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Synthesis and Evaluation of N^6-Substituted Apioadenosines as Potential Adenosine A_3 Receptor Modulators

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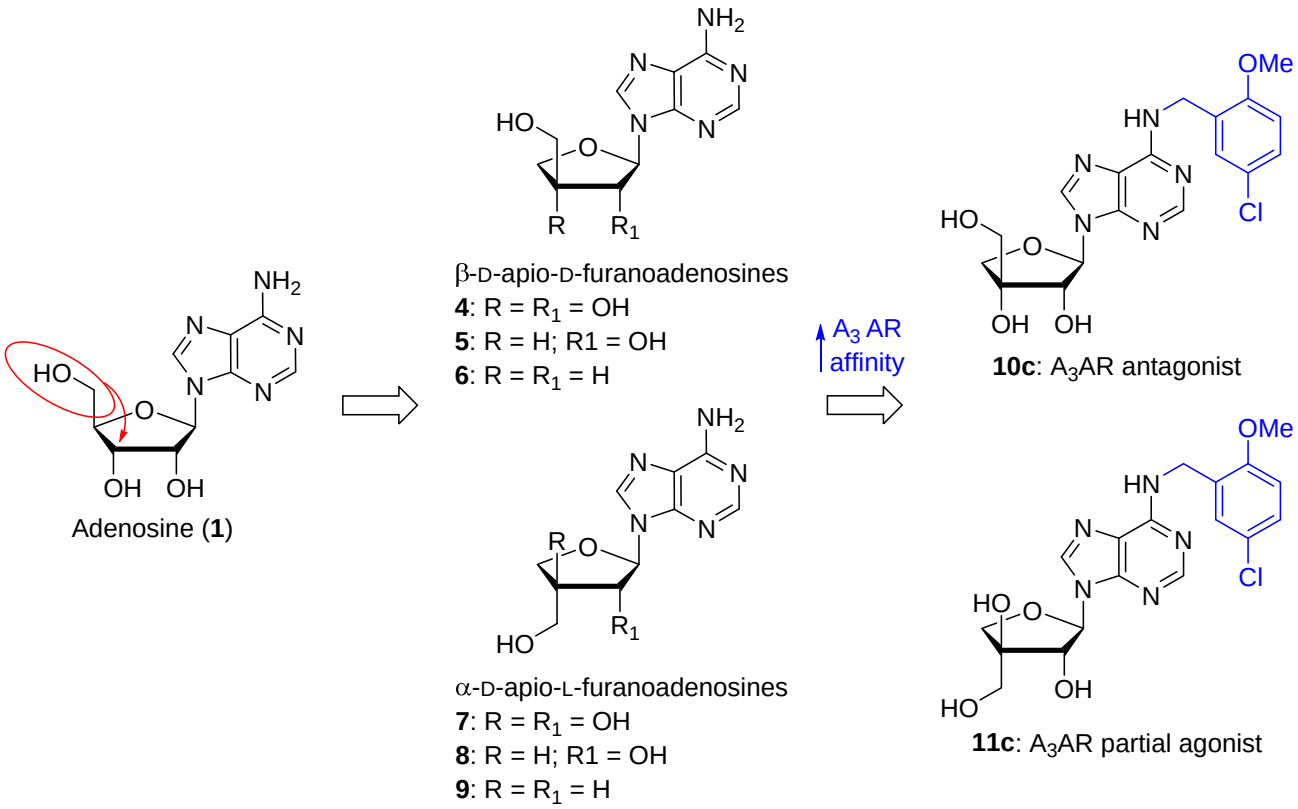
Abbreviations: AR, adenosine receptor; BAIB, bis-acetoxyiodobenzene; cAMP, adenosine 3′,5′-cyclic phosphate; CDI, carbonyldiimidazole; Cl-IB-MECA = 2-chloro-N^6-(3-iodobenzyl)-5□-*N*-methylcarboxamidoadenosine; CHO, Chinese hamster ovary; DMF, *N*,*N*-dimethylformamide; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; GPCR, G protein-coupled receptor; HEK, human embryonic kidney; I-AB-MECA, N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyl-uronamide; NECA, 5′-*N*-ethylcarboxamidoadenosine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRMS, high resolution mass spectroscopy; NMR, nuclear magnetic resonance; *R*-PIA, N^6-R-phenylisopropyladenosine; TBDMS, *tert*-butyldimethylsilyl; TCA, trichloroacetic acid; TEMPO, (2,2,6,6-tetramethylpyperidin-1yl)-oxyl; THF, tetrahydrofuran; TLC, thin layer chromatography; TM, transmembrane helical domain.

ABSTRACT

Adenosine receptors (ARs) trigger signal transduction pathways inside the cell when activated by extracellular adenosine. Selective modulation of the A_3AR subtype may be beneficial in controlling diseases such as colorectal cancer and rheumatoid arthritis. Here, we report the synthesis and evaluation of β-D-apio-D-furano- and α-D-apio-L-furanoadenosines and derivatives thereof. Introduction of a 2-methoxy-5-chlorobenzyl group at N^6 of β-D-apio-D-furanoadenosine afforded an A_3AR antagonist (**10c**, K_i = 0.98 μM), while a similar modification of an α-D-apio-L-furanoadenosine gave rise to a partial agonist (**11c**, K_i = 3.07 μM). The structural basis for this difference was examined by docking to an A_3AR model; the antagonist lacked a crucial interaction with Thr94.



INTRODUCTION

Transmembrane receptors coupled to G-proteins (heterotrimeric guanine nucleotide binding proteins) constitute a large receptor family, referred to as G protein-coupled receptors (GPCRs) or seven-transmembrane domain (7TM) receptors. Triggered by messenger molecules or signals outside the cell, GPCRs activate different signal transduction pathways inside the cell and, ultimately, cellular responses. The extracellular messengers range from photons to biogenic amines and other neurotransmitters to proteins. GPCRs are ubiquitous and involved in processes varying from directed chemotaxis[1] of small organisms (e.g. searching food for survival) to triggering apoptosis (programmed cell death) in large animals. They are the targets of almost 50% of marketed active pharmaceutical ingredients.[2]

GPCRs activated by extracellular adenosine are classified as adenosine receptors (ARs), which are divided in four different subtypes, i.e. A_1, A_{2A}, A_{2B} and A_3 ARs.[3,4] The amino acid sequence similarity between the human (h) A_3AR and hA_1, hA_{2A}, hA_{2B} AR is 54 %, 48 % and 44 %, respectively.[5] The ARs use different signaling pathways; the A_1 AR and A_3 AR are preferentially coupled to Gi-proteins, and upon activation lead to adenylate cyclase inhibition, while the A_{2A} AR and A_{2B} AR are preferentially coupled to Gs-proteins and lead to adenylate cyclase activation. In some cells (e.g., mast cells), the A_{2B}AR is dually coupled to Gs and Gq and consequently also mobilizes calcium and activates phospholipase C and MAPK. [3,6]

Besides adenosine (**1**) itself, which is used clinically for the treatment of supraventricular tachycardia and in myocardial perfusion imaging,[7] only one AR-specific agent, the A_{2A}AR agonist regedenoson, has so far been approved by the FDA. However, a relatively large group of AR ligands is currently under clinical evaluation.[8]

With the exception of compound **2**, which was reported in the mid-1980s as being inactive at A_1 and A_2ARs,[9] and the recently reported carbocyclic analogue **3**,[10] a weak A_3AR agonist, 4′-hydroxymethyl transposed nucleosides have not been investigated as AR ligands.

This led us to employ a new and convenient method for the synthesis of apionucleosides from 1,2-*O*-isopropylidene-α-L-threose,[11] for the construction of suitably modified 9-(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenines (aka β-D-apio-D-furanoadenosines) (**4**-**6**) and 9-(3-*C*-hydroxymethyl-α-L-threofuranosyl)adenines (aka α-D-apio-L-furanoadenosines) (**7**-**9**) as potential A_3AR modulators (Figure 1).

On the one hand, we envisaged to substitute the N^6 position of β-D-apio-D-furanoadenosine **4** with substituted benzyl groups known to enhance A_3AR affinity;[12] on the other hand we planned to substitute the well-known ethylcarboxamide moiety in place of the 4′-CH_2OH group.[13] We decided to introduce a benzyl moiety at the N^6 position and an N-alkylcarboxamide moiety at the 5′ position because this combination was shown to be conducive to selectivity at the A_3AR. For example, N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine and its 2-chloro analogue (Cl-IB-MECA) display K_i values at A_3AR of ~1 nM and ≥50-fold selectivity for this subtype.[14] Subsequently, an N^6-(3-chloro-5-methoxybenzyl) substitution was shown to be beneficial for A_3AR selectivity,[12] and we incorporated this moiety in the current target compounds.

However, synthetic problems in introducing an ethylcarboxamide moiety motivated us to introduce a *N*-methyl or *N*-ethylcarbamoyloxymethyl group instead. Analogue modifications were carried out on α-D-apio-L-furanoadenosine **7**.

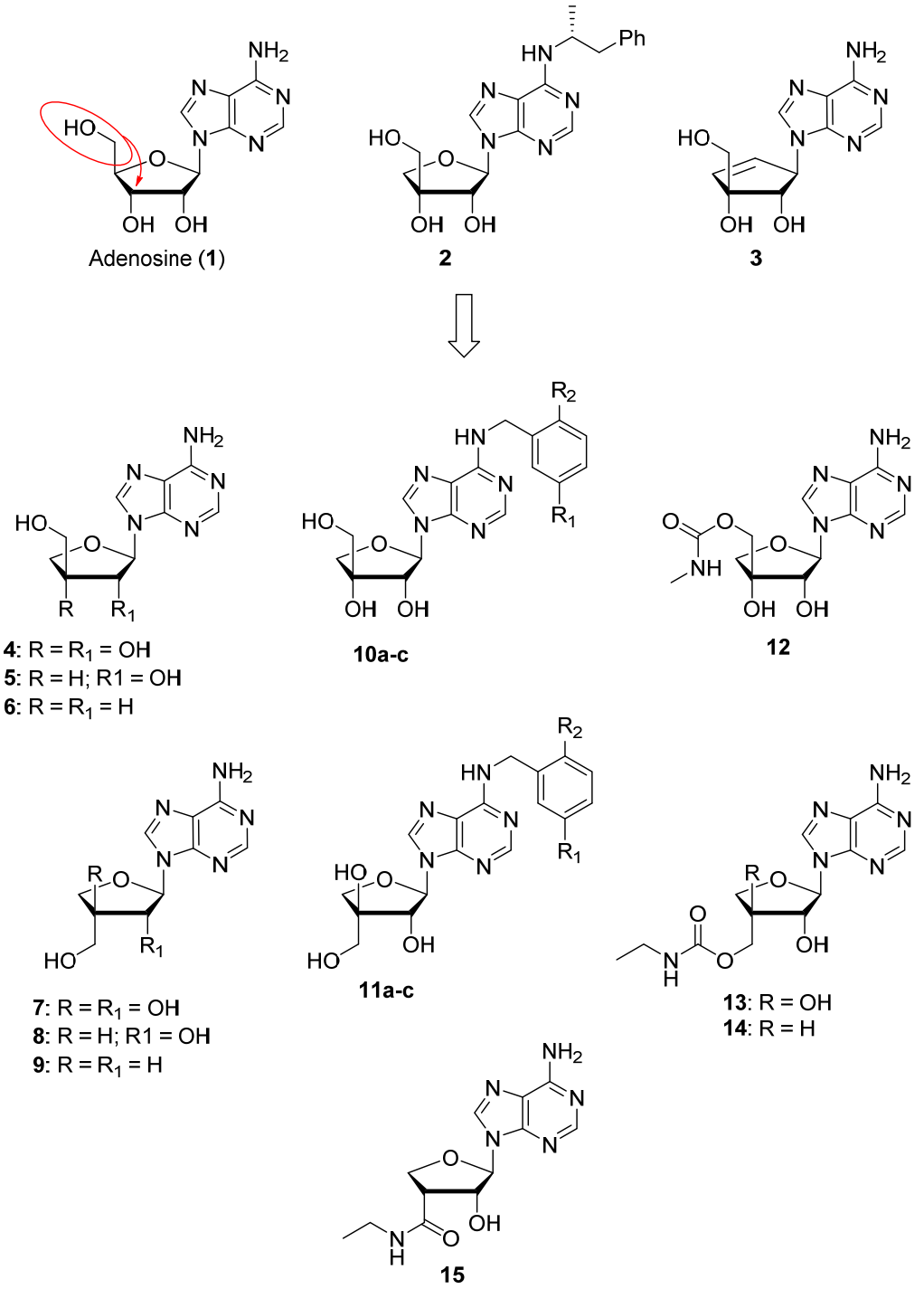
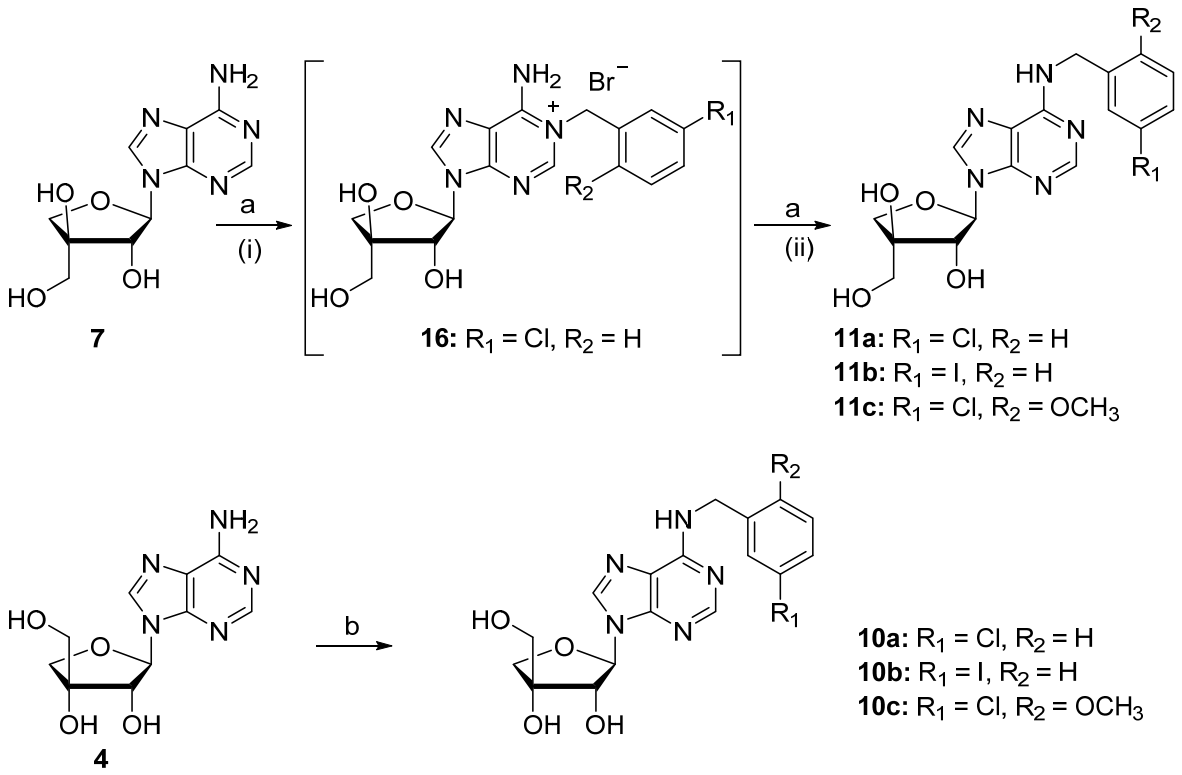


Figure 1. Apio-type nucleosides previously tested for modulation of ARs (**1-3**) and target analogues of the present study (**4-15**).

RESULTS AND DISCUSSION

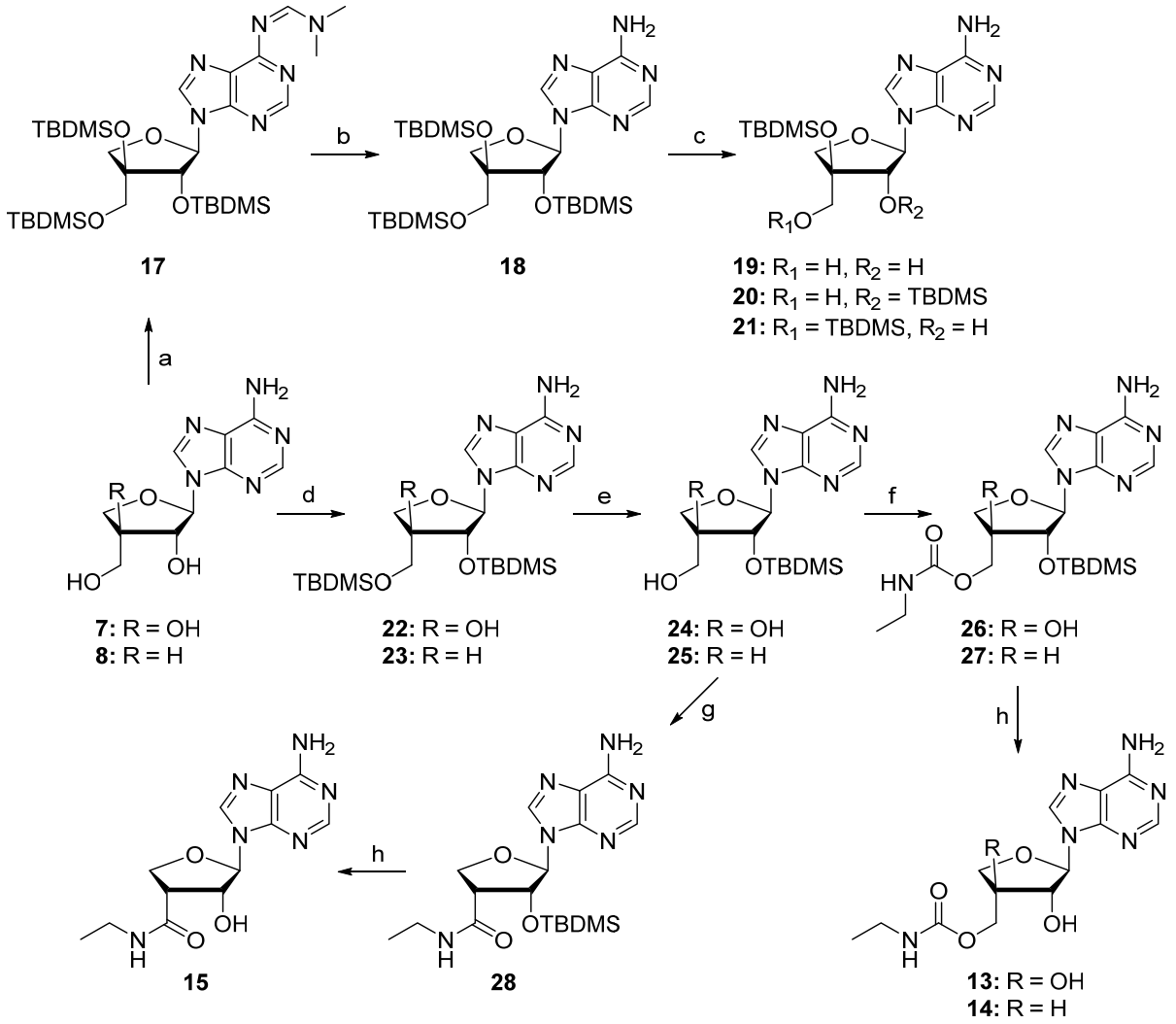
Chemistry

The β-D-apio-D-furanoadenosines **4-6** and the α-D-apio-L-furanoadenosines **7-9** were prepared by microwave assisted synthesis as described in ref. [11].



Scheme 1. Synthesis of N^6 substituted apioadenosines. *Reagents and conditions:* (a) (i) appropriate benzyl bromide, DMF, 50 °C, 48 h; (ii) 25% NH_4OH, 50 °C, 48 h or 90 °C, 3 h, 20-56% over two steps; (b) (i) appropriate benzyl bromide, DMF, rt, 48 h; (ii) 25% NH_4OH, 50 °C, 24 h, 8-21% over two steps.

The L-*threo* analogue **7** was used as a model substrate for N^6-derivatisation reactions. Treatment of **7** with the appropriate benzyl bromide first afforded the N^1-benzyl derivative, which was isolated in the case of the 3-chlorobenzyl derivative **16** (Scheme 1). Upon prolonged heating with ammonia solution, the N^1-benzyl intermediates were converted to the desired N^6-benzylated compounds **11a-11c** via Dimroth rearrangement.[14] Although in many cases this method suffered from low yields, it allowed gaining fast access to the target molecules. Using similar conditions β-D-apio-D-furanoadenosine **4** was benzylated at N^6 affording the desired derivatives in low yield after purification by RP-HPLC or preparative TLC. In the case of **10c**, using excess of the benzyl bromide led to degradation of the starting material and afforded bis-(2-methoxy-5-chlorobenzyl)adenine as observed by HRMS. By limiting the amount of this benzyl bromide to one equivalent, **10c** could be obtained in 21% yield.



Scheme 2. Synthesis of sugar modified α-D-apio-L-furanoadenosines. *Reagents and conditions*: (a) TBDMSCl, imidazole, DMF, 100 °C, 3 days, 80%; (b) 7N NH_3 in MeOH, rt, 18 h, 73%; (c) HF.pyridine, pyridine, THF, rt or TCA-H_2O, THF, rt; (d) TBDMSCl, imidazole, DMF, rt, 18 h, 75-83%; (e) **22**, TCA-H_2O, THF, 0°C, 1 h, rt, 1 h, 87%; **23**, HF.pyridine, pyridine, THF, 0°C, 1 h, rt, 1 h, 82%; (f) (i) CDI, THF, rt, 3h; (ii) $EtNH_2$, rt, 16 h, 55-95%; (g) (i) $RuCl_3$, $NaIO_4$, AcCN-CCl_4-H_2O, rt, 7 h; (ii) CDI, THF, rt, 3 h; (iii) $EtNH_2$, rt, 18 h, 9% over three steps; (h) NH_4F, MeOH, 50 °C, 48 h, 70-94%.