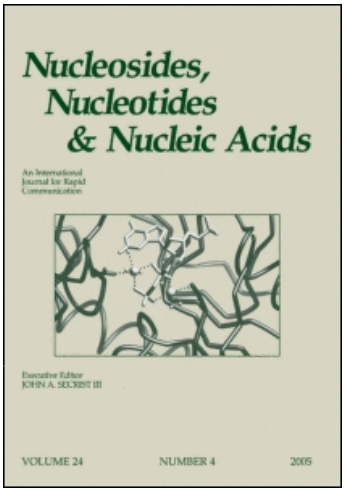

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Van Calenbergh, Serge , IJzerman, Adriaan P. and Herdewijn, Piet(1998) '3'-Amidated 3'-Deoxyxylofuranose Analogues of N^6-Cyclopentyladenosines: a New Class of Non-Xanthine Antagonists at the Adenosine A_1 Receptor.', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1571 — 1576
To link to this Article: DOI: 10.1080/07328319808004688
URL: http://dx.doi.org/10.1080/07328319808004688

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1571-1576 (1998)

3'-AMIDATED 3'-DEOXYXYLOFURANOSE ANALOGUES OF N^6-CYCLOPENTYLADENOSINES: A NEW CLASS OF NON-XANTHINE ANTAGONISTS AT THE ADENOSINE A_1 RECEPTOR.

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ABSTRACT : Full adenosine A_1 receptor agonists like CPA and other N^6-substituted adenosine analogs have previously been shown to become partial agonists upon deletion of the 3'-hydroxyl moiety. The present study further explored the C-3' site for modification. The modest affinity at A_1 and A_{2a} receptors found in the 3'-amido-3'-deoxyxylofuranosyladenine series prompted us to synthesize the corresponding N^6-cyclopentyl derivatives, which proved to exhibit potent antagonistic behaviour at the A_1 receptors. This represents a new perspective in the purinergic field.

Recently, Nyce and Metzger developed a phosphorothioate 21-deoxynucleotide sequence complementary to the m-RNA that codes for adenosine A_1 receptor protein in rabbit airway smooth muscle tissue in order to block synthesis of the receptor.[1] Rabbits treated with an aerosol solution of this antisense DNA developed less bronchoconstriction when challenged with adenosine or dust-mite allergen. These findings indicate that the adenosine A_1 receptor is an important mediator of airway obstruction and inflammation and elegantly illustrate the potential role of adenosine receptors as targets for drug design.

The effect of numerous modifications of the adenosine scaffold on affinity and intrinsic activity has been investigated. In general, substitution at the N^6 position of the adenine moiety of adenosine, for example N^6-cyclohexyladenosine (CHA) and N^6-cyclopentyladenosine (CPA), enhances the affinity for the A_1 receptor.[2] Ligands for the A_1 and A_{2a} receptors have also been found in the xanthine series (e.g., 1,3-dipropyl-8-

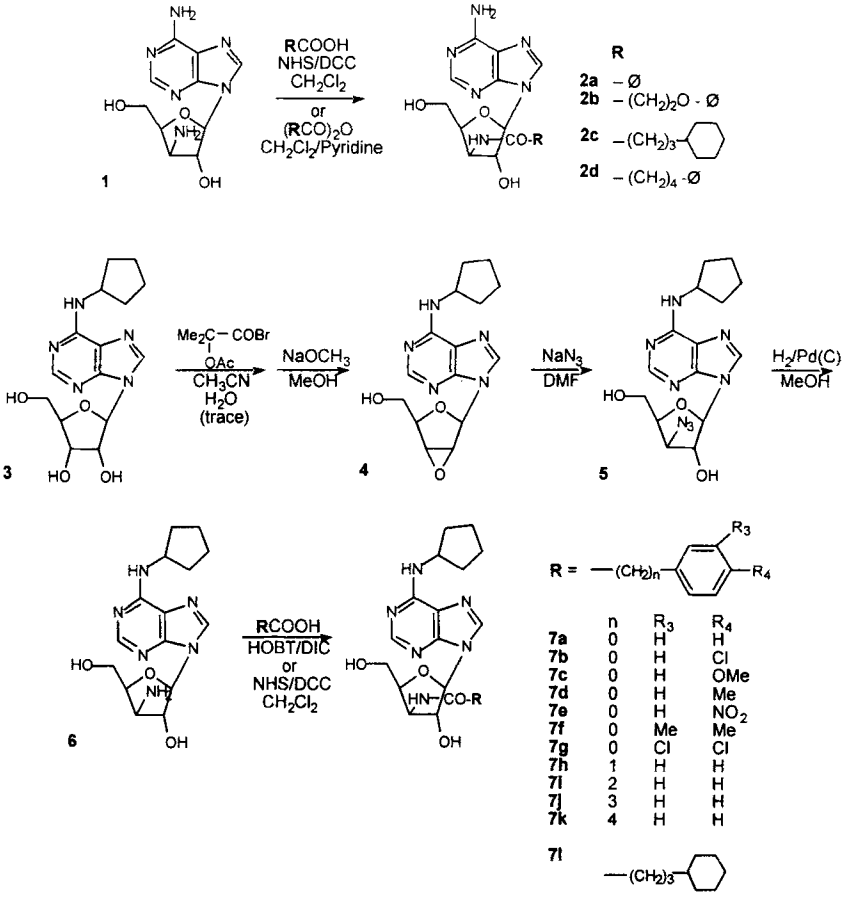
cyclopentylxanthine (CPX) as antagonist for A_1)[3] and triazoloquinazoline series (e.g., CGS 15943,[4] an antagonist with moderate selectivity for A_{2a}).

Except for a certain freedom of substitution at the 5'-position, for instance by a 5'-uronamide moiety, A_1 (and A_2) receptors are generally proposed to require an unchanged ribose moiety for adenosine agonist activity. The secondary hydroxyl groups of the ribose moiety are believed to be determinants for the intrinsic activity of adenosine receptor agonists. Most modifications at the 2' and 3' positions of the sugar ring or inversions of chiral centers at these positions were found to abolish adenosine receptor binding.[5] Removal of the 2'- and 3'-hydroxyl groups of N^6-substituted adenosine derivatives was shown to result in partial agonists,[6] or, in the case of the 2',3'-dideoxy analog of CHA, antagonist properties.[7] Such changes also affect affinity, whereby the drop in A_1 affinity by deletion of the 2'-hydroxyl moiety is more pronounced than the decrease caused by removal of the 3'-group.[5,6] This inspired us to explore other possible modifications at the C-3' position. Therefore, a series of 3'-amido-3'-deoxyxylofuranosyl adenines (**2a–d**) together with a number of 3'-amido-3'-deoxyadenosines[8] (formulae not shown) were synthesized. These compounds were tested *in vitro* for their affinity for the adenosine A_1 and A_{2a} receptors. The affinities found in the series of the xylo-analogs prompted us to prepare the compounds (**7a–l**) in which this favorable 3'-modification is combined with a N^6-cyclopentyl ring.

The synthesis of compounds **2a–d** and **7a–l** was performed according to Scheme 1 using known procedures. Full experimental details will be published elsewhere.[9]

Biological Results and Discussion

All modified adenosine and CPA analogs were tested in radioligand binding assays to determine their affinities toward adenosine A_1 and A_{2a} receptors in rat brain cortex and rat striatum, respectively (Table 1). The affinity of the 3'-amido-3'-deoxyadenosines (mentioned in reference 8) for these receptors was low: only marginal (i.e., $\leq$ 10 %) displacement of radioligand is observed at 10 μM concentration (results not shown). The 3'-amidated 3'-deoxyxylofuranosyl counterparts (**2a–d**) proved to be better tolerated at these receptors and displayed a K_i of 3–17 μM at A_1 and 2–12 μM at A_{2a} receptors.

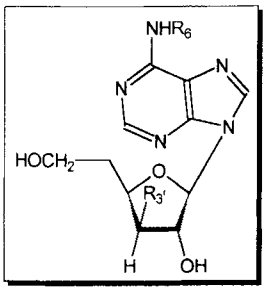


Scheme 1

Here, we demonstrate the possibility to escape from the necessity of conserving the ribosyl configuration of adenosine for affinity.

Drastic (18–31-fold) improvement in potency towards A_1 receptors was obtained with the N^6-cycopentyl congeners, *i.e.* **7a**,**k**,**l**, suggesting the compatibility of our carbohydrate modification with this typical N^6-substituent. A series of structural analogs of the benzamido derivative (**7a**), provides a number of insights into structure–activity relationships with regard to adenosine receptor binding. Varying substituents in *para* position of the benzamido moiety shows the following trend on A_1 receptor affinity : NO_2

Table 1. Affinities [K_i Values (standard errors of the mean) in the Presence and Absence of GTP] and GTP Shifts of the (N^6-substituted) 3'-Deoxyadenosine Analogues



			K_i (μM)			K_i (μM)
cmpd	R_6	$R_{3'}$	A_1 - GTP	A_1 + GTP	GTP shift	A_{2a}
	H	H	7.12 ± 3.62	15.2 ± 2.0	2.1 ± 1.4	–[a]
2a	H	NHCO-Ph	10.9 ± 2.8	11.1 ± 0.4	1.1 ± 0.3	2.47 ± 0.52
2b	H	NHCO-$(CH_2)_2$O-Ph	16.9 ± 1.6	12.6 ± 2.0	0.74 ± 0.05	11.8 ± 3.0
2c	H	NHCO-$(CH_2)_3C_6H_{11}$	3.59 ● 0.10	2.68 ± 0.04	0.75 ± 0.03	1.77 ± 0.48
2d	H	NHCO-$(CH_2)_4$-Ph	7.84 ± 0.37	5.74 ± 0.58	0.73 ± 0.04	4.66 ± 1.35
	cyclopentyl	H	0.11 ± 0.03	0.47 ± 0.04	4.3 ± 1.2	18.6 ± 6.8
5	cyclopentyl	N_3	8.47 ± 3.48	11.2 ± 1.5	1.4 ± 0.3	2.41 ± 0.52
7a	cyclopentyl	NHCO-Ph	0.40 ± 0.13	0.31 ± 0.05	0.80 ± 0.13	2.76 ± 1.19
7b	cyclopentyl	NHCO-Ph-*p*Cl	0.11 ± 0.09	0.081 ± 0.033	0.87 ± 0.29	0.58 ± 0.13
7c	cyclopentyl	NHCO-Ph-*p*OMe	0.20 ± 0.13	0.13 ± 0.02	0.77 ± 0.30	0.77–2.45
7d	cyclopentyl	NHCO-Ph-*p*Me	0.083 ± 0.015	0.068 ± 0.010	0.84 ± 0.19	0.79–2.52
7e	cyclopentyl	NHCO-Ph-*p*NO_2	0.82 ± 0.31	0.74 ± 0.22	0.91 ± 0.08	6.85 ± 2.39
7f	cyclopentyl	NHCO-Ph-3,4-diMe	0.0236 ± 0.0025	0.0174 ± 0.0042	0.73 ● 0.11	3.68 ± 1.50
7g	cyclopentyl	NHCO-Ph-3,4-diCl	0.0325 ± 0.0031	0.0237 ± 0.0014	0.73 ± 0.09	2.02 ± 0.98
7h	cyclopentyl	NHCO-CH_2-Ph	0.85 ± 0.29	0.77 ± 0.24	0.95 ± 0.31	(50%)[b]
7i	cyclopentyl	NHCO-$(CH_2)_2$-Ph	0.37 ± 0.12	0.35 ± 0.12	0.99 ± 0.36	7.94 ± 4.48
7j	cyclopentyl	NHCO-$(CH_2)_3$-Ph	0.13 ± 0.08	0.10 ± 0.02	0.94 ± 0.38	0.65 ± 0.16
7k	cyclopentyl	NHCO-$(CH_2)_4$-Ph	0.24 ± 0.03	0.20 ± 0.03	0.83 ± 0.16	4.23 ± 0.94
7l	cyclopentyl	NHCO-$(CH_2)_3$-C_6H_{11}	0.19 ± 0.11	0.15 ± 0.09	0.80 ± 0.03	(74%)[b]

[a] –, not determined because the addition of deoxycoformycin, necessary to prevent degradation of this compound by adenosine deaminase, disturbed the binding assay. This was not the case for the A_1 receptor assay. [b] Percentage radioligand bound to the receptor in the presence of 10^{-5} M ligand.

< H < OMe < Cl ≤ Me. Adding a further chloro or methyl group in *meta* on the aromatic ring of **7b** and **7d** respectively, to give **7g** and **7f**, further increases activity.

Optimization of the spacer between the phenyl ring and the amido functionality reveals a length of three methylenes to be the most favorable. The 3'-azido intermediate **5** displays a relatively poor affinity for A_1 receptors, suggesting the importance of the amide moiety for effective binding at these receptors. For all analogs synthesized, the GTP shift for the A_1 receptor is nearly 1. The absence of a GTP-induced shift in binding *vs* radiolabeled DPCPX (1,3-dipropyl-8-cyclopentylxanthine) antagonist strongly suggests the 3'-amido xylosyl CPA analogs to be full antagonists for the A_1 receptor. This fact proves that by suitable sugar modification, it is possible to turn a full (CPA) or partial agonist (3'-deoxy-CPA) into an antagonist. As expected, the effect of the N^6-cyclopentyl substituent is negligible at the A_{2a} receptors (for those compounds for which comparable data are available). With the exception of compound **5**, the selectivity for A_1 *vs* A_{2a} receptors was thus significantly improved (e.g., **7f**, the most potent A_1 ligand of this series was found to be 160 times selective *vs* A_{2a}).

The crystal structure of compound **7a**[9] reveals the orientation of the ribose group relative to the adenine ring system. The compound appears to be in the so-called *syn* conformation stabilized by a hydrogen bond formed between N3 and the 5'-OH group (distance N3···H: 2.0 Å, angle N3···H-O: 176°). Generally it is assumed that the *anti* conformation (where the ribose group is turned away from the adenine ring) is essential for agonistic activity.[10] Although certainly not conclusive the *syn/anti* aspect may explain the antagonistic properties of this new series of compounds. Other studies have systematically addressed N9-substitution other than ribose.[5,11,12] Taylor *et al* synthesized ribose-modified analogs of adenosine.[5] Of relevance to the present study were compounds that did not display agonistic activity *in vivo*, but retained some affinity towards adenosine receptors as determined in radioligand binding studies. A 9-(2-tetrahydrofuran) substituent proved acceptable with modest adenosine A_1 receptor affinity (K_i value for N^6-(2-phenylethyl)-9-(2-tetrahydrofuran)adenine of 1.5 μM). Olsson and coworkers explored methyl, ethyl, 2-hydroxyethyl, cyclopentyl, phenyl and 2-tetrahydrofuran substituents at N-9.[11] In this study N^6-cyclopentyl-9-ethyladenine was most potent with a K_i value of 440 nM. More recently, Jacobson and colleagues investigated the relationship

between adenosine A_3 receptor affinity and N9 substitution.[12] For comparison adenosine A_1 receptor affinities were also included in this study, showing that again a 2-tetrahydrofuran substituent yielded moderate affinity for this receptor subtype. From the present study we conclude that N^6-cyclopentylxylofuranosyl adenines bearing a substituent of considerably larger size at the 3'-position can be accommodated as well, and may even lead to derivatives with enhanced potencies in the lower nanomolar range.

REFERENCES

1 Nyce, J. W.; Metzger, W. J. *Nature* **1997**, *385*, 721-725.

2 Moos, W. H.; Szotek, D. S.; Bruns, R. F. *J. Med. Chem.* **1985**, *28*, 1383-1384.

3 Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. *Naunyn-Schmiedberg's Arch. Pharmacol.* **1987**, *335*, 59-63.

4 Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; Stone, G. A.; Desai, M.; Williams, M. *J. Med. Chem.* **1988**, *31*, 1014-1020.

5 Taylor, M. D.; Moos, W. H.; Hamilton, H. W.; Szotek, D. S., Patt, W. C.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Heffner, T. G.; Mertz, T. E. *J. Med. Chem.* **1986**, *29*, 346–353.

6 van der Wenden, E. M.; von Frijtag Drabbe Künzel, J. K.; Mathôt, R. A. A.; Danhof, M.; IJzerman, A. P.; Soudijn, W. *J. Med. Chem.* **1995**, *38*, 4000–4006.

7 Lohse, M. J.; Klotz, K.-N.; Diekmann, E.; Friedrich, K.; Schwabe, U. *Eur. J. Pharmacol.* **1988**, *156*, 157–160.

8 Soenens, J.; François, G ; Van den Eeckhout, E.; Herdewijn, P. *Nucleos. Nucleot.* **1995**, *14*, 409–411.

9 Van Calenbergh, S.; von Frijtag Drabbe Künzel, J. K.; Blaton, N. M.; Peeters, O. M.; Rozenski, J.; Van Aerschot, A.; De Deukeleire, D.; IJzerman, A. P.; Herdewijn, P. submitted to *J. Med. Chem.*

10 Van der Wenden, E. M.; Hartog-Witte, H. R.; Roelen, H. C. P. F.; von Frijtag Drabbe Künzel, J. K.; Pirovano, I. M.; Mathôt, R. A. A.; Danhof, M.; Van Aerschot, A.; Lidaks, M. J.; IJzerman, A. P.; Soudijn, W. *Eur. J. Pharmacol.-Mol. Pharm. Sect.* **1995**, *290*, 189-199.

11 Thompson, R. D.; Secunda, S.; Daly, J. W.; Olsson, R. A. *J. Med. Chem.* **1991**, *34*, 2877-2882.

12 Jacobson, K.A.; Siddiqi, S.M.; Olah, M.E.; Ji, X-d.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G.L.; Kim, H.O. *J. Med. Chem.* **1995**, *38*, 1729-1735.