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A Highly Potent Non-Nucleoside Adenosine Deaminase Inhibitor: Efficient Drug Discovery by Intentional Lead Hybridization

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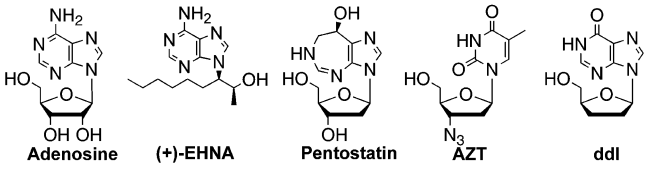
Adenosine deaminase (EC 3.5.4.4; ADA) is a key enzyme in purine metabolism and catalyzes the irreversible deamination of adenosine to inosine. ADA is known to be ubiquitous to almost all human tissues, and ADA abnormalities have been reported in a wide variety of diseases,[1−3] including rheumatoid arthritis.[4] In recent years, the role of adenosine in the attenuation of inflammation has become increasingly clear,[5] and it is also believed that ecto-ADA has an extra-enzymatic function via binding with CD26 on the surface of activated lymphocytes and metabolizing adenosine released at inflamed sites.[6] It is thus considered that an ADA inhibitor may change the concentration of adenosine specifically at inflamed sites and have potential as an antiinflammatory drug with few side effects. We disclose herein the rapid discovery of a novel, highly potent non-nucleoside ADA inhibitor based on the rational hybridization of two structurally distinct lead compounds.

A number of ADA inhibitors have been reported to date, for example, (+)-EHNA[7] and pentostatin.[8] However, all are nucleoside analogues and have many problems, such as poor pharmacokinetics[9] and/or severe toxicity.[10] Therefore, ADA inhibitors free of these problems are of major interest and are expected to have potential use for many clinical conditions. We reasoned that the unfavorable properties of the known inhibitors could be improved by changing the nucleoside framework to a non-nucleoside framework.

It is extremely difficult to modify and replace natural templates, such as nucleosides, peptides, and steroids, by synthetic non-natural frameworks. A very large body of work on this issue has been described, and most efforts have failed to overcome the difficulties associated with the natural template. In particular, the conversion of a nucleoside to a non-nucleoside is extremely difficult and has hardly been reported. As a result, many nucleoside analogues are currently used as medicines, for example, the antivirals AZT[11] and ddI[12] and the ADA inhibitor pentostatin.



We recently reported the discovery of the non-nucleoside ADA inhibitor **1** (K_i = 5.9 μM, human ADA) (Figure 1) by rational design.[13] While this compound is relatively weak in terms of ADA inhibitory activity, it displays good pharmacokinetics and low-toxicity. Hence, it was selected as a first lead compound. Random screening of the Fujisawa chemical library was used to discover a second lead compound **2** (K_i = 1.2 μM) (Figure 1). This compound displayed cytotoxicity, but it also showed moderate ADA inhibitory activity and had a unique structure. The structure of **2** is completely different from adenosine and all known ADA inhibitors. Because the ADA binding mode of this compound could not be predicted by docking studies, we predicted a major conformational change induced by binding of **2** to the enzyme.

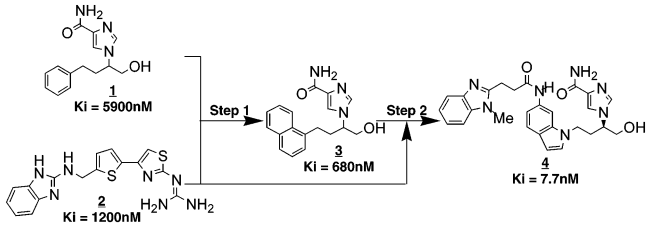


Figure 1. Lead hybridization process for discovery of compound **4**.

Crystal structure analysis of a **2**/ADA complex[14] revealed a major conformational change in the enzyme, as hypothesized above, and the presence of newly formed hydrophobic cavities in the active site (Figure 2A, B, and C). This compound utilizes previously unknown binding pockets in the enzyme that are spatially distinct from the pockets occupied by substrate analogues. A narrow planar hydrophobic space (F1) is occupied by the thiazole and thiophene rings, and the comparatively large hydrophobic space (F2) is occupied by the benzimidazole ring (Figure 2B and C).

Compound **1** binds to known binding pocket in a manner similar to that of substrate analogues, without changing the structure of the enzyme, as shown by molecular modeling.[13] However, the crystal structure of the **1**/ADA complex[14] revealed that the enzyme had also undergone conformational change and newly formed hydrophobic cavities, the same as in the **2**/ADA complex, were also present, but were unused in the binding of **1** (Figure 2B and C). Despite the structural changes to the active site, the structure of **1** bound to the active site was as expected based on docking studies except for the orientation of the phenyl ring (Figure 2A and B). Therefore, we performed conformational analysis around the phenyl rotation axis based on the crystal structure of the **1**/ADA complex. The most stable conformation is distorted 90° around the phenyl rotation axis from the active conformation. This observation provided us with a clue to overcome a difficulty that we faced in the next design step.

Although traditional optimization could be performed separately based on **1** and **2**, we reasoned from the following considerations that hybridization of these two leads should lead to a more active inhibitor. The hydrophobic spaces of F1 and F2 are used in binding of **2**, but not for **1**. Furthermore, the thiazole ring of **2** is located close to the phenyl ring of **1** (Figure 2B and C). These suggested that extension of the molecule from the phenyl ring of **1** to the F1 and F2 spaces might be effective to gain more favorable van der Waals or hydrophobic interactions. However, this is not straightforward because the F1 space and the phenyl face of **1** are in a perpendicular relationship (Figure 2C). We therefore considered that hybridization could be realized intentionally by the following two steps.

For the first step, we planned to rotate the phenyl ring of **1** toward the narrow hydrophobic space (F1). The phenyl ring in the crystal

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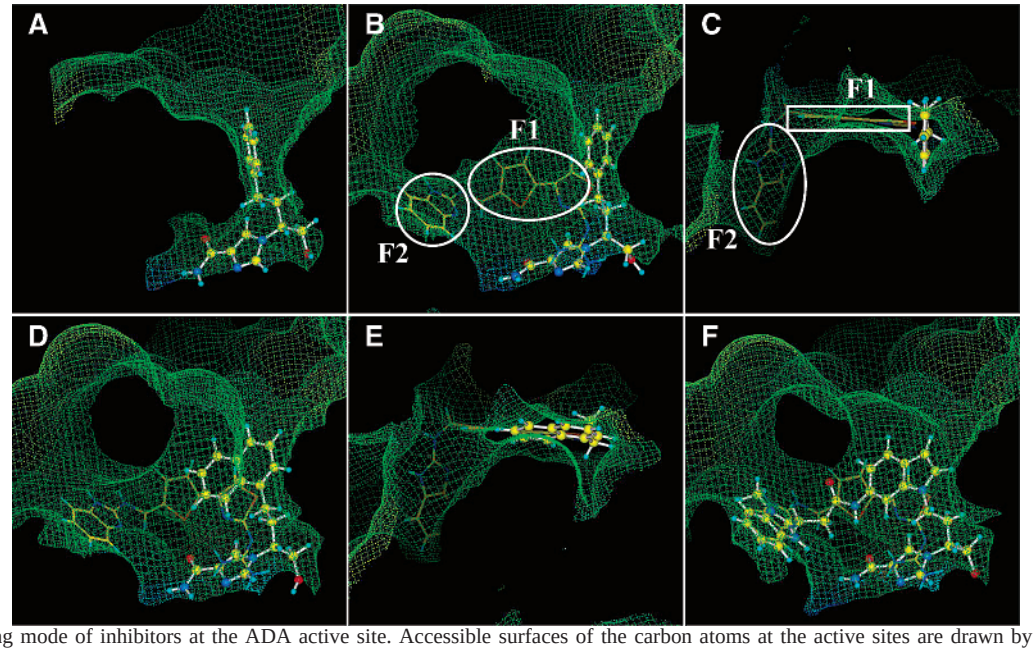

Figure 2. Binding mode of inhibitors at the ADA active site. Accessible surfaces of the carbon atoms at the active sites are drawn by mesh. The upper portion of figures, (A), (B), (D), and (F) is the active-site entrance (solvent region), and other nonmeshed black regions are occupied by protein. (A) Simulated binding mode of **1** to the active site of the substrate analogue/ADA complex. (B) Binding orientations of **1** and **2** superimposed onto the active-site surface of the **2**/ADA complex. (C) View from the active-site entrance of (B). (D) Binding orientations of **3** and **2** superimposed onto the active-site surface of the **3**/ADA complex. (E) View from the active-site entrance of (D). (F) Binding orientations of **4** and **2** superimposed onto the active-site surface of the **4**/ADA complex.

structure is adjacent to the wall of the active-site entrance, in particular, the ortho position of the phenyl ring, and there is no space to introduce a substituent. Therefore, it was considered that a compound containing a substituent in the ortho position of the phenyl ring would not bind to the active site. On the other hand, we appreciated from the docking simulation of **1** that the conformation of the phenyl ring was distorted 90° from that in the crystal structure and that location toward the F1 space was likely to be the most stable. We hypothesized that the phenyl face could rotate and would nicely fit the F1 space by introducing a hindered planar moiety into this position. Hence, a bicyclic naphthyl ring was introduced in place of the phenyl ring of **1**, and the resulting compound **3** was 9-fold more potent ($K_i = 0.68\ \mu M$) than **1** (Figure 1). A crystal structure of the **3**/ADA complex[14] confirmed our design hypothesis (Figure 2D and E).

For the second step, we aimed to use both F1 and F2 hydrophobic spaces by introducing the benzimidazole portion of **2** to **3**. On the basis of the crystal structures of the **2** and **3**/ADA complexes, it was surmised that the substituent should extend from the 6- or 7-position of the naphthyl ring. Due to synthetic ease, the naphthyl ring was changed to an indolyl ring. The resulting hybrid compound **4** was designed as shown in Figure 1. In consideration of the narrow planar space available at F1 and conformational restriction of the designed compound, an amide group was introduced at the 6-position of the indolyl ring to extend the molecule to the F2 region effectively. Furthermore, in consideration of the observation that F1 and F2 are distorted by about 90° by the connection, a two-methylene chain was inserted as a spacer to give flexibility in the connection of the indolyl ring with the benzimidazole. The activity of the newly designed **4** ($K_i = 7.7$ nM) was drastically improved compared to that of the two lead compounds. The crystal structure of the **4**/ADA complex[14] verified the basis of our rational design (Figure 2F). Moreover, compound **4** is expected to avoid the toxic liabilities of nucleosides (not cytotoxic up to a concentration of 1 μM).

In summary, we adopted a new approach to discover a novel highly potent non-nucleoside ADA inhibitor and achieved rapidly and efficiently not only a drastic improvement in activity but also a drastic change in the basic skeletal framework by rational lead hybridization based on our hypothesis. This new method should be applicable not only to many types of enzymes but also to a plethora of potential new drug targets and should provide a method for efficient drug discovery.

Supporting Information Available: Experimental procedures, additional figure and spectral data for **1**−**4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The coordinates of the four complexes have been deposited with the Protein Data Bank: **2**/ADA; 1NDV, **1**/ADA; 1NDW, **3**/ADA; 1NDY and **4**/ADA; 1NDZ.

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