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2-(*N*-Acyl) and 2-*N*-acyl-N^6-substituted analogues of adenosine and their affinity at the human adenosine receptors

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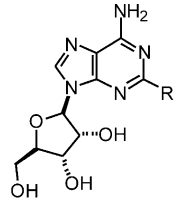
Abstract—A series of 2-(*N*-acyl) and 2-(*N*-acyl)-N^6-alkyladenosine analogues have been synthesized from the intermediate 2-amino-6-chloroadenosine derivatives (**2b** and **7**) and evaluated for their affinity at the human A_1, A_{2A}, and A_3 receptors. We found that 2-(*N*-acyl) derivatives of adenosine showed relatively low affinity at A_{2A} and A_3 receptors, while the N^6-cyclopentyl substituent in **4h** and **4i** induced high potency [A_1 (K_i) = 20.7 and 31.8 nM respectively] at the A_1 receptor and resulted therefore in increased selectivity for this subtype. The general synthetic methods and their binding studies are presented herein.

1. Introduction

Adenosine mediates its physiological effects through four G protein-coupled receptors named A_1, A_{2A}, A_{2B}, and A_3.[1] A large number of agonists with high affinity at all but the A_{2B} subtype have been developed over the years. Many compounds originally thought to be selective for the A_1 or A_{2A} subtypes later turned out to be also potent agonists at the recently discovered A_3 receptor. Here we study the potential of 2-(*N*-acyl) adenosine derivatives to serve as leads for novel A_1 selective agonists.

Agonists substituted at C-2 position are usually known for their selectivity at adenosine A_{2A} receptor.[2,3] Various modifications at the C-2 position of adenosine have been performed by several research groups using C–C bond formation[2a–c] or by nucleophilic replacement of C-2 halogen atom with amines,[2d] alcohols[2e] or thiols.[2f] Condensation reaction of appropriately modified bases and protected sugar derivatives have also been applied to prepare C-2 modified adenosines.[3] Such functional groups at the C-2 position or combination of modifications at C-2 and N^6 positions have been shown to result in different activity profiles and selectivity at the four known adenosine receptor subtypes.[2,3] The striking difference in selectivity between 2-*N*-modified adenosines



1a-c

1a: R = NH-$(CH_2)_2$-C_6H_4-*p*-$(CH_2)_2CO_2H$
1b: R = NH-N=CH-cyclohexyl
1c: R = N=N-NH-CO-NH-C_6H_5

Figure 1. 2-(*N*-Substituted)-adenosine derivatives.

1a and **b** (A_2 agonists)[2d,e] and **1c** and its N^6-cyclopentyl derivative (A_1 agonists)[4] suggest the proper combination of substituent at C-2 and N^6 position yields selective agonists for the A_1 receptor (Fig. 1).[4] In this communication we report the synthesis of novel adenosine derivatives carrying the *N*-acyl side chain at the C-2 position and their evaluation at the human adenosine receptors.

2. Results and discussion

Although there are several reports on the 2-*N* substituted adenosine derivatives in the literature,[2d,e,4] the 2-*N*-acyl analogues of adenosine have not been evaluated for their affinity at any known adenosine receptor.

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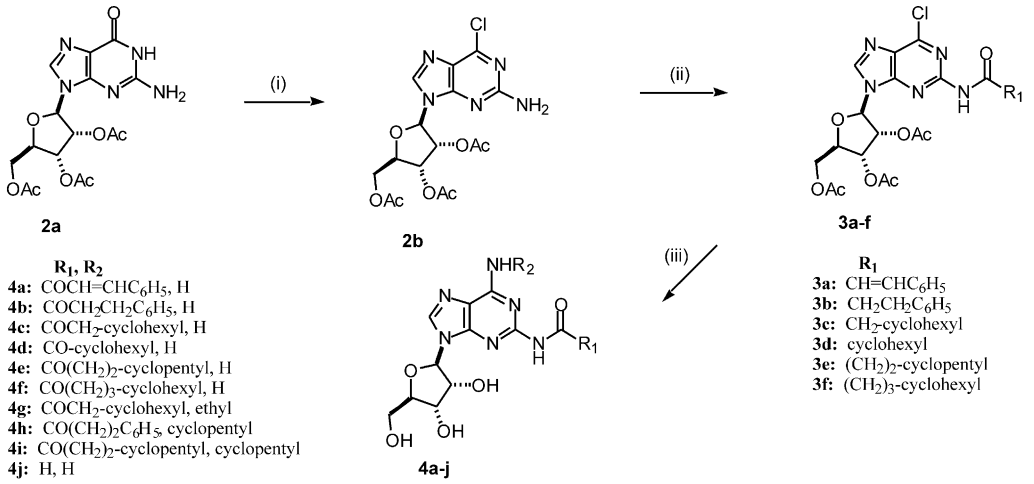
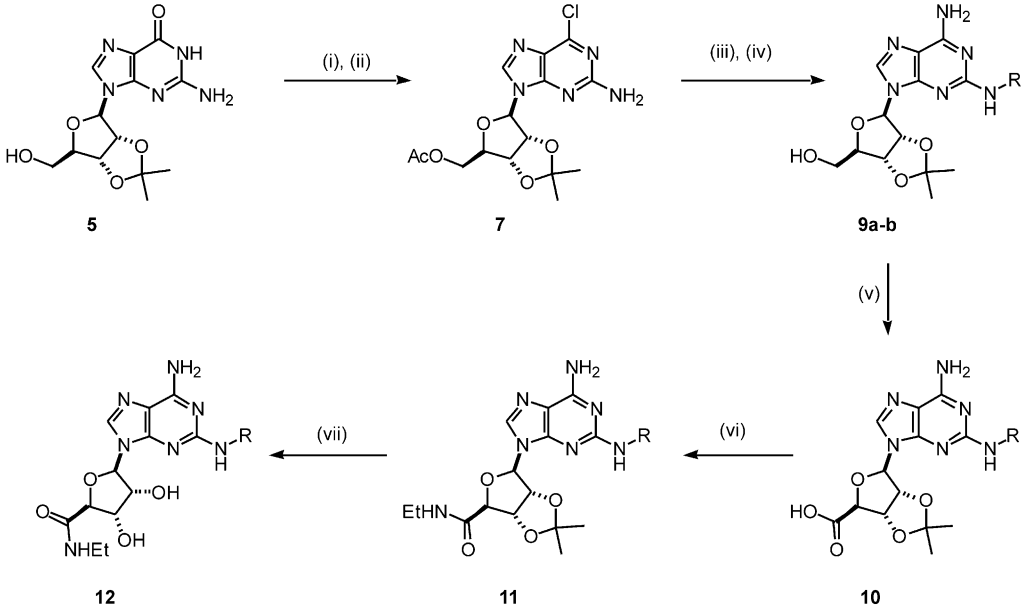
The main objective of current work is to develop a general synthetic method which will give an easy access to 2-(*N*-acyl)adenosines compared to the known literature methods.[5,6] The synthetic routes to obtain 2-(*N*-acyl) substituted (**4a–f**), 2,6-disubstituted derivatives of adenosine (**4g–i**) and 5′-carboxamide analogue **12** from the intermediates **2b** and **7** are depicted in Schemes 1 and 2. Treatment of 2′,3′,5′-tri-*O*-acetoxyguanosine (**2a**) with phosphorous oxychloride[7] and tetraethyl ammonium chloride in dry acetonitrile and dry *N,N*-dimethylaniline at 100 °C gave the intermediate 2-amino-6-chloroadenosine (**2b**), which was acylated using various acid chlorides in the presence of pyridine and 4-dimethylamino-pyridine (DMAP) in methylene chloride to furnish 2-(*N*-acyl)-6-chloroadenosines (**3a–f**). Nucleophilic displacement of the chlorine atom and cleavage of tri-*O*-acetyl groups using 2M solution of ammonia in ethanol at 90–100 °C in a sealed tube furnished nucleosides **4a–f**. Similarly, reaction of **3c** with ethylamine in ethanol (2M) provided 2-[*N*-(2-cyclohexylacetyl)]-N^6-ethyl adenosine (**4g**). N^6-Cyclopentyl derivatives **4h–i** were prepared from **3b** and **e** by reacting with cyclopentyl amine in ethanol. Minor amount of amide cleavage product 2-aminoadenosine (**4j**) was also isolated when the compounds **3a–f** were heated with amines in ethanol for a longer time or >100 °C.



Scheme 1. Reagents and conditions: (i) $POCl_3$, Et_4NCl, *N,N*-dimethylaniline, dry MeCN, 100 °C, 10 min, **2b** = 95%; (ii) DMAP, R_1COCl, pyridine, methylene chloride, rt (60 °C for **3a**), overnight; (iii) amine, EtOH, sealed tube, 90–100 °C, 12–24 h.



Scheme 2. Reagents and conditions: (i) Ac_2O, DMAP, Et_3N, MeCN, 8 h, **6a** = 87%, **6b** = 9%; (ii) $POCl_3$, DMA, 15 min, **7** = 79%; (iii) DMAP, pyridine, cyclopentyl-CH_2CH_2COCl, CH_2Cl_2, **8** = 87%; (iv) NH_3–EtOH (2N), 90 °C, 24 h, sealed tube, **9a** = 33%, **9b** = 50%; (v) BAIB, TEMPO, MeCN–H_2O (1:1), rt, 24 h, **10** = 49%; (vi) EDAC, CH_2Cl_2, DMF, $EtNH_2$ in THF, rt, 24 h, **11** = 35%; (vii) TFA–H_2O (1:4), rt, 1 h, **12** = 60%.

Synthesis of 5′-carboxamide derivative **12** is shown in Scheme 2. *O*-Acetylation[8] of the commercially available nucleoside (**5**) was carried out using acetic anhydride, DMAP and triethylamine in acetonitrile to give 2′,3′-*O*-isopropylidene-5′-*O*-acetylguanosine (**6a**) as a major product and minor amount of 2′,3′-*O*-isopropylidene-2-*N*-5′-*O*-diacetylguanosine (**6b**). The next two steps chlorination and acylation for the synthesis of **7** and **8** are similar to that of Scheme 1. Although amination reaction of 2′,3′-*O*-isopropylidene-5′-*O*-acetyl-2-[*N*-(2-cyclopentyl-propanoyl)]-6-chloroadenosine (**8**) with ammonia in ethanol was carried out using analogous conditions described above, it produced mixture of **9a** and amide cleaved product **9b** which were isolated by silica gel column chromatography. Oxidation of **9a** with [bis(acetoxy)-iodo] benzene (BAIB) and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in MeCN–H_2O provided 5′-carboxylic acid **10** in 49% yield.[9] Coupling reaction of ethylamine and acid **10** using EDAC and triethylamine gave amide (**11**) which on treatment with TFA–water (1:4) gave 2-[*N*-(2-cyclopentylpropanoyl)]-NECA (**12**).

3. Pharmacology

The affinity of the novel compounds at A_1, A_{2A}, and A_3 receptors was determined in binding studies according to the methods described earlier.[10] In order to get additional information about the relative A_{2B} affinity, activation of adenylyl cyclase in membranes form CHO cells with stably transfected human A_{2B} receptors was measured as described.[10] All compounds exhibit very low potency at this receptor subtype as at 100 μM they showed less than 20% of the maximal NECA-signal (data not shown). The binding data are summarized in Table 1. Since the data showed some degree of selectivity for the A_1 subtype for most of the compounds presented in this study, the K_i value of the highly potent prototypical A_1 agonist 2-chloro-N^6-cyclopentyladenosine (CCPA) is included in Table 1 as reference.

Compound **4d** with a 2-*N*-cyclohexanoyl substituent exhibits the lowest A_1 affinity whereas all compounds with the cyclohexyl separated from the carbonyl by at least one methylene group (**4c**,**f**) show increased A_1 affinity. The number of methylene groups (1–3) and the size of the cycloalkyl (hexyl or pentyl) appears to play a minor role as for each of the compounds **4c**, **e** and **f** virtually identical K_i-values at the A_1, A_{2A}, and A_3 subtypes, respectively, were measured. Replacement of the terminal cyclopentyl in the 2-substituent of **4e** for a phenyl ring (**4b**) results in an about 2-fold decrease of affinity at all three receptor subtypes. An 6-fold increase in A_1 affinity was achieved by the introduction of a double bond in the 2-substituent of **4b** with a resulting 58-fold A_1 selectivity compared to A_{2A}. Although the resulting compound **4a** is 3-fold more potent at A_3 compared to **4b** it also shows a 12-fold A_1 selectivity versus A_3.

In addition to the novel 2-(*N*-acyl) substituents simultaneous modification of the N^6- and the 5′-position were probed. First, the effect of the introduction of an N^6-ethyl group in compound **4c** was tested. The resulting compound **4g** shows little change in the A_1 K_i-value, an about three-fold loss of A_{2A} affinity and an increased A_3 affinity. These changes are comparable to the effects of an N^6-ethyl group in a series of 2-alkynyl derivatives of adenosine described recently by Volpini et al.[2b] Of all the compounds investigated in this study **4g** is the only one with a submicromolar A_3 affinity. All other compounds have strikingly similar A_3 K_i-values in the range of about 1–3 μM.

N^6-Substitution with a cyclopentyl ring like in CCPA has long been known to increase the A_1 affinity of adenosine derivatives. Such an effect may not necessarily be observed with concomitant substitution of the purine ring of adenosine. The N^6-cyclopentyl substituted compound **4h** is the most potent A_1 agonist in this study with a K_i-value of 20.7 nM. It is over 20-fold more potent than the corresponding unsubstituted compound **4b** and 25-fold less potent than prototypical A_1 agonist CCPA. That evidence, although limited, suggests the 2-chloro or other 2-halo groups contribute significantly to the affinity of 2-halo-N^6-cycloalkyl adenosines for the A_1 receptor.[11,12] A 8-fold increase in A_1 affinity was found for compound **4i** compared to **4e** with the unsubstituted N^6-position. Compound **4h** showed 60-fold selectivity versus the A_3 subtype which matches the selectivity of CCPA. The selectivity of **4h** and **4i**

Table 1. Affinities of the adenosine analogues **4a–i** and **12** in radioligand binding assays at human A_1, A_{2A} and A_3 adenosine receptors

Compd	$K_i(A_1)$[a] (nM)	$K_i(A_{2A})$[b] (nM)	$K_i(A_3)$[c] (nM)	A_{2a}/A_1	A_3/A_1
CCPA[d]	0.83 (0.55–1.25)	2270 (1950–2660)	42.3 (32.1–55.8)	2730	50
4a	70.7 (39.1–128)	4100 (2630–6390)	900 (739–1100)	58	13
4b	432 (335–556)	4570 (3540–5900)	2710 (2260–3270)	11	6
4c	342 (264–444)	2410 (1870–3100)	1230 (860–1,780)	7	4
4d	1620 (1360–1940)	1780 (1430–2230)	2760 (2600–2930)	1.1	1.7
4e	252 (228–278)	2000 (1070–3760)	1040 (936–1140)	8	4
4f	733 (423–1270)	1940 (1590–2360)	1060 (652–1710)	3	1.4
4g	207 (159–270)	7750 (5000–12,000)	181 (130–252)	37	0.9
4h	20.7 (12.4–34.5)	5660 (3780–8480)	1250 (963–1630)	273	60
4i	31.8 (23.9–42.3)	2180 (1860–2560)	1410 (1040–1920)	69	44
12	5450 (3750–7940)	10,800 (8910–13,000)	1010 (533–1900)	2	0.2

[a] Displacement of specific [^{3}H]CCPA binding in CHO cells stably transfected with human recombinant A_1 adenosine receptor, expressed as K_i (nM).
[b] Displacement of specific [^{3}H]NECA binding in CHO cells stably transfected with human recombinant A_{2A} adenosine receptor, expressed as K_i (nM).
[c] Displacement of specific [^{3}H]NECA binding in HEK cells stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (nM).
[d] Data from ref 10. All data are geometric means with 95% confidence intervals in parantheses.

compared to A_{2A} is 273 and 69-fold, respectively. In a recently published series of 2-alkynyl derivatives of adenosine it was shown that a 5′-*N*-carboxamido modification has only a minor effect on the affinity at A_1, A_{2A}, and A_3 receptors compared to the unmodified ribose.[2b] Compound **12** bears such a 5′-modified ribose and exhibits an unchanged affinity at A_3, however, the affinity was 22-fold and 5-fold reduced at A_1 and A_{2A} respectively, versus compound **4e** with the intact ribose.

4. Conclusion

To summarize, we synthesized a series of 2-*N*-acyl analogues of adenosine and evaluated their ability in radioligand binding assays at human A_1, A_{2a} and A_3 adenosine receptors. The compounds we present in this study show various degrees of potency at the human A_1 adenosine receptor and weaker potency than the CCPA. However, the most potent compound **4h**, which is 25-fold weaker than CCPA, binds with a K_i-value of 20.7 nM and is 273-fold selective versus A_{2A} and also showed 60-fold selectivity versus the A_3 subtype, which is slightly better than the selectivity of CCPA as one of the most potent and selective A_1 agonists known. Overall, we present novel 2-substituted adenosine derivatives with high affinity at the A_1 receptor and marked selectivity for this adenosine receptor subtype. We hope to use these findings to design novel A_1 selective agonists.

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