Bioorganic & Medicinal Chemistry Letters 19 (2009) 2877–2879

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Enhanced activity or resistance of adenosine derivatives towards adenosine deaminase-catalyzed deamination: Influence of ribose modifications

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ARTICLE INFO

Article history:
Received 23 December 2008
Revised 14 March 2009
Accepted 19 March 2009
Available online 24 March 2009

Keywords:
Adenosine deaminase
Adenosine analogues
Adenosine derivatives
Molecular docking

ABSTRACT

The effect of the presence of the 1′-*C*-methyl group and 2′,3′-*O*-substitution in the adenosine structure on ADA activity has been investigated by modeling studies. Results show that the 2′- and 3′-*O*- substituents are harbored in a quite large cavity of intermediate polarity, whereas the 1′-*C*-substituent clashes against Ala180 distorting the architecture of the catalytic centre. Globally, the study emphasizes the ability of ADA to transform a large set of 2′,3′-*O*-substituted adenosine analogues as well as the opportunity to design 1′-*C*-substituted adenosine derivatives resistant to ADA-catalyzed deamination.

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Adenosine deaminase (ADA, EC 3.5.4.4) catalyzes a rapid and irreversible conversion of adenosine **1** to inosine **2** (Fig. 1).[1] The enzyme is also able to deaminate a wide range of structurally diverse purine nucleosides[2] and plays a key role in purine metabolic pathways as well as in mammalian immune system development. It is well known that the sugar moiety plays a critical role in ADA binding and activation, determining the optimal conformation for enzyme recognition[3] and that substitutions at the 2′- and 3′-*O*-position are compatible with ADA activity.[4] In this respect, modifications of the hydroxy groups at the 2′ and 3′ positions would increase the specificity, preventing recognition by the biological targets for which the contacts realized by such hydroxy groups are mandatory, as in the case of adenosine receptors.[5] The effect of the substitution of the 1′ -hydrogen of adenosine **1** has been reported only recently and this modification seems to be responsible for the lack of the enzymatic activity.[6,7]

In light of the above considerations, in the present study we have analyzed a small set of adenosine derivatives (Fig. 2) modified at the ribose moiety in order to investigate in more detail the properties of the enzymatic cavity which harbors the ribose moiety.[8] The effect of substitution different from an isopropylidene moiety at the 2′ and 3′ positions (compound **3**) on ADA activity was evaluated by preparing compounds **4** and **5**.[9,10] [9*H*-(1-deoxy-3,4-*O*-isopropylidene-β-D-psicofuranosyl)adenine **6** has also been synthesized[11] (Scheme 1) and lack of ADA activity on this 1′-*C*-methyl-substitued nucleoside has been confirmed.[12]

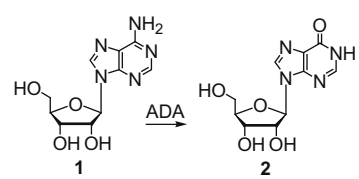

Figure 1. ADA-catalyzed deamination of adenosine **1** to inosine **2**.

Our synthesis utilized 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-1-deoxy-D-psicofuranoses **7a**/**7b** and N-benzoylade-

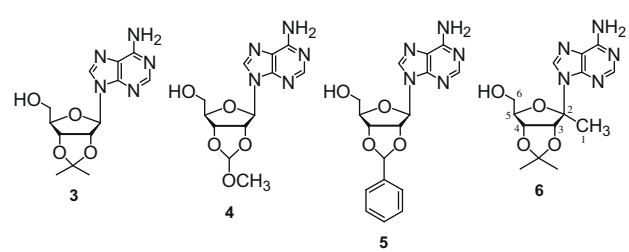

Figure 2. Modified nucleosides described in this study. **3**, **4** and **5** are substrates for ADA, that conversely is unable to catalyze the deamination of 1′-*C*-methyl analogue **6**.

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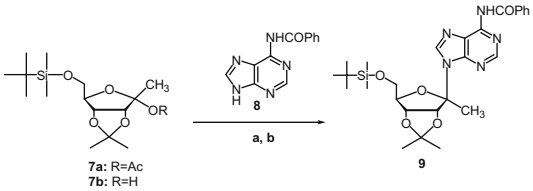
doi:10.1016/j.bmcl.2009.03.084



Scheme 1. Synthesis of nucleoside **9**: (a) coupling of acetate **7a** with N^6-benzoyl adenine (**8**) in the presence of $EtAlCl_2$; (b) Mitsunobu reaction of psicofuranose **7b**.

nine (**8**) under Mitsunobu or Lewis acid promoted coupling conditions (Scheme 1). Briefly, reaction of the anomeric mixture of acetates **7a** (1:2, α-acetate/β-acetate) with N^6-benzoyladenine (**8**) in the presence of $EtAlCl_2$ afforded nucleoside **9** as an anomeric mixture (1:2 α/β, 85%). After chromatographic separation, compound **9** was isolated in 53% yield. The β-configuration of the nucleosidic bond in compound **9** was established analyzing the results of NOE experiments.[13] Finally, the required nucleoside **6** was obtained after debenzoylation and desilylation (see Supplementary data). The overall yield of **6** from 2-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene -1-deoxy-D-psicofuranose (**7a**) can be considered satisfactory (42%), taking into account steps involving anomeric mixtures of intermediates.

The synthesis of intermediate **9** has also been undertaken from anomerically pure psicofuranose **7b**[7] using the Mitsunobu reaction.[14] Although this synthetic methodology has found a wide number of applications,[15] no examples have been provided for the formation of the β-glycosidic bond of a nucleoside in a de novo synthesis, i.e. coupling the anomeric 1-hydroxyl group of a ribose derivative with a purine or pyrimidine. Due to the stereochemical control of the reaction, the expected inversion of configuration of **7b**, that bears an α-hydroxyl, afforded the required β-nucleoside **9**. However, an extensive purification from other by-products was required and yields of compound **9** were only 20–25%. Finally, lack of activity of ADA on the 1′-*C*-methyl nucleoside **6** was confirmed, whereas compounds **4** and **5** were substrates for the enzyme. In particular, ADA activity with 2′,3′-*O*-methoxymethylidene-adenosine (**4**) was higher than that with adenosine (**1**) and 2′,3′-*O*-isopropylidene adenosine **3** (Table 1).

Computational studies were carried out with a view to rationalize the above enzymatic results. Firstly, conformational analyses were performed by MD simulations in water to investigate whether the designed modifications alter the conformational profile of such derivatives rendering them unable to be recognized by ADA.[16] Table 1 collects relevant conformational descriptors for such compounds. It is worth noting that the modifications in 2′ and 3′ with formation of a fused dioxolane ring slightly influence the furanose geometry.

In fact, the puckering angle[17] shifts from 144° (${}_1{}^2$T) to 72° (${}_0{}^4$T) and the pseudorotation angle ranges from −15° to about −60° in derivatives **3–6**. The maximum out-of-plane pucker values (ν_{max}) of **3**–**6** are very similar to that of adenosine (60°) and this suggests that the fused ring does not flatten the ribose. The computational results are in line with NMR pseudorotational analysis of 1′-C-methyl nucleosides related to compound **6** and confirm a 'distorted' anti-position of the purine ring.[18] Moreover, the glycosidic torsion (χ) assumes antiperiplanar geometries in all examined compounds.

The only conformational difference is seen in the angle between the plane of the purine base and that of the furanose (ring packing) which is significantly lower in the derivatives **3**, **4** and **5**. Conversely, the 1′-*C*-methyl derivative **6** shows an angle quite identical to that of adenosine, probably due to the steric hindrance at 1′. Also this conformational difference is confirmed by NOE contact pattern, which suggests that the 1′-*C*-methyl causes a torsion of the

Table 1
Enzymatic data, conformational properties and docking scores for adenosine analogues **3**, **4**, **5**, **6** and adenosine (**1**)

Compound	Specific activity	Time of conversion	Glycosidic torsion (χ)	Ring packing	Puckering classification	Pseudororation angle	Electrostatic score	van der Waals score
1	1.00	15	183.83	119.39	${}_1{}^2$T	−14.99	−21.89	−36.96
3	0.90	35	190.97	70.07	${}_0{}^4$T	−58.39	−19.59	−41.26
4	2.00	20	160.94	63.88	${}_0{}^4$T/${}_4$E	−57.68	−25.67	−42.92
5	0.90	60	181.41	65.68	${}_0{}^4$T	−56.57	−18.13	−48.06
6	0.00	–	177.93	120.80	${}_0{}^4$T	−58.02	−14.79	−46.13

Specific activities are expressed as μmol/min/mg enzyme, times of conversion correspond to the minutes for 100% conversion, and docking scores are expressed in kcal/mol.

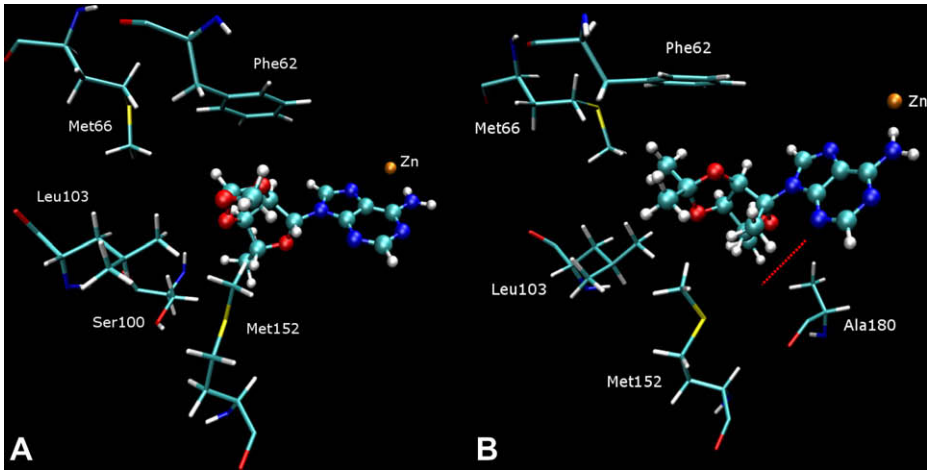


Figure 3. Main contacts stabilizing the computed complexes between ADA and compound **4** (Panel A) and **6** (Panel B). The dashed red line in Panel B enlightens the putative steric clashes between enzyme and **6** responsible for the resistance of this derivative to ADA deamination.

purine base relative to the sugar ring different from that found for 2′,3′-*O*-isopropylidenadenosine **3**.[13] These results indicate that the examined modifications do not heavily constrain the conformational behaviour of the examined compounds, which can assume an optimal conformation to interact with the enzyme.

Docking studies were performed using the crystal structure of bovine adenosine deaminase (PDB Id: 1KRM).[19,20] The computed complexes allowed us to rationalize the differences in deamination reactivity for the considered compounds. Figure 3A reports the complex obtained for the methoxymethylidene derivative **4**, showing that the enzymatic cavity can accommodate it without loosing the pivotal catalytic interactions. The pocket, which harbors the ribose, can be subdivided in two regions. The first more polar region contacts the 5′ hydroxy group, which is involved in strong H–bonds with His14, Asp16 and Ser100. The second more hydrophobic subpocket accommodates the 2′ and 3′ hydroxy groups which do not realize direct polar interactions with related enzyme residues. Hence, it comes as no surprise that the transformation of 2′ and 3′ hydroxy groups in the corresponding dioxolane derivatives does not affect the substrate recognition by ADA. Moreover, docking results reveal that fully apolar substitutions (as seen in **3** and **5**) can realize several hydrophobic and ππ interactions The high specific activity of the 2′,3′-dioxolane derivative **4** suggests that the methoxymethylidene group is able to stabilize polar contacts in the above subcavity. The overall results from 2′,3′-*O*-substituted adenosines suggest that the region harbouring 2′ and 3′ hydroxyl groups can accept moieties with a wide range of polarity and a steric hindrance comparable to that of a phenyl ring without significant alterations of the catalytic activity.

Figure 3B shows the main interactions stabilizing the putative complex between **6** and the enzyme. The 1′-*C*-methyl group that characterizes compound **6** undergoes an apparent unfavourable steric interaction with the side chain of Ala180, thus distorting the fine architecture of the catalytic site. This particular feature can explain why this analogue can occupy the ADA binding pocket (as also suggested by docking scores, see below) without being transformed and thus behaving as a competitive inhibitor.

Among the examined docking scores, Table 1 reports the electrostatic and the van der Waals interaction energies as computed by VEGA. It is worth remembering that docking simulations can model only the recognition phase between enzymes and substrates, whereas the catalytic phase cannot be simulated. Nonetheless, the analysis of compiled scores unveils an interesting correlation between specific activities and electrostatic energies. This confirms that ionic contacts play a key role in the catalytic centre. In contrast, conversion times are in line with apolar contacts which do not enhance the enzymatic efficiency, but reinforce the complex stability, thus hampering the product egress and the enzymatic turn-over. The derivative **6** shows reduced electrostatic energy and significant Van der Waals interactions, confirming that it can occupy the enzyme as a competitive inhibitor.

In summary**,** the present study describes the synthesis of adenosine analogues suitably designed to explore the interaction abilities of the pocket harboring the furanose ring. The enzymatic results evidence that ADA aptly transforms substrates largely modified at the 2′- and 3′-*O*-positions, whereas *C*-modification at the 1′-position prevents the ADA-catalyzed deamination. Docking analyses unveil that the cavity accommodating 2′,3′-*O*-substituents is markedly large and quite apolar, whereas the 1′-*C*-methyl bumps against Ala180 distorting the catalytic centre. The latter observation constitutes an encouraging premise to investigate other 1′-*C*-substituted nucleosides, potentially endowed with interesting biological activities.[21]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.084.

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12. A preliminary account on the synthesis of compound **6** and related ADA activity has been presented at the International Roundtable on Nucleosides, Nucleotides and Nucleic Acids (Bern, Switzerland, September 3–7, 2006). A short paper reporting the above results has also been published (Ciuffreda, P.; Alessandrini, L.; Santaniello, E. *Nucleosides Nucleotides Nucleic Acids* **2007**, *26*, 1311). A detailed description of the synthesis of **6** is now available as Supplementary data.
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21. For a recent example, see: Sommadossi, J.-P.; Gosselin, G.; Storer, R.; Egan J. WO 037028, 2006 *Chem. Abstr.* **2006**, *144*, 331664.