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2-Nitro Analogues of Adenosine and 1-Deazaadenosine: Synthesis and Binding Studies at the Adenosine A_1, A_{2A} and A_3 Receptor Subtypes

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Abstract—The influence of nitro substituents on the properties of adenosine and 1-deazaadenosine was studied. Combination of a nitro group at the 2-position with several N^6 substituents such as cyclopentyl and *m*-iodobenzyl gave a series of analogues with good adenosine receptor affinity, showing directable selectivity for the A_1, A_{2A} and A_3 adenosine receptor subtypes.

Regulation of adenosine receptor affinity is an expanding target for drug development.[1,2] Due to its low in vivo stability adenosine itself has only limited therapeutic applications which requires the availability of selective and metabolically stable ligands for the A_1, A_{2A} A_{2B} or A_3 adenosine receptor subtypes. Modification of adenosine is a subtle process, and only variation of the adenosine 2, N^6, and/or 5′ position was shown to produce selective agonists. From the numerous analogues that have been prepared, it can be deduced that substituents on the adenosine 2-position such as chlorine, have a positive effect on receptor affinity and selectivity.[3–5] To further explore this trend we have chosen for a nitro-group at the adenosine 2-position. Nitro-substituents in (hetero)aromatic ring systems are widely used in pharmacology and in particular nitro substituted imidazoles and furans have found a variety of clinical applications.[6] Previous to our work[7,8] the appearance of nitro substituents in purines, both from synthetical and biological point of view, was limited to only a few examples.[9,10] In this study the effect of a nitro-group at the (1-deaza)-adenosine 2-position in combination with receptor selective N^6 substituents on the affinity at the adenosine A_1, A_{2A} and A_3 receptor will be described.

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Chemistry

Functionalization of adenosines at the 2-position by direct aromatic substitution is not a general process. For instance halogenation reactions take place exclusively at the purine 8-position. To obtain C-2 substituted adenosines, 2,6-dichloropurines or guanosine are commonly used as starting materials.[10–14] Recently we have shown that nitration of a number of 6-substituted nucleosides using a mixture of tetrabutylammonium nitrate (TBAN) and trifluoroacetic anhydride (TFAA), efficiently results in the formation of 2-nitropurine and 2-nitro-deazapurine nucleosides.[7,8] In the present study this nitration strategy was applied for the preparation of a series of receptor ligands.

In a first attempt towards a short synthetic route for 2-nitroadenosine, penta-acetylated adenosine **1** was nitrated with TBAN/TFAA and the acetate protecting groups of the corresponding 2-nitro product **2** were removed with potassium cyanide in methanol (Scheme 1). In particular removal of the second *N*-acetyl group from the 6-position was rather slow and 2-methoxyadenosine **3** was formed as the main product via nucleophilic substitution of the nitro group. This side reaction also occurred during deprotection of nitrated tetra-acetyl N^6-cyclopentyladenosine **5**, resulting in the formation of 2-methoxy-CPA **6**.

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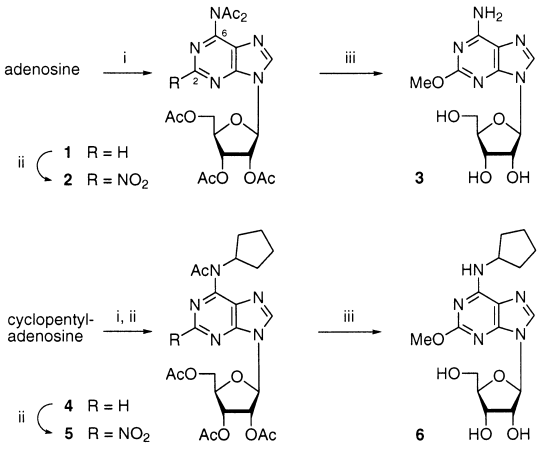
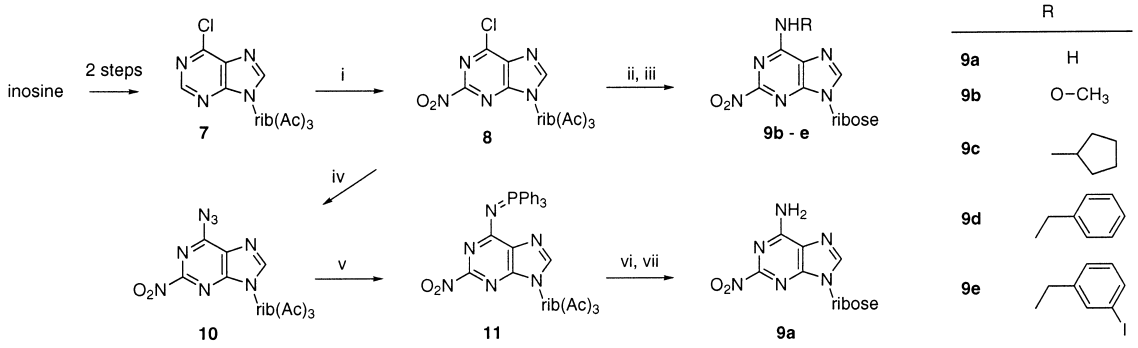


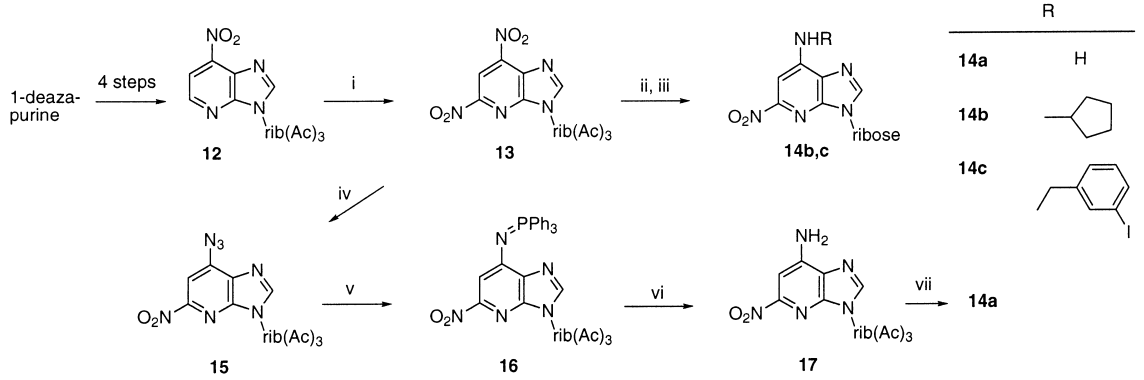
Scheme 1. (i) Acetic anhydride, DMAP, reflux (**1**: 79%; **4**: 70%); (ii) 1.5 equiv TBAN/TFAA, DCM, 0 °C (**2**: 55%; **5**: 48%); (iii) 1.2 equiv KCN, MeOH, 48 h, rt (**3**: 62%; **6**: 73%).

An alternative synthetic procedure for 2-nitroadenosines starts with tri-*O*-acetyl protected 6-chloropurine-riboside **7** which is readily available from inosine (Scheme 2).[15] Crystalline 6-chloro-2-nitro derivative **8** was obtained in 65–71% overall yield based on inosine.[8] Introduction of a nitro substituent in the purine ring had a strong accelerating effect on substitution reactions at the 6-position. With almost equimolar amounts of nitrogen nucleophiles and reaction temperatures below 0 °C the 6-aminated purines were obtained without affecting the acetates or the nitro group. Removal of the acetate protecting groups from the sugar part with potassium cyanide in methanol gave adenosine analogues **9b–9e**.[16] Shorter reaction times reduced competing methanolysis of the nitro group, as was described in Scheme 1, to a minor process.

For the preparation of the parent compound **2**-nitroadenosine **9a**, a three-step procedure was developed to avoid the use of ammonia as a nucleophile.[17] Substitution of **8** with sodium azide, subsequent reaction of the azide with triphenylphosphine and acid hydrolysis of the corresponding iminophosphorane **11** produced 2-nitroadenosine **9a**.[16] For the synthesis of 2-nitro-1-deazaadenosines **14a–c**, 6-nitro-derivative **12** (purine numbering) was used (Scheme 3). This precursor for 1-deazaadenosine was prepared via an efficient literature procedure by electrophilic nitration of 1-deazapurine-3-oxide followed by regioselective ribosylation.[18] Introduction of a second nitrogroup in **12** was accomplished with TBAN/TFAA to give 2,6-dinitro-1-deazapurine riboside **13**.[7] The 6-nitro substituent in this electron-poor ring system displayed good leaving group properties and nucleophilic substitution with amines proceeded exclusively at this position. 1-Deaza-adenosine analogues **14b** and **14c**[19] were obtained in good overall yield from reaction with cyclopentylamine and *m*-iodobenzyl-



Scheme 2. (i) 1.7 equiv TBAN/TFAA, DCM, 0 °C, 65–71% (3 steps); (ii) 1.2 equiv RNH_2, TEA, DMF, 0 °C; (iii) KCN, MeOH, 2 h, rt (**9b**: 42%; **9c**: 53%; **9d**: 46%; **9e**: 56%, 2 steps); (iv) 1.0 equiv NaN_3, DMF, −18 °C; (v) PPh_3, DCM, rt; (vi) HOAc:H_2O 3:1, 45 °C, 64%, 3 steps; (vii) KCN, methanol, 2 h, rt, 80%.



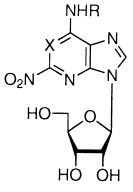
Scheme 3. (i) 1.5 equiv TBAN/TFAA, DCM, 0 °C, 73%; (ii) 1.2 equiv RNH_2, TEA, DMF, 6 h, rt; (iii) KCN, MeOH, 5 h, rt (**14b**: 60%; **14c**: 68% (2 steps); (iv) 1.1 equiv NaN_3, DMF, 0 °C; (v) PPh_3, DCM, rt; (vi) HOAc:H_2O 3:1, 40 °C, 51%, 3 steps; (vii) 0.3 equiv KCN, MeOH, 5 h, rt, 92%.

amine respectively. Conversion of **13** into the parent compound 2-nitro-1-deazaadenosine **14a**[19] was performed in three steps via the azide **15** and the iminophosphorane **16**, analogous to the sequence described for **9a**. X-ray analysis of 2-nitro-1-deazaadenosine triacetate **17**[7] confirmed the regioselectivity of the S_NAr reaction.

Biological Evaluation

Next the affinity of the 2-nitro adenosine derivatives for the adenosine A_1, A_{2A} and A_3 receptors was studied. Receptor affinities were determined by radioligand binding studies according to previously reported protocols and are given in nanomolar concentrations or as percentage displacement at a single concentration of 10 μM.[20–22] The results of the binding studies (Table 1) show that the reference agonist for the adenosine A_1 receptor, N^6-cyclopentyladenosine (CPA), which has affinities of 5.9 and 580 nM for adenosine A_1 and A_{2A} receptors, respectively,[23] can be compared to 2-nitro-derivative **9c**. Both ligands show selectivity for A_1 relative to A_{2A} and A_3 receptors and the K_i value is in the same range as for the reference compound CPA. Similarly, **14b** is the 2-nitro equivalent of 1-deaza-N^6-cyclopentyladenosine. In this case the two compounds are more or less equipotent, since 1-deaza-N^6-cyclopentyladenosine has K_i values of 100 nM and 10 μM for adenosine A_1 and A_{2A} receptors, respectively.[3] From these values it appears that the introduction of the 2-nitro substituent is fairly well tolerated by the A_1 receptor.

Table 1. Adenosine receptor affinities (K_i values±SEM) as determined in radioligand binding studies



9 (X = N)
14 (X = CH)

Compounds	R	A_1 (K_i, nM)[a]	A_{2A} (K_i, nM)[b]	A_3 (K_i, nM)[c]
9a	H	344±16	286±112	202±103
9b	Methoxy	1160±570	9%[d]	35.8±27.8
9c	Cyclopentyl	47.1±3.4	3510±1940	222±145
9d	Benzyl	1420±240	20%[d]	163±43
9e	3-I-Benzyl	138±30	1440±790	12.0±3.7
14a	H	646±150	437±147	216±89
14b	Cyclopentyl	52.6±6.8	52%[d]	340±54
14c	3-I-Benzyl	110±36	208±22	9.8±3.2
CPA		5.9	580	120
1-Deaza-CPA		100	10100	—
3-I-Benzyladenosine		79	340	28

[a]Displacement of [^{3}H]DPCPX from rat cortical membranes.
[b]Displacement of [^{3}H]ZM241,385 from rat striatal membranes.
[c]Displacement of [^{125}I]-ABMECA from human A_3 receptors expressed on HEK293 cells.
[d]% Displacement at 10 μM.

The A_3 receptor also seems to accommodate the nitro-substituent very well. When **9d** is compared to the analogous compound lacking the nitro-group, an increase in affinity was observed. N^6-Benzyladenosine was shown to have a K_i value of 550 nM versus. 163 nM for the 2-nitro substituted counterpart **9d**. Introduction of the 3-iodobenzyl enhanced both affinity and selectivity for the A_3 receptor: 28 nM versus 12 nM.[24] The highest affinity for the A_3 receptor was obtained for 1-deaza-analogue **14c**: $K_i = 9.8$ nM. The corresponding material without the 2-nitro substituent is not known to us. In conclusion, introduction of the 2-nitro group, a substituent with outspoken physico-chemical characteristics, affected receptor affinities only marginally. Further work on transformation reactions of the nitronucleosides into new receptor ligands is currently underway.

References and Notes

1. Poulsen, S.-A.; Quinn, R. J. *Bioorg. Med. Chem.* **1998**, *6*, 619.
2. Siddiqui, S. M.; Jacobsen, K. A.; Esker, J. L.; Olah, M. E.; Ji, X.; Melman, N.; Tiwari, K. N.; Secrist, J. A., III; Schneller, S. W.; Cristalli, G.; Stiles, G. L.; Johnson, C. R.; IJzerman, A. P. *J. Med. Chem.* **1995**, *38*, 1174.
3. Vittori, S.; Lorenzen, A.; Stannek, C.; Costanzi, S.; Volpini, R.; IJzerman, A. P.; Von Frijtag Drabbe Künzel, J. K.; Cristalli, G. *J. Med. Chem.* **2000**, *43*, 250.
4. Ha, S. B.; Melman, N.; Jacobson, K. A.; Nair, V. *Bioorg. Med. Chem. Letters* **1997**, *7*, 3085.
5. Knutsen, L. J. S.; Lau, J.; Petersen, H.; Thomsen, C.; Weis, J. U.; Shalmi, M.; Judge, M. E.; Hansen, A. J.; Sheardown, M. J. *J. Med. Chem.* **1999**, *42*, 3463.
6. Siim, B. G.; Denny, W. A.; Wilson, W. R. *Oncology Research* **1997**, *9*, 357.
7. Deghati, P. Y. F.; Bieräugel, H.; Wanner, M. J.; Koomen, G.-J. *Tetrahedron Lett.* **2000**, *41*, 569.
8. Deghati, P. Y. F.; Wanner, M. J.; Koomen, G.-J. *Tetrahedron Lett.* **2000**, *41*, 1291.
9. Nitroinosine was obtained in low yield from diazotation of guanosine: Shapiro, R. S.; Pohl, S. H. *Biochemistry* **1968**, *7*, 448.
10. A 6-nitro prodrug of F-ddI was formed as a side product from a diazotation reaction: Driscoll, J. S.; Siddiqui, M. A.; Ford, H., Jr.; Kelley, J. A.; Roth, J. S.; Mitsuya, H.; Tanaka, M.; Marquez, V. E. *J. Med. Chem.* **1996**, *39*, 1619.
11. Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2601.
12. Nair, V.; Sells, T. B. *Tetrahedron Lett.* **1990**, *31*, 807.
13. Adah, S. A.; Nair, V. *Tetrahedron* **1997**, *53*, 6747.
14. Kumamoto, H.; Tanaka, H.; Tsukioka, R.; Ishida, Y.; Nakamura, A.; Kimura, S.; Hayakawa, H.; Kato, K.; Miyasaka, T. *J. Org. Chem.* **1999**, *64*, 7773.
15. Buck, I. M.; Reese, C. B. *J. Chem. Soc. Perkin Trans. 1* **1990**, 293.
16. Selected data: **9a**: mp 218–220 °C; ^{1}H NMR (d_6-DMSO) δ 8.67 (s, 1H, H8), 8.31 (bs, NH_2), 5.92 (d, 1H, $J = 5.9$ Hz, H1′); HRMS obs. mass 313.0895, calcd for $C_{10}H_{13}O_6N_6$ (M + 1) 313.0901; **9b**: yellow needles, mp 106–108 °C; ^{1}H NMR (d_6-DMSO) δ 12.02 (bs, NH), 8.75 (s, 1H, H8), 5.96 (m, 1H, H1′), 3.84 (s, 3H, CH_3O); HRMS obs. mass 343.1002, calcd for $C_{11}H_{15}O_7N_6$ (M + 1) 343.1002; **9c**: mp 206–209 °C; ^{1}H NMR (d_6-DMSO) δ 8.80 (bs, NH), 8.67 (s, 1H, H8), 5.92 (d, 1H, $J = 5.6$ Hz, H1′), 5.1 and 4.5 (m, 1H, CHN); HRMS obs. mass 381.1532, calcd for $C_{15}H_{21}O_6N_6$ (M + 1) 381.1507; **9d**: mp 107–109 °C; ^{1}H NMR (d_6-DMSO) δ (two rotamers, ratio 80:20) 9.35 and 9.25 (m, NH), 8.70 (s, 1H, H8), 5.94 (d, 1H,

$J = 5.7$ Hz, H1′), 5.25 and 4.73 (m, 2H, CH_2N); M + 1 (FAB): 402; **9e**: mp 105–107 °C; ^{1}H NMR (d_6-DMSO) δ (two rotamers, ratio 80:20) 9.35 and 9.25 (m, NH), 8.71 (s, 1H, H8), 5.94 (d, 1H, $J = 5.7$ Hz, H1′), 5.19 and 4.68 (br, 2H, CH_2N); M + 1 (FAB): 529.1.

17. With ammonia as a nucleophile partial aminolysis of the acetates and substitution of the nitro-group take place.

18. Cristalli, G.; Franchetti, P.; Grifantini, M.; Vittori, S.; Martelli, S.; Bordoni, T.; Geroni, C. *J. Med. Chem.* **1987**, *30*, 1686.

19. Selected data: **14a**: mp 269–273 °C; ^{1}H NMR (d_6-DMSO) δ 8.62 (s, 1H, H8), 7.40 (s, 1H, H1); 7.27 (bs, NH_2), 5.96 (d, 1H, $J = 6.1$ Hz, H1′); HRMS obs. mass 312.0939, calcd for $C_{11}H_{14}O_6N_5$ (M + 1) 313.0955; **14b**: mp 216–218 °C; ^{1}H NMR (d_6-DMSO) δ 8.63 (s, 1H, H8); 7.60 (d, 1H, $J = 7.4$ Hz, NH), 7.35 (s, 1H, H1), 5.97 (d, 1H, $J = 6.0$ Hz, H1′), 4.5–4.2 (m, 1H, CHN); HRMS obs. mass 380.1568, calcd for $C_{16}H_{22}O_6N_5$ (M + 1) 381.1573; **14c**: mp 110–112 °C; ^{1}H NMR (d_6-DMSO) δ 8.68 (s, 1H, H8); 8.32 (br, NH), 7.32 (bs, 1H, H1), 5.98 (d, 1H, $J = 6.0$ Hz, H1′), 4.8 (br, 2H, CH_2N); HRMS obs. mass 528.0395, calcd for $C_{18}H_{19}O_6N_5$ I (M + 1) 528.0367.

20. Adenosine A_1 receptor affinities were determined on rat cortical membranes with [^{3}H]DPCPX as radioligand according to a protocol published previously: Pirovano, I. M.; IJzerman, A. P.; Van Galen, P. J. M.; Soudijn, W. *Eur. J. Pharmacol.* **1989**, *172*, 185.

21. Adenosine A_{2A} receptor affinities were determined on rat striatal membranes with [^{3}H]ZM 241,385 as radioligand according to a recently reported protocol: Gao, Z.-G.; Jiang, Q.; Jacobson, K. A.; IJzerman, A. P. *Biochem. Pharmacol.* in press.

22. Adenosine A_3 receptor affinities were determined on membranes prepared from HEK293 cells stably transfected with human A_3 receptor cDNA, with [^{125}I]-ABMECA as radioligand: Van Muijlwijk-Koezen, J. E.; Timmerman, H.; Link, R.; Van der Goot, H.; IJzerman, A. P. *J. Med. Chem.* **1998**, *41*, 3987.

23. Van der Wenden, E. M.; Carnielli, M.; Roelen, H. C. P. F.; Lorenzen, A.; Von Frijtag Drabbe Künzel, J. K.; IJzerman, A. P. *J. Med. Chem.* **1998**, *41*, 102.

24. Van Tilburg, E. W.; Von Frijtag Drabbe Künzel, J. K.; De Groote, M.; Vollinga, R. C.; Lorenzen, A.; IJzerman, A. P. *J. Med. Chem.* **1999**, *42*, 1393.