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Design, Synthesis and Evaluation of N^6-Substituted 2-Aminoadenosine-5'-*N*-methylcarboxamides as A_3 Adenosine Receptor Agonists

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A series of N^6-substituted 2-aminoadenosine-5'-*N*-methylcarboxamides were synthesized from the versatile intermediate, O^6-(benzotriazol-1-yl)-2-amino-2',3'-*O*-isopropylideneinosine-5'-*N*-methylcarboxamide (**1**). These compounds were evaluated as A_3 adenosine receptor agonists in terms of their potency and receptor sub-type selectivity in a cAMP accumulation assay and a number of potent and selective compounds were identified. One such compound, 2-amino-N^6-(3-chlorobenzyl)adenosine-5'-*N*-methylcarboxamide (**3i**), was over 500-fold selective for the A_3AR over the other three adenosine receptor subtypes. This represents a significantly greater selectivity profile compared with the prototypical IB-MECA.

Introduction

Nitrogen-containing heterocycles are ubiquitous in nature, including purine nucleosides such as adenosine and guanosine. Adenosine is involved in a host of physiological processes through interaction with adenosine receptors, of which there are four distinct receptor subtypes, namely A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs).[1,2] This family A or rhodopsin-like purinergic G protein-coupled receptor (GPCR) mediates a number of important biological functions and agonists acting specifically at the A_3AR have been shown to be involved in cancer, inflammation and cardioprotection.[3] Many pharmacologically relevant A_3AR agonists are known and have a diverse range of potential therapeutic applications. Selected examples are shown in Figure 1. IB-MECA is currently in clinical trials for psoriasis, rheumatoid arthritis, dry eye disease and glaucoma. The structurally related Cl-IB-MECA has potential indications for hepatitis and liver cancer.[4] Meanwhile, CP-532,903 has anti-inflammatory effects and is involved in myocardial ischemia[5] and reducing superoxide production in damaged tissues.[6]

With the recent emergence of GPCR crystal structure determination and the realisation that the seven transmembrane (7TM) topology is common throughout many family A GPCRs, such as the CXCR4 chemokine,[7] D3 dopamine,[8] and β1 and β2 adrenergic receptors,[9,10] a new frontier has opened up in GPCR drug discovery. Highlighting this paradigm shift, the A_{2A}AR crystal structure with a bound antagonist (ZM241385) has been reported.[11] Increasingly, this has resulted in greater insights aimed at exploiting this information through chemical modulation of putative small molecules targeting this receptor, by the identification of selective A_{2A}AR agonists, such as UK-432097 (Fig. 1).[12,13] A common feature of these molecules is the incorporation of bulky groups substituted through the 2-position, typically with retention of the N^6-amino group present in the parent molecule, adenosine, as seen by CGS-21680.[14] However, retention of a 2-amino group, such as that present in guanosine, has not been explored to determine the selectivity profile and affinity for this series of molecules at the four distinct adenosine receptor subtypes. We envisaged retaining an N^6-bulky substituent known to give rise to A_3AR selectivity, whilst the added 2-amino group present could potentially lead to the identification of an A_3AR selective molecule. Another modification tolerated in known A_{2A}AR agonists is that of the incorporation of an 5'-ethylcarboxamido group,[12,13] whereas tuning the selectivity towards the A_3AR might be achieved by adaptation of the 5'-position. From these scaffolds and other known A_3AR agonists, a few key qualities demonstrate improvements in A_3AR selectivity over the other subtypes. A significant feature that imparts selectivity towards A_3AR specificity is the inclusion of a 5'-*N*-methylcarboxamido moiety, as demonstrated in Figure 1. Incorporation of an N^6-substitution comprising benzyl functionality imbued with a 3-halo moiety gives enhanced A_3AR selectivity as well. The addition of a 2-substituted halogen, typically a chloro group engenders even greater activity at the A_3AR, however anything bulkier such as pyrazolo or alkyne linked groups as demonstrated by Regadenoson and Apadenoson impart A_{2A}AR selectivity.[15-17] Whilst the effect of a range of 2-substituents on adenosine receptor affinity has been studied,[18] there is a paucity of information on the influence of 2-amino and substituted 2-amino groups. Our study addresses this shortfall in understanding.

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Electronic Supplementary Information (ESI) available: Experimental methods, adenosine receptor agonist synthesis, cAMP assay. See DOI: 10.1039/c000000x/

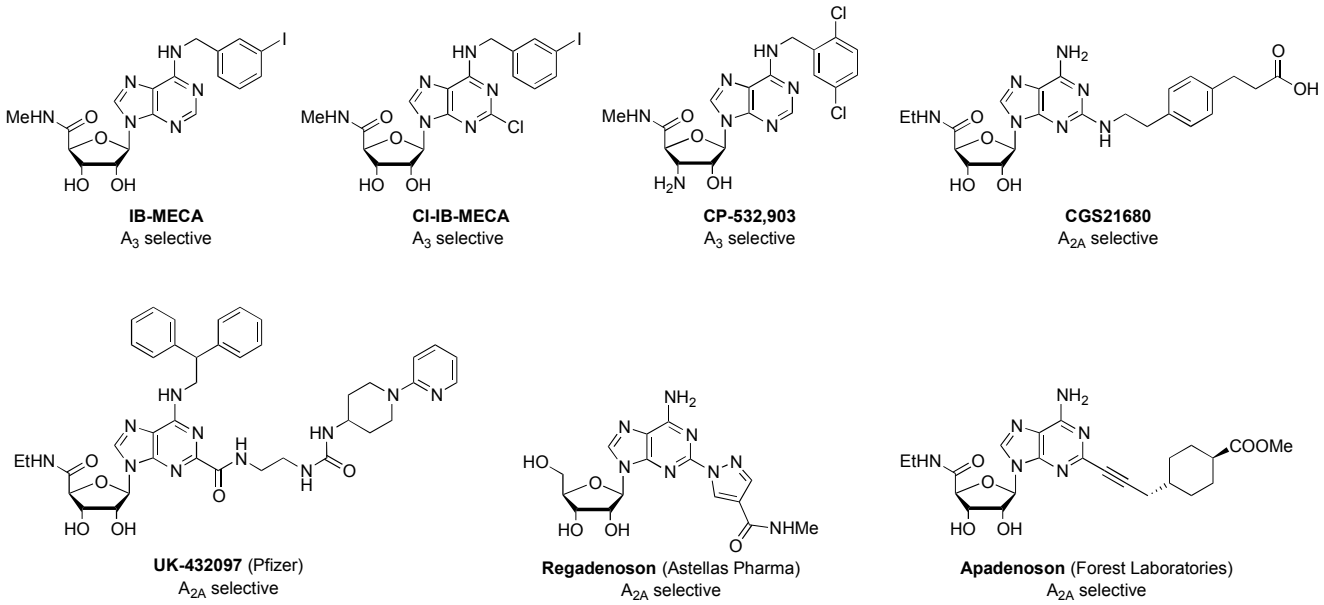


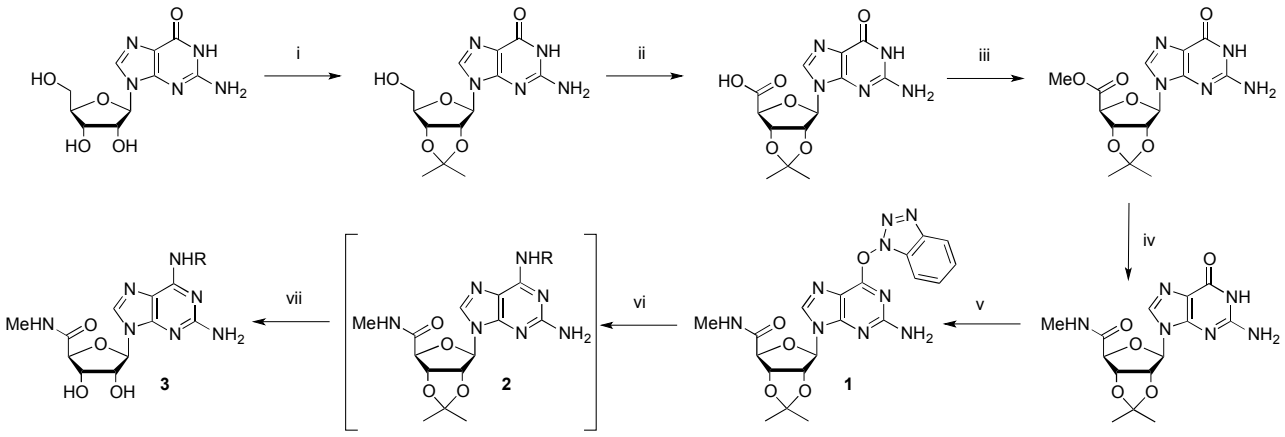
Fig. 1 Pharmacologically relevant AR agonists

Chemistry

As part of our ongoing program to develop potent and selective adenosine agonists, we recently described the synthesis of O^6-(benzotriazol-1-yl)-2-amino-2',3'-*O*-isopropylideneinsoine-5'-*N*-methylcarboxamide (**1**) using a phosphonium-mediated coupling approach via C−OH activation of guanosine over 5 steps.[19,20]

Firstly, the 2',3'-diol moiety present in guanosine was protected as the corresponding 2',3'-isopropylidene group with acetone, *p*-tosic acid and dimethoxypropane, greatly improving solubility for further chemical manipulation (Scheme 1). Oxidation using TEMPO and BAIB afforded the corresponding 5'-carboxylic acid. The 5'-methylcarboxamide was subsequently achieved via esterification with thionyl chloride in methanol followed by nucleophilic displacement with $MeNH_2$. Finally, reaction with BOP, in the presence of DBU introduced the desired O^6-benzotriazol-1-yl moiety (compound **1**). This versatile intermediate retains the highly A_3AR selective 5'-*N*-methylcarboxamido functionality and is set up for nucleophilic attack at the 6-position with its incorporation of a labile O^6-(bentriazol-1-yl) motif.

A host of nitrogen-bearing nucleophiles were selected to demonstrate the pharmacological character of this class of N^6-substituted, 2-aminoadenosine-5'-*N*-methylcarboxamides. Firstly, N^6-substitution occurred by reaction of **1** with various amines in the presence of a non-nucleophilic organic tertiary amine base (DIPEA) in *t*-BuOH to give the 2',3'-*O*-isopropylidene protected N^6-substituted adenosine-5'-*N*-methylcarboxamides (**2a**–**l**). Secondly, deprotection of the diol took place under hot aqueous acidic conditions; aided by the inclusion of MeCN, to facilitate solubilisation of the reagents. This generated N^6-substituted 2-aminoadenosine-5'-*N*-methylcarboxamides (**3a**–**l**) in moderate to good yields (ranging from 58–78% over 2 steps). These compounds were subsequently evaluated in a cAMP functional assay (Table 1).

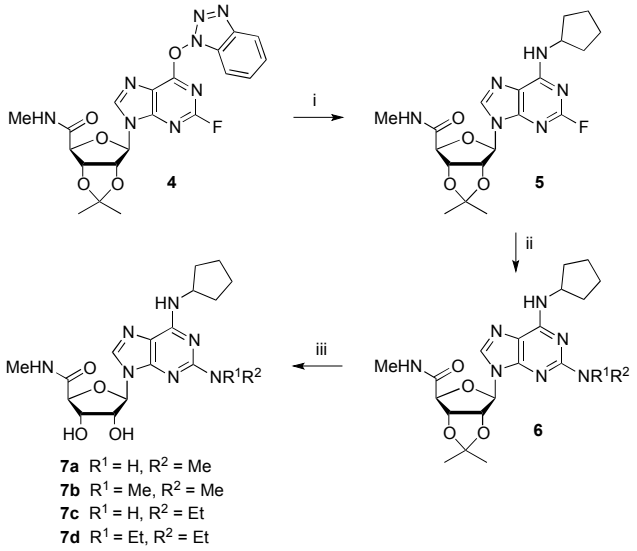


Scheme 1. $(CH_3)_2C(OCH_3)_2$, 25 °C, 16 h; 94%;[19] (ii) TEMPO, BAIB MeCN/H_2O (1:1), 25 °C, 16 h, 72%;[19] (iii) $SOCl_2$, MeOH 0 °C → 25 °C, 16 h, 61 %;[19] (iv) 2.0 M $MeNH_2$ in THF, MeOH/DMF (9:1), 100 °C, MW, 2 h, 100%;[20] (v) BOP, DBU, MeCN, 25 °C, 16 h, 92%;[20] (vi) R–NH_2, *t*-BuOH, DIPEA, 80 °C, 2 h; (vii) MeCN/1M HCl (4:1), 60 °C, 1 h, 58–78%.

A small series of *N*-alkyl and *N*,*N*-dialkylamino analogs were prepared in order to assess the influence of these substituents on AR potency and receptor subtype selectivity. These compounds were prepared from O^6-(benzotriazol-1-yl)-2-fluoroinosine derivative **4** (Scheme 2). Installation of the N^6-cyclopentyl group was achieved by reaction of O^6-(benzotriazol-1-yl)-2-fluoro-2',3'-*O*-isopropylideneinosine-5'-*N*-methylcarboxamide (**4**) with cyclopentyl amine in *t*-BuOH with DIPEA. A second substitution at the 2-position was introduced by heating the required amine at 150 °C under microwave conditions, displacing the fluorine. Deprotection of the 2',3'-*O*-isopropylidene was achieved with 1M HCl and MeCN to give N^6-(cyclopentyl), 2-*N*-substituted adenosine-5'-*N*-methylcarboxamides (**7**).

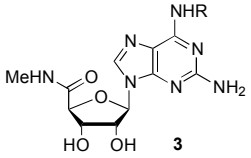
Pharmacology

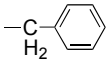
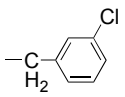
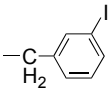
In general the N^6-substituted 2-aminoadenosine-5'-*N*-methylcarboxamides demonstrated A_3AR selectivity (Table 1). In some cases, large selectivity increases were seen, with four compounds showing more than 100-fold selectivity for the A_3AR over all of the other receptor subtypes (namely compounds **3a**, **3b**, **3i** and **3j**). The 2-aminoadenosine-5'-*N*-methylcarboxamides with alkyl groups in the N^6-position (compounds **3a**–**d**) had relatively similar potencies with pEC_{50}'s ranging from 7.4–8.3.



Scheme 2. Reagents and conditions: (i) CyclopentylNH$_2$, *t*-BuOH, DIPEA, 80 °C; (ii) R$_2$NH or RNH$_2$, *t*-BuOH, 150 °C (iii) MeCN/1M HCl (4:1), 60 °C, 48–72%.

Table 1. pEC_{50} data (cAMP accumulation assay) of compounds **3a-l** at all AR subtypes



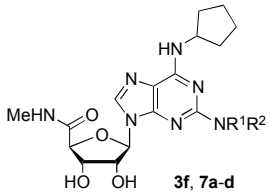
	R	pEC_{50} A_1AR	pEC_{50} A_{2A}AR	pEC_{50} A_{2B}AR	pEC_{50} A_3AR	A_3/A_1	A_3/A_{2A}	A_3/A_{2B}
NECA	–	8.9 ± 0.2	7.9 ± 0.2	8.5 ± 0.3	8.5 ± 0.2	0	4	1
IB-MECA	–	7.5 ± 0.2	7.1 ± 0.1	7.5 ± 0.2	9.3 ± 0.2	63	158	63
CGS21680	–	6.3 ± 0.2	7.9 ± 0.1	7.4 ± 0.2	8.0 ± 0.3	50	1	4
3a	–Me	5.4 ± 0.3	< 5	5.8 ± 0.4	8.3 ± 0.2	794	> 1995	316
3b	–Et	5.6 ± 0.2	< 5	5.8 ± 0.3	8.0 ± 0.2	251	> 1000	158
3c	–Pr	6.5 ± 0.1	< 5	5.3 ± 0.1	8.0 ± 0.2	32	> 1000	501
3d	–Bu	6.1 ± 0.2	< 5	5.9 ± 0.3	7.4 ± 0.2	20	> 251	32
3e		7.2 ± 0.1	5.6 ± 0.3	6.7 ± 0.3	8.3 ± 0.2	13	501	40
3f		7.4 ± 0.1	5.9 ± 0.2	6.1 ± 0.3	7.8 ± 0.4	3	79	50
3g		7.2 ± 0.1	6.1 ± 0.2	5.6 ± 0.2	6.9 ± 0.2	1	6	20
3h		5.2 ± 0.2	5.5 ± 0.2	6.9 ± 0.2	7.5 ± 0.4	200	100	4
3i		6.5 ± 0.1	6.4 ± 0.2	6.4 ± 0.3	9.2 ± 0.4	501	631	631
3j		6.6 ± 0.2	6.6 ± 0.3	6.8 ± 0.3	8.8 ± 0.3	158	158	100
3k	–CH_2CH_2Ph	6.5 ± 0.2	7.1 ± 0.1	6.8 ± 0.3	8.4 ± 0.3	79	20	40
3l	–CH_2CHPh_2	6.4 ± 0.2	6.1 ± 0.2	6.7 ± 0.2	8.3 ± 0.3	79	158	40

N^6-methyl containing **3a**, exhibited ~800 fold selectivity for A_3AR over the A_1AR and 300-fold selectivity over A_{2B}AR, exhibiting the highest potency of the alkyl chain derivatives with a pEC_{50} of 8.3. Increasing the carbon chain from methyl to butyl (**3a**–**d**), maintained activity, but decreased the A_3AR character of these molecules resulting in a reduction in selectivity. In terms of the saturated cycloalkyl N^6-substituents (**3e**–**g**), the cyclobutyl (**3e**), had a marked effect in terms of A_3AR over A_{2A}AR selectivity, with a 500-fold increase and a pEC_{50} of 8.3, comparable with the best of the *n*-alkyl derivatives, namely **3a**. As ring size increased from cyclobutyl to cyclohexyl, A_3AR potency decreased (pEC_{50} for **3e**, **3f** and **3g** = 8.3, 7.8 and 6.9, respectively). A corresponding reduction in A_3AR selectivity versus all other receptor subtypes was generally observed with increasing ring size. Unsurprisingly, the N^6-cyclopentyl group in **3f** did not display great selectivity between A_3AR and A_1AR, as N^6-cyclopentyladenosine (CPA) is a potent A_1AR selective molecule. However, **3f** did show a striking difference in A_{2A}AR and A_{2B}AR affinity, with 79-fold and 50-fold A_3AR selectivity, respectively. The inclusion of N^6-benzyl (**3h**) demonstrated the typical increased A_3AR selectivity profile expected, known when benzyl moieties are incorporated into adenosine compounds at the N^6-position. This however, was coupled with relatively modest activity with a measured pEC_{50} of 7.5. 2-amino-N^6-(3-chlorobenzyl)adenosine-5'-*N*-methylcarboxamide (**3i**) showed a substantial increase in pEC_{50} to 9.2, which is commensurate with IB-MECA's activity.

Interestingly, the A_3AR selectivity escalated significantly with 501-, 631- and 631-fold selectivity over the A_1, A_{2A} and A_{2B}AR respectively. This also equates to an 8-, 4- and 10-fold increase at the A_1, A_{2A} and A_{2B}AR, respectively, over IB-MECA, thus representing a highly selective molecule. The 3-iodobenzyl derivative (**3j**), was also highly A_3AR selective and more selective than IB-MECA with a slight reduction in potency (pEC_{50} = 8.8). Bulkier substituents attached at the N^6-position, as exhibited by the phenethyl (**3k**) and diphenylethyl (**3l**) compounds had similar potency to NECA (pEC_{50} = 8.4, for **3k** and pEC_{50} = 8.3, for **3l**, compared with NECA, pEC_{50} = 8.5). Unlike NECA however, **3k** and **3l** were 40-fold and 79-fold selective over A_1 and A_{2B}AR for the A_3AR. A_{2A}AR selectivity was even greater for **3l**, demonstrating a ~150-fold selectivity profile, compared with 20-fold for **3k**. This series of N^6-substituted, 2-aminoadenosine-5'-*N*-methylcarboxamides, personified by **3i** are highly efficacious A_3AR selective molecules, worthy of further study.

To determine the effect of the 2-amino group on potency and selectivity a small series of N^2-substitued molecules were prepared (Scheme 2). Installation of a substituent at the N^2-position greatly reduced activity in comparison to the parent **3f**, indicating the absolute necessity for retention of the 2-amino group for A_3AR selectivity. The *N*-methyl (**7a**) and *N*-ethyl (**7c**) had very similar affinities at the A_1AR and the A_3AR, but only modest A_3AR selectivity. The *N*,*N*-dimethyl (**7b**) and *N*,*N*-diethyl (**7d**) had further reduced activity.

Table 2. pEC_{50} (cAMP accumulation assay) of 2-*N*-substituted N^6-(cyclopentyl)adenosine-5'-*N*-methylcarboxamides at all AR subtypes



3f, **7a-d**

	R^1	R^2	pEC_{50} A_1AR	pEC_{50} A_{2A}AR	pEC_{50} A_{2B}AR	pEC_{50} A_3AR	A_3/A_1	A_3/A_{2A}	A_3/A_{2B}
NECA	–	–	8.9 ± 0.2	7.9 ± 0.2	8.5 ± 0.3	8.5 ± 0.2	0	4	1
IB-MECA	–	–	7.5 ± 0.2	7.1 ± 0.1	7.5 ± 0.2	9.3 ± 0.2	63	158	63
CGS21680	–	–	6.3 ± 0.2	7.9 ± 0.1	7.4 ± 0.2	8.0 ± 0.3	50	1	4
3f	H	H	7.4 ± 0.1	5.9 ± 0.2	6.1 ± 0.3	7.8 ± 0.4	3	79	50
7a	H	Me	6.4 ± 0.1	5.4 ± 0.2	< 5	7.0 ± 0.2	4	40	> 100
7b	Me	Me	< 5	5.3 ± 0.4	< 5	6.2 ± 0.3	> 16	8	> 16
7c	H	Et	6.3 ± 0.2	6.3 ± 0.1	< 5	7.4 ± 0.2	8	8	> 251
7d	Et	Et	< 5	< 5	< 5	5.6 ± 0.3	> 4	> 4	> 4

Conclusion

A series of N^6-substituted 2-aminoadenosine-5'-*N*-methylcarboxamides were designed and synthesised and their pharmacological properties were evaluated in a cAMP accumulation assay. A number of these compounds proved to be potent, selective A_3AR agonists and in particular 2-amino-N^6-(3-chlorobenzyl)adenosine-5'-*N*-methylcarboxamide (**3i**) and 2-amino-N^6-(3-iodobenzyl)adenosine-5'-*N*-methylcarboxamide (**3j**) showed massive selectivity increases over the other three receptor subtypes. These compounds also had similar potency, but much greater selectivity over IB-MECA, the classic selective A_3AR agonist.

Acknowledgments

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Graphical Abstract