10 eqiuv 1-hexyne, 0.13 equiv CuI, 0.13 equiv $(Ph_3P)_2PdCl_2$, 0.25 equiv Ph_3P, 2:1 Et_2NH/DMF, 3 h, 120 °C Biotage Initiator™ microwave (46%).

prepare both the reversed amide and *N*-bonded heterocyclic series and favourable selectivities in the *cis*-dihydroxylation reaction.

To start exploring the two 4′-aza-carbocyclic nucleoside series, the reversed amide analogue of HENECA and *N*-bonded heterocyclic analogues of GW328267X were selected as the initial targets for synthesis. Starting from (1*R*,3*S*)-4-cyclopentene-1,3-diol monoacetate, palladium(0) catalysed allylic coupling reactions with the sodium anions of the purine derivatives **1a–c** provided the products coupled at the 9-position in good yield, Scheme 1.[16] Small amounts of an isomeric product, presumed to be the 7-substituted purine analogues, were identified in the crude reaction mixtures from these allylic coupling reactions and were removed upon purification. Subsequent acylation to prepare the ethyl carbonate **2a** proceeded smoothly with ethyl chloroformate. Similarly, for the 6-substituted-amino analogues, acylation with 3-ethoxycarbonylbenzotriazole-1-oxide was found to give improved yields of the carbonates **2b,c**.[17] Starting from **2b** to prepare the reversed amide series, a second palladium(0) catalysed allylic coupling reaction

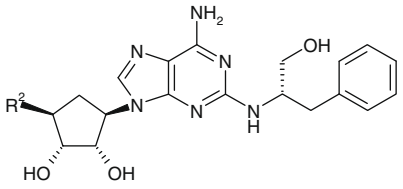


Scheme 2. Synthesis of 4′-aza-carbocyclic *N*-bonded heterocyclic nucleosides. Reagents and conditions: (i) 1.1 equiv *N*-heterocycle (R^2–H), 0.05 equiv $(Ph_3P)_4Pd$, 0.15 equiv Ph_3P, THF, 3 h, rt (27–95%); (ii) 15 mol % OsO_4, 2 equiv NMMO, 10:1 THF/H_2O, 18 h, rt (20–71%); (iii) 5 equiv (*S*)-phenylalaninol, 1,2-dichlorobenzene, 3 h, 240 °C Biotage Initiator™ microwave (10–33%); (iv) 5 equiv histamine derivative, DMSO, 8 h, 160 °C (31%), or 5 equiv histamine derivative, 1 equiv NaI, 1:1 CH_3CN/NMP, 1–2 h, 200–240 °C Biotage Initiator™ microwave (27–42%); v) 1.1 equiv *N*-heterocycle, 0.05 equiv $Pd_2(dba)_3$, 15 mol % Ph_3P, THF, 1 h, 50 °C (68–72%); (vi) 1.1 equiv amine, 1.2 equiv *i*-Pr_2NEt, THF, 2–24 h, 35–50 °C (70–94%).

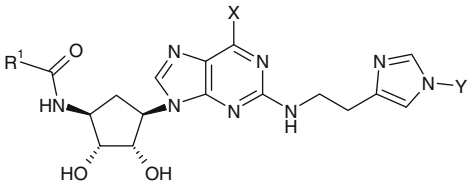
with bis-(*tert*-butoxycarbonyl)-amine as the nucleophile introduced the 4′-aza functionality with the anticipated high level of regio and stereoselectivity. Dihydroxylation of the 2,3-double bond then proceeded under steric control with a high degree of facial selectivity to give the intermediate **3b** bearing all the key functionality of the targeted compounds. To prepare the reversed amide analogue of HENECA, treatment of **3b** with trifluoroacetic acid removed the *tert*-butoxycarbonyl (Boc) and 4,4′-dimethoxybenzhydryl (DMBH) protecting groups from the two amino residues. Acylation of the free base form of the diamino intermediate gave the 4′-propionamide **4** which then underwent a Sonogashira coupling with 1-hexyne to give the targeted product **5**. Similarly to prepare the *N*-bonded heterocyclic analogues of GW328267X the ethyl carbonate **2**b underwent palladium(0) catalysed allylic coupling reactions with a series of *N–H* heterocycles to give the corresponding N-allylated analogues, as shown in Scheme 2 with the substituents detailed in Table 1. Isolated yields for these N-allylation reactions ranged from good for the pyrazole analogues (76–95%), to modest in the case of the pyridine-2-one (47%) and indazole (27%) examples. In an analogous fashion to the preparation of **5**, dihydroxylation of the N-allylated heterocycles proceeded with a high level of facial selectivity to give the intermediates **6b**. Finally, S_NAr reactions to introduce (*S*)-phenylalaninol into the purine 2-position of the intermediates **6b** occurred under elevated temperatures which also resulted in the removal of the DMBH protecting groups to give directly the targeted products **7**–**12**. The modest yields observed in these final coupling reactions were not improved upon if a two-step approach was followed in which the DMBH group was removed prior to displacement of the 2-chloro substituent.

To further explore the 4′-aza carbocyclic nucleosides, examples bearing histamine 2-substituents were selected for synthesis in both the reversed amide and *N*-bonded heterocyclic series. This selection was based upon the NECA analogues having been reported to be highly potent and selective adenosine A_{2A} receptor agonists with good solubility profiles.[18] The reversed amide examples were prepared by an analogous sequence to that described above as outlined in Scheme 3 with the substituents detailed in Table 1. Starting from **3a**, displacement of the 6-chloro substituent with either 2,2-diphenylethylamine or 1-ethylpropylamine was followed by Boc deprotection to give the 4′-primary amines **13c,d**. Subsequent acylation of **13c,d** with a range of acid chlorides

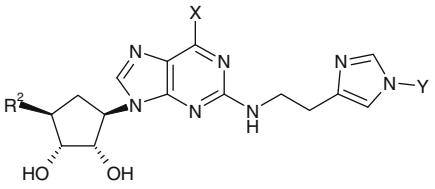
Table 1
Purinergic P1 receptor binding and functional data



General structure **7**-**12**



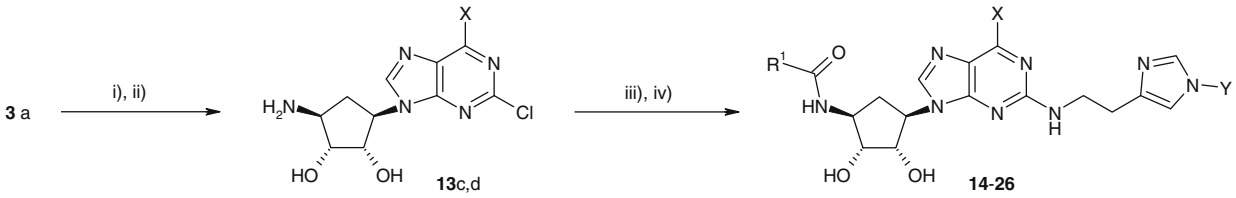
General structure **14**-**26**



General structure **28**, **29**, **31**-**33**

Compound	R^1 (reversed amide)/R^2 (*N*-bonded heterocycle)	X	Y	A_{2A} K_i (nM)	Neutrophil IC_{50} (nM)	A_1 EC_{50} (nM)	A_{2B} EC_{50} (nM)	A_3 EC_{50} (nM)
NECA				25	16	30	38	63
CGS21680				21	19	>10,000	>10,000	613
GW328267				2.3	0.4	882	51	4.2[a]
5	Et			18	12	>10,000	>10,000	105
7	1-Pyrazolyl			702	nd	nd	nd	nd
8	4-Methyl-1-pyrazolyl			229	56	>10,000	>10,000	449[a]
9	4-Ethyl-1-pyrazolyl			182	62	>10,000	>10,000	776[a]
10	4-Phenyl-1-pyrazolyl			>10,000	nd	nd	nd	nd
11	1-Indazolyl			5406	nd	nd	nd	nd
12	1-Pyridin-2-one			5491	nd	nd	nd	nd
14	Et	DPEA	H	5.2	5.9	>10,000	>10,000	139
15	Et	DPEA	Me	4.6	4.7	>10,000	nd	42
16	Et	DPEA	Et	5.2	5.0	nd	nd	44
17	Et	EPA	Et	5.4	2.7	>10,000	9866	1640
18	Et	EPA	*i*-Pr	8.0	8.3	>10,000	nd	2520
19	*n*-Pr	DPEA	*i*-Pr	39	160	nd	nd	nd
20	*i*-Pr	DPEA	*i*-Pr	27	67	nd	nd	nd
21	*tert*-Bu	DPEA	*i*-Pr	382	542	nd	nd	nd
22	CycloPr	DPEA	*i*-Pr	16	60	>10,000	nd	357[a]
23	CycloBu	DPEA	*i*-Pr	7.9	40	>10,000	nd	480[b]
24	CH_2Ph	DPEA	*i*-Pr	1666	nd	nd	nd	nd
25	CH_2CH_2Ph	DPEA	*i*-Pr	5472	nd	nd	nd	nd
26	Et	DPEA	CH_2CO_2H	97	nd	nd	nd	nd
28	4-Ethyl-1-pyrazolyl	DPEA	Et	146	nd	nd	nd	nd
29	5-Methyl-2-tetrazolyl	DPEA	*i*-Pr	38	101	nd	nd	nd
31	4-Ethyl-1-pyrazolyl	EPA	Et	34	169	>10,000	nd	>10,000
32	4-Hydroxymethyl-1-pyrazolyl	DPEA	Et	5.4	5.3	4100	>10,000	789[a]
33	2-(4-Ethyl-1,2,3-triazolyl)	DPEA	Et	48	nd	nd	nd	nd

All data are expressed as means from duplicates derived from 1 to 5 separate experiments.
All functional assay EC_{50} data reflect agonist activity unless otherwise indicated.
nd; not determined.
[a] Most potent activity of the compound determined to be >50% antagonism of the I-AB-MECA response.
[b] Most potent activity determined to be a partial agonism, producing 48% the maximal response of I-AB-MECA.



Scheme 3. Synthesis of the 2-histamine 4′-aza-carbocyclic reversed amide nucleosides **14–26**. Reagents and conditions: (i) 1.1 equiv 2,2-diphenylethylamine or 1-ethylpropylamine, 1.2 equiv *i*-Pr_2NEt, THF, 24 h, 50 °C (91–95%); (ii) 2:1 CH_2Cl_2/CF_3CO_2H, 18 h, rt (74–75%); (iii) 1 equiv acid chloride ($R^1C(O)Cl$), 5 equiv *i*-Pr_2NEt, THF, 3 h, rt (>90%); (iv) for the preparation of **14–25**: 5 equiv histamine derivative, 1,2-dichlorobenzene, 1–2 h, 200–240 °C Biotage Initiator™ microwave (19–48%), or to prepare **26**: 5 equiv histamine derivative, 1 equiv NaI, 1:1 CH_3CN/NMP, 1 h, 200 °C Biotage Initiator™ microwave (41%).

and then displacement of the 2-chlorosubstituent with a series of histamine derivatives (Y = H, Me, Et, *i*-Pr and $CH_2CO_2C_6H_{11}$) gave the products **14–26**.[19] The carboxylic acid example **26** being isolated directly from the S_NAr reaction following an in situ hydrolysis of the histamine acetic acid cyclohexyl ester derivative. Synthesis of the *N*-bonded heterocyclic analogues is shown in Scheme 2 with the substituents detailed in Table 1. Starting from the 6-DPEA substituted carbonate **2c**, 4-ethylpyrazole and 5-methyltetrazole were introduced by allylic coupling reactions using the $(Ph_3P)_4Pd/Ph_3P$ catalyst system to give the intermediates **27c**. In the case of 5-methyltetrazole a single regioisomer was obtained exhibiting spectral properties consistent with the 2-substituted derivative. Only approximately 10% of an isomeric product was present in the crude allylic coupling reaction mixture for this example, consistent with formation of a small amount of the 1-substituted tetrazole. Dihydroxylation of the intermediates **27c** was then followed by S_NAr reactions to deliver the products **28** and **29**. Similarly the 2,6-dichlorocarbonate **2a** underwent allylic coupling reactions with 4-ethylpyrazole, 4-hydroxymethylpyrazole and 4-ethyl-1,2,3-triazole to give the intermediates **30** using a $Pd_2(dba)_3/Ph_3P$ catalyst protocol. For the 1,2,3-triazole example again predominantly a single product was obtained in the allylic coupling reaction and determined to be the 2-substituted regioisomer by NMR spectroscopy. Dihydroxylation and S_NAr reactions with the intermediates **30** then yielded the targeted products **31–33**.

To assess the activity of the compounds against the human adenosine A_{2A} receptor: affinity was measured in a radioligand binding assay versus [^{3}H]-NECA with membranes produced from SF21 cells coexpressing recombinant human adenosine A_{2A} receptors and the human G protein subunits $G\alpha s_2$, β_4 andg_2. Functional adenosine A_{2A} activity was assessed by the ability to inhibit fMLP induced release of reactive oxygen species from isolated human neutrophils.[20] Selectivity versus the other human P1 purinoceptors was assessed in a series of functional assays: GTPγS for the adenosine A_1 and A_3 receptors expressed in Chinese hamster ovary (CHO) cells, in both agonist and antagonist modes versus the ligands NECA and I-AB- MECA, respectively; and with a CRE-luciferase reporter gene assay measuring cAMP production in CHO cells expressing the adenosine A_{2B} receptor, with NECA as the reference agonist. All the data are shown in Table 1.

Comparison of the data generated for the reversed amide HENECA analogue **5** with the human data reported for the parent compound shows: a similar high level of affinity for, and functional activity at, the adenosine A_{2A} receptor; a comparable high level of selectivity over the adenosine A_1 and A_{2B} receptors; and modest 10-fold selectivity over the adenosine A_3 receptor.[21] In contrast, the most active 4-methyl and 4-ethylpyrazole *N*-bonded heterocyclic analogues **8** and **9** of GW328267X are 100-fold less active adenosine A_{2A} receptor agonists compared to the parent compound. However, **8** and **9** do possess similar P1 receptor selectivity profiles to GW328267X, including antagonist activity against the I-AB-MECA activation of the adenosine A_3 receptor. The pyrazole 4-methyl and 4-ethyl substituents proved more effective than the unsubstituted example **7**. The larger phenyl residue in **10** was not tolerated and neither was the fusion of the phenyl ring in the indazole example **11**. Incorporation of pyridin-2-one in example **12**, as a cyclic reversed amide analogue, showed little affinity for the adenosine A_{2A} receptor. The weak activity of **12** is also consistent with the requirement for an imino-nitrogen adjacent to the site of attachment to the carbocyclic ring for the *N*-bonded heterocyclic series, as hypothesised from the electron density analysis.

Analysis of the data from the 2-histamine reversed-amide analogues showed all the propionamide examples **14–18** to be potent adenosine A_{2A} receptor agonists with both the 2,2-diphenylethylamino (DPEA) and 1-ethylpropylamino (EPA) purine 6-substituents. High functional selectivity over the other P1 receptors was also observed, in particular for the 6-EPA substituted examples **17** and **18** where greater than 100-fold selectivity was determined over the closely related adenosine A_3 receptor. The nature of the imidazole substituent Y, as methyl, ethyl, isopropyl or in the unsubstituted form in examples **14–18** was seen to have little impact on the overall P1 receptor profiles for these examples. Increasing the size of the amide group reduced the affinity and functional activity at the adenosine A_{2A} receptor: the single homologation to the *n*-propyl, isopropyl and cyclopropyl analogues **19**, **20** and **22** resulted in a 10–30-fold drop in functional potency relative to the propionamide. Interestingly, for the larger cyclopropyl and cyclobutyl analogues **22** and **23** this also resulted in a drop in intrinsic efficacy at the adenosine A_3 receptor. These derivatives exhibited partial agonist and antagonist adenosine A_3 activities, respectively, relative to the fuller agonism seen for the propionamide examples **14** and **15**. The histamine acetic acid derivative **26**, with a carboxylic acid residue in a similar position to that of CGS21680, surprisingly showed a 20-fold drop in affinity towards the adenosine A_{2A} receptor relative to the alkyl substituted analogues **15** and **16**. Assessing the data for the *N*-bonded heterocyclic 2-histamine analogues: the methyl and ethyl substituted pyrazole, 1,2,3-triazole and tetrazole examples **28**, **29**, **31** and **33** all showed only modest affinity for the adenosine A_{2A} receptor which translated into a similar level of functional activity for the two examples tested. Of greater interest was the 4-hydroxymethylpyrazole analogue **32** which exhibited sub 10 nM adenosine A_{2A} receptor affinity and functional activity. In terms of selectivity, greater than 100-fold selectivity was observed over the other P1 purinoceptors for **32**, which again exhibited antagonist activity at the adenosine A_3 receptor. Presumably the increase in activity and selectivity for compound **32**, relative to the other *N*-bonded heterocyclic derivatives, is as a consequence of a favourable interaction of the hydroxymethyl residue of the pyrazole moiety with the adenosine A_{2A} receptor.

Interspecies cross-reactivity variation has been described for a number of purinergic P1 receptor ligands as a consequence of variations in the amino acid receptor sequences.[22] Additionally, our primary interest related to the potential for adenosine A_{2A} receptor agonists as anti-inflammatory agents, where consistent responses can be established across a wide range of human inflammatory cell

Table 2
Comparison of the inhibition of TNF-α release in rat and human LPS stimulated PBMC assays

Compound	PBMC IC_{50} (nM)	
	Human	Rat
NECA	40	187
CGS21680	58	83
GW328267	1.3	2.9
17	4.7	255
18	5.9	458
32	21	3591

All data are expressed as means from 2 to 5 separate experiments.

types.[3] In contrast, reproducing these A_{2A} responses in the equivalent inflammatory cell types of lower species was found to be problematic. After reviewing several test systems an equivalent adenosine A_{2A} dependant response was established for the inhibition of the LPS induced release of tumour necrosis factor alpha (TNF-α) from isolated human and rat peripheral blood mononuclear cells (PBMC).[23] Using this test system it was possible to assess the extent of any cross-reactivity differences between human and rat as a consequence of receptor sequence variation. Cross-reactivity data for the most interesting 4′-azacarbocyclic nucleoside examples and the reference compounds are shown in Table 2. The three reference compounds exhibited modest 2–5-fold shifts to lower IC_{50} values in the rat relative to the human PBMC assay. In contrast, the reversed amide analogues **17** and **18** demonstrated 54- and 78-fold shifts in activity, respectively. Even more dramatic was the 171-fold shift observed for the *N*-bonded heterocyclic example **32**. These data highlight that an understanding of interspecies cross-reactivity, as a consequence of amino acid sequence differences, will be an important parameter for the preclinical optimisation of both the *N*-bonded heterocycle and reversed amide 4′-aza carbocyclic nucleoside series.

In summary, reversed amide and *N*-bonded heterocycle 4′-aza carbocyclic nucleosides were identified as scaffolds of interest through in silico electron density comparisons with the known adenosine A_{2A} receptor agonist NECA. An efficient synthetic route to these two 4′-aza carbocyclic nucleoside series is described which utilises two successive palladium(0) allylic coupling reactions as the key transformations. Following this approach, highly potent and selective agonists of the human adenosine A_{2A} receptor have been identified in both the *N*-bonded heterocycle and reversed amide series by employing established purine substitution patterns. Using these established purine substitution patterns the propionamides **14**–**18** and the 4-hydroxymethylpyrazole **32** were determined to be the most potent and selective examples from the 4′-reversed amide and 4′-*N*-bonded heterocyclic series, respectively. An assessment of the interspecies variability highlighted relatively poor rat cross-reactivity for the most potent examples at the human adenosine A_{2A} receptor from both 4′-aza carbocyclic nucleoside series. The optimisation of these two 4′-aza carbocyclic nucleoside series as lung-targeted adenosine A_{2A} receptor agonists for use as inhaled anti-inflammatory agents will be the subject of future publications.

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