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Design and synthesis of 3′-ureidoadenosine-5′-uronamides: effects of the 3′-ureido group on binding to the A_3 adenosine receptor

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Abstract—On the basis of high binding affinity at the A_3 adenosine receptor of 3′-aminoadenosine derivatives with hydrogen bonding donor ability, novel 3′-ureidoadenosine analogues were synthesized from 1,2:5,6-di-*O*-isopropylidene-D-glucose in order to lead to stronger hydrogen bonding than the corresponding 3′-aminoadenosine derivatives. However, the synthesized 3′-ureidoadenosine analogues were totally devoid of binding affinity, because 3′-urea moiety caused steric and electrostatic repulsions at the binding site of the A_3 adenosine receptor, leading to conformational distortion.

1. Introduction

Adenosine is an endogenous material and regulates many physiological functions through the four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3),[1] among which the A_3 adenosine receptor is the most recently identified subtype.[2]

The A_3 adenosine receptor is negatively coupled to adenylyl cyclase and positively coupled to phospholipase C, resulting in the increase of the Ca^{2+} level.[1] The selective agonism of A_3 adenosine receptors has been associated with anticancer activity,[3] cardioprotective activity,[4,5] and cerebroprotective activity,[6,7] while selective antagonists[8] for A_3 adenosine receptors are of potential clinical use for inflammation and asthma. Thus, the A_3 adenosine receptors have been promising targets for the development of clinically useful agents.

A number of N^6- and/or 2-substituted adenosine derivatives have been synthesized and evaluated for A_3 adenosine receptor agonistic activity.[9–11]

Among these compounds, N^6-(3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine (**1a**, IB-MECA) and 2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine (**1b**, Cl-IB-MECA) were found to be highly selective full agonists with high binding affinities (K_i = 1.8 ± 0.7, 1.4 ± 0.3 nM, respectively), at the human A_3 adenosine receptor (Fig. 1).[11,12] Fishman et al. have reported that IB-MECA (**1a**) exhibited anticancer activity by down regulating the Wnt signaling pathway.[3] This compound is now undergoing Phase II clinical trials as an anticancer agent. The 2-chloro analogue of IB-MECA (**1a**), Cl-IB-MECA (**1b**) also exhibited high binding affinity to the A_3 adenosine receptor, but due to its in vivo toxicity, is being widely used as a pharmacological tool, instead of being developed as a clinically useful agent.[12,13] Recently, on the basis of high binding affinity and selectivity of Cl-IB-MECA, we have reported the synthesis of the 3′-fluoro analogue of Cl-IB-MECA to determine if the 3′-hydroxyl group of Cl-IB-MECA acts as a hydrogen bonding donor or acceptor upon binding to the binding site of the A_3 adenosine receptor. From this

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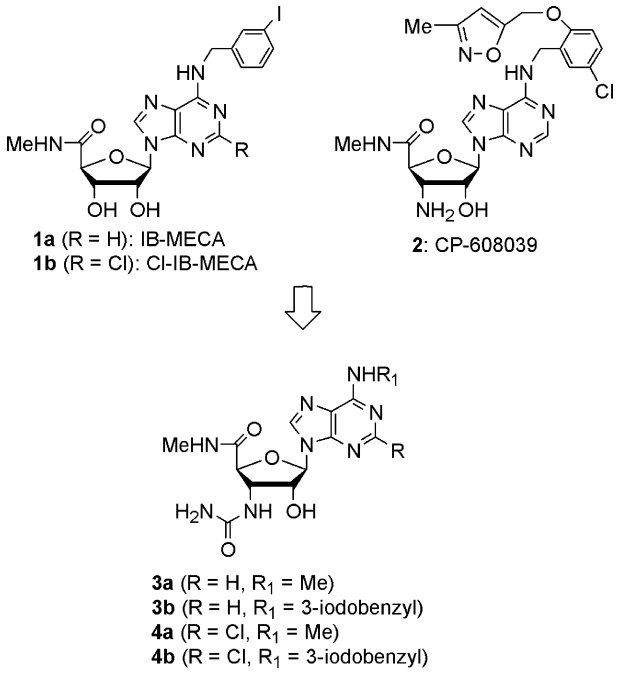


Figure 1. The rationale for the design of the desired nucleosides.

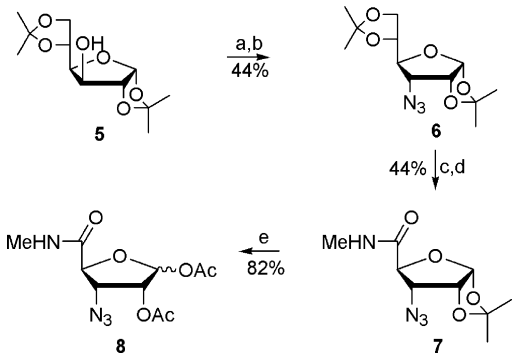
study, we concluded that the 3′-hydroxyl group might act as hydrogen bonding donor, but not acceptor.[14] This result was independently confirmed with 3′-aminoadenosine analogues, among which CP-608039 (**2**)[15] was reported to be a highly selective agonist of the human A_3 adenosine receptor, indicating that the 3′-amino group might play a key role as hydrogen bonding donor in the binding site of the human A_3 adenosine receptor.

Thus, on the basis of these results, it was very interesting to design 3′-ureidoadenosine analogues, **3** and **4** since the 3′-ureido moiety may form stronger hydrogen bonds in the binding site than the corresponding 3′-amino- or 3′-hydroxy-substituted nucleosides (Fig. 1). It is also of interest to find out whether the larger 3′-ureido group can be tolerated in the active site in comparison with smaller substituents that is the 3′-hydroxy or 3′-amino groups. Herein, we wish to report the efficient synthesis of novel 3′-ureidoadenosine derivatives and their effects on binding to the A_3 adenosine receptor.

2. Results and discussion

2.1. Synthesis

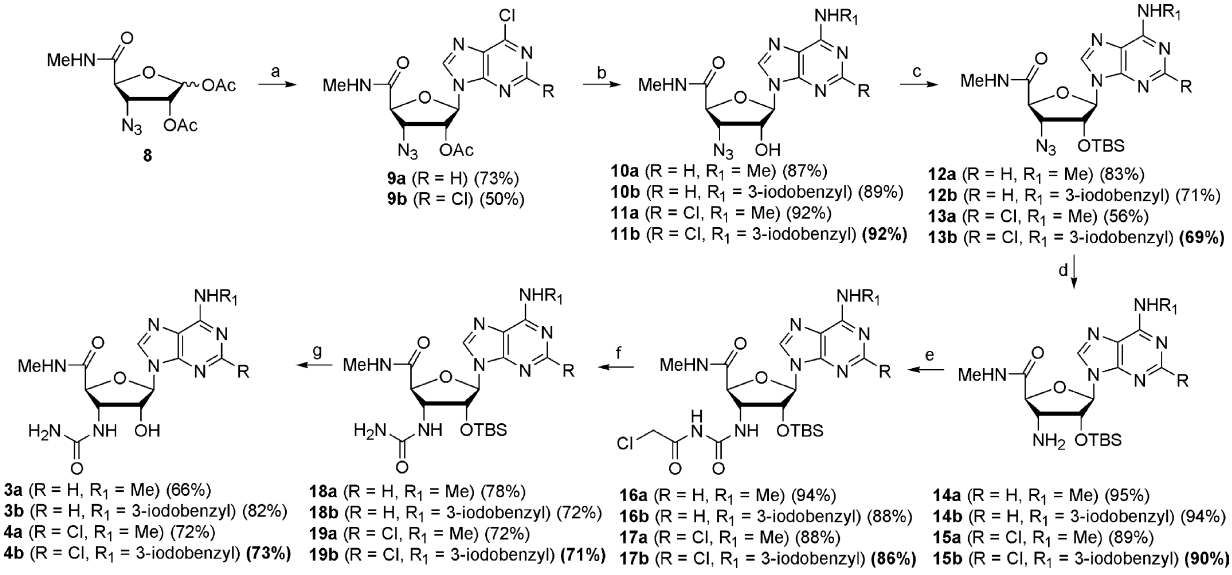
The target nucleosides **3** and **4** were synthesized starting from 1,2:5,6-di-*O*-isopropylidene-D-glucose via the glycosyl donor, 3-deoxy-3-azidosugar **8**, which was synthesized by the known procedure[15] (Scheme 1). 1,2:5,6-Di-*O*-isopropylidene-D-glucose (**5**) was treated with trifluoromethanesulfonic anhydride in pyridine to give the triflate, which reacted with sodium azide in DMF at room temperature to afford azido sugar **6**. Selective hydrolysis of 5,6-acetonide in **6** using 75% aqueous acetic acid followed by the treatment of the resulting diol with $NaIO_4/RuCl_3$ in $CCl_4/CH_3CN/H_2O$ (2/2/3) produced acid derivative. Without purification, acid derivative was treated with oxalyl chloride to give the activated ester, which was converted to methyl amide **7** by treating with methylamine in methylene chloride. Hydrolysis of the 1,2-acetonide group in **7** using 85% formic acid followed by acetylation of the resulting diol with acetic anhydride in pyridine afforded the key intermediate **8**.[15]



Scheme 1. Reagents and conditions: (a) Tf_2O, pyridine, 0 °C, 1 h; (b) NaN_3, DMF, rt, 48 h; (c) (i) 75% AcOH, 55 °C, 1.5 h; (ii) $NaIO_4$, $RuCl_3{\cdot}H_2O$, $CCl_4/CH_3CN/H_2O$, rt, 4 h; (d) (i) $(COCl)_2$, DMF, CH_2Cl_2, rt, 16 h; (ii) 2 M CH_3NH_2, CH_2Cl_2, 0 °C, 3 h; (e) (i) 85% HCO_2H, 60 °C, 1.5 h; (ii) Ac_2O, pyridine, rt, 16 h.

The glycosyl donor **8** was utilized for the synthesis of the target nucleosides, **3** and **4**, as shown in Scheme 2. Condensation of **8** with silylated 6-chloropurine and 2,6-dichloropurine in the presence of TMSOTf as a Lewis acid catalyst afforded the protected nucleosides **9a**[15] and **9b**, respectively. 6-Chloropurine derivative **9a** was treated with methylamine and 3-iodobenzylamine to give the N^6-substituted nucleosides, **10a** and **10b**, respectively. The acetyl protecting groups of **9a** and **9b** were removed in the process and replaced with TBS ethers in **12a** and **12b**, respectively, because of the facile migration of a 2′-acetyl group to the 3′-ureido group. Reduction of azido group of **12a** and **12b** was achieved using triphenylphosphine and ammonium hydroxide in aqueous solution to yield amino derivatives, **14a** and **14b**, respectively.

For the introduction of a urea moiety at the 3′-position, the amino derivatives, **14a** and **14b** were treated with chloroacetyl isocyanate in DMF to yield 3′-chloroacetyl urea derivatives, **16a** and **16b**, which were smoothly converted to the 3′-ureido derivatives, **18a** and **18b**, respectively, by the treatment with sodium methoxide.[16] As mentioned above, a 2′-acetyl or benzoyl group would easily migrate to the 3′-ureido group upon treating 3′-chloroacetyl urea derivatives, **16a** and **16b** with sodium methoxide, giving *N*-acetyl- or *N*-benzoylureido derivatives as sole products. This migration was prevented by using a TBS group as a protecting group. Finally, removal of the TBS group of **18a** and **18b** using tetra-*n*-butylammonium fluoride in THF afforded the final 3′-ureidoadenosine derivatives, **3a** and **3b**, respectively. Using a similar strategy, 2,6-dichloropurine derivative **9b** was converted to other 2-chloro-3-ureidoadenosine derivatives, **4a**[17] and **4b**, respectively.



Scheme 2. Reagents and conditions: (a) silylated 6-chloropurine or 2,6-dichloropurine, TMSOTf, $C_2H_4Cl_2$, 0–60 °C, 2 h; (b) $MeNH_2$, 1,4-dioxane, rt, 4 h or 3-iodobezylamine hydrochloride, Et_3N, EtOH, 50 °C, 18 h, then NaOMe, MeOH, rt, 2 h; (c) TBSCl, imidazole, DMF, rt, 24 h; (d) Ph_3P, NH_4OH/H_2O, THF, rt, 18 h; (e) chloroacetyl isocyanate, DMF, 0 °C, 3 h; (f) NaOMe, MeOH, rt, 18 h; (g) TBAF, THF, rt, 4 h.

3. Binding affinity at the adenosine receptors

The synthesized nucleosides, **3a**, **3b**, **4a**, and **4b** were subjected to competitive radioligand binding assays.[18] All of A_3 adenosine receptor experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human A_3 adenosine receptor using [^{125}I]I-AB-MECA (N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyl-uronamide) (1.0 nM) as radioligand.[18,19] Binding at A_1 and A_{2A} adenosine receptors was carried out using [^{3}H]*R*-PIA (*R*-N^6-[2-phenylisopropyl]adenosine) (2.0 nM, recombinant human A_1 AR) or [^{3}H]CGS21680 (2-[*p*-(carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine) (10 nM, recombinant human A_{2A} AR) as radioligands.[18]

None of the tested compounds displayed measurable binding affinities to A_1 and A_{2A} adenosine receptors, while they exhibited at best very weak affinity at the A_3 adenosine receptor (Table 1).

To explain the structural basis for the lack of interaction of 3′-ureidoadenosine analogues with the human A_3 adenosine receptor, a receptor docking study[10] of N^6-methyl-3′-ureidoadenosine-5′-uronamide (**3a**) was performed. From the result of a conformational search using MOPAC PM3 calculation, the lowest energy conformer of **3a** displayed intramolecular hydrogen bonding between the 3′-carbonyl oxygen of ureido group and the 5′-amino group, and between the 3′-amino and 2′-hydroxyl groups.

When the lowest energy conformer of **3a** was superimposed onto the NECA hA_3 adenosine receptor complex[10] to establish the ribose binding position, there was steric repulsion in the starting geometry between the 3′-substituent and the side chain of H272 (7.43) of the receptor and electrostatic repulsion between the 3′-carbonyl and backbone carbonyl oxygen atom of S271 (7.42) of the receptor. This docking model showing steric and electrostatic interference indicated conformational distortion of the ribose moiety of **3a**, possibly explaining the lack of binding affinity of **3a**. Thus, if a less sterically bulky group having strong hydrogen bonding ability, such as hydroxylamine, is introduced, binding affinity to the A_3 adenosine receptor might be restored.

Table 1. Binding affinity of the final nucleosides at human A_1, A_{2A}, and A_3 adenosine receptors[a]

Compound number	K_i (nM) or % inhibition at 10 μM[b]		
	hA_1	hA_{2A}	hA_3
IB-MECA (**1a**)	51.2 ± 5.1	2910 ± 580	1.8 ± 0.7
Cl-IB-MECA (**1b**)	222 ± 22	5360 ± 2470	1.4 ± 0.3
3a	<3%	<3%	<3%
3b	<3%	<3%	9%
4a	<3%	<3%	8%
4b	<3%	3%	24%

[a] Values from this study are means ± SEM, $N = 3$.
[b] Binding using [^{3}H]*R*-PIA (A_1 AR), [^{3}H]CGS21680 (A_{2A} AR), or [^{125}I]I-AB-MECA (A_3 AR), unless noted.

4. Conclusions

We have accomplished the synthesis of novel 3′-ureidoadenosine derivatives, starting from 1,2:5,6-di-*O*-ispropylidene-D-glucose. The key 3′-urea moiety was introduced upon reacting the 3′-amino derivative with chloroacetyl urea followed by treating with sodium methoxide. Although we did not discover new, potent A_3 adenosine receptor agonists, using molecular modeling we revealed unfavorable steric and electrostatic interactions likely to occur upon binding of the 3′-ureido derivatives in the agonist binding site of the A_3

adenosine receptor, which will provide valuable information about the identification of binding site of the A_3 adenosine receptor.

Acknowledgements

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16. To a stirred solution of **14a** (250 mg, 0.59 mmol) in anhydrous DMF (10 mL) was added chloroacetyl isocyanate (0.055 mL, 0.65 mmol) at 0 °C. After being stirred for 2 h at 0 °C, the reaction mixture was evaporated and the residue was purified by silica gel column chromatography (Hex/EtOAc = 1/4) to give **16a** (300 mg, 94%) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.92 (d, 1H, J = 5.1 Hz), 8.53 (d, 1H, J = 4.6 Hz), 8.47 (s, 1H), 7.96 (s, 1H), 6.71 (br s, 1H), 6.51 (br s, 1H), 6.26 (d, 1H, J = 4.2 Hz), 5.96 (d, 1H, J = 5.7 Hz), 5.01 (t, 1H, J = 7.9 Hz), 4.74–4.81 (m, 2H), 4.13 (s, 2H), 3.29 (br s, 3H), 2.98 (d, 1H, J = 4.7 Hz), 0.86 (s, 9H), 0.00 (s, 3H), −0.20 (s, 3H); IR (KBr): 3296, 2953, 1703, 1626, 1536, 1237, 1155, 840, 756 cm^{-1}; FAB-MS *m/z*: 542 $[M+H]^+$ Anal. Calcd for $C_{21}H_{33}ClN_8O_5Si$: C, 46.62; H, 6.15; N, 20.71. Found: C, 46.63; H, 6.03; N, 20.32. To a stirred solution of **16a** (300 mg, 0.55 mmol) in MeOH (10 mL) was added 28% NaOMe (0.27 mL) at rt and the reaction mixture was stirred for 18 h at rt and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 7/1) to give **18a** (200 mg, 78%) as a foam: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69 (br s, 1H), 8.37 (s, 1H), 7.79 (s, 1H), 5.95 (br s, 1H), 5.78 (d, 1H, J = 7.2), 5.37 (br s, 1H), 4.95 (t, 1H, J = 7.4 Hz), 4.74 (d, 1H, J = 2.1 Hz), 4.05 (m, 1H), 3.23 (br s, 3H), 2.95 (d, 1H, J = 5.0 Hz), 0.78 (s, 9H), −0.13 (s, 3H), −0.32 (s, 3H); IR (KBr): 3301, 2933, 1668, 1625, 1377, 1252, 1116, 840, 755 cm^{-1}; FAB-MS *m/z*: 465 $[M+H]^+$. Anal. Calcd for $C_{19}H_{32}N_8O_4Si$: C, 49.12; H, 6.94; N, 24.12. Found: C, 48.88; H, 7.04; N, 23.92.
17. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.36 (d, 1H, J = 4.8 Hz), 8.27 (d, 1H, J = 4.6 Hz), 6.32 (d, 1H, J = 4.2 Hz), 6.24 (d, 1H, J = 7.0 Hz), 5.94 (d, 1H, J = 2.6 Hz), 5.76 (s, 2H), 4.35–4.41 (m, 2H), 4.23 (d, 1H, J = 6.1 Hz), 2.90 (d, 1H, J = 4.5 Hz), 2.64 (d, 1H, J = 4.6 Hz); IR (KBr): 3423, 1666, 1630, 1534, 1356, 1306, 1084, 936, 636 cm^{-1}; FAB-MS *m/z*: 385 $[M+H]^+$. Anal. Calcd for $C_{13}H_{17}ClN_8O_4$: C, 40.58; H, 4.45; N, 29.12. Found: C, 40.59; H, 4.32; N, 29.11.
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