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# Structure-Based De Novo Design of Non-nucleoside Adenosine Deaminase Inhibitors

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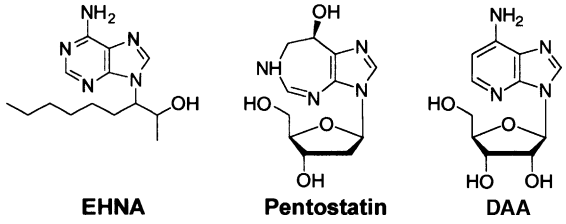
**Abstract**—We searched for non-nucleoside inhibitors of adenosine deaminase by rational structure-based de novo design and succeeded in the discovery of 1-(1-hydroxy-4-phenyl-2-butyl)imidazole-4-carboxamide (FR221647: $K_i = 5.9\,\mu M$ to human ADA) as a novel inhibitor with moderate activity and good pharmacokinetics compared with the known inhibitors pentostatin and EHNA.


Adenosine deaminase (ADA, E.C. 3.5.4.4) is a key enzyme in purine metabolism that catalyzes the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. ADA plays a central role to maintain the immune system, but the physiological role in different tissues has not yet been fully clarified. Recently, it has been shown that ADA is involved in T-cell activation,[1] and it is well known that heritable ADA deficiency is associated with severe combined immunodeficiency disease (SCID),[2] and both enzyme-replacement therapy and gene therapy have been used to treat patients of this rare disease. ADA abnormalities have also been reported in a variety of other diseases such as acquired immunodeficiency syndrome,[3] rheumatoid arthritis,[4] anemia,[5] lymphomas and leukemia.[6] Therefore, compounds that modulate ADA activity have potential as medicines.

A number of ADA inhibitors have been reported to date,[7] and they are classified into two types, ground-state inhibitors and transition-state inhibitors. *Erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA)[8] and 2′-deoxycoformycin (pentostatin)[9] are representative examples of ground-state and transition-state inhibitors, respectively (Scheme 1).




**Scheme 1.** Chemical structures of known ADA inhibitors.

While both compounds possess very potent inhibitory activity toward ADA, they have a number of problems as medicines such as severe toxicity,[10] poor oral absorption and rapid metabolism.[11] Due to these problems, pentostatin is only used as a medicine for lymphoma and leukemia.

ADA inhibitors with reduced toxicity and improved pharmacokinetics are eagerly desired, since they are expected to have potential to be used for many other clinical conditions, for example transplantation, rheumatism, nephritis, diabetes, hypertension, ischemic injury and so on. The unfavorable properties of the

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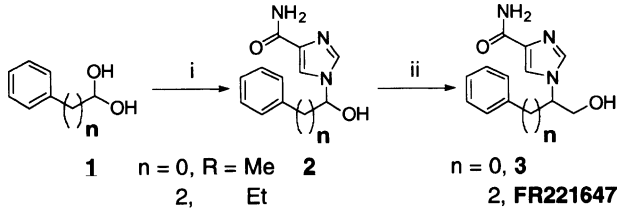
known inhibitors are deemed to be derived from the nucleoside framework of these compounds. Therefore, we speculated that ADA inhibitors without a nucleoside framework would improve these properties, hence we have carried out a rational de novo design of non-nucleoside inhibitors, employing the crystal structures of ADA with several nucleoside inhibitors (see ref 12 for the detailed process) as the basis of the design. In this paper, we describe the successful discovery of a novel, non-nucleoside ADA inhibitor.

## Results and Discussion

In order to discover a first good candidate of ADA inhibitor, it is necessary to obtain a good medicinal lead compound. Therefore, we set the initial goal of this research as creation of a lead compound efficiently which has at least μM order activity and a structure which is amenable to synthetic modification. An important prerequisite to effective design of non-nucleoside inhibitors of ADA is information leading to the identification of chemical structures that are different from adenosine, but retaining the essential sites for interaction with ADA, such as by hydrogen bonds. Judging from the crystal structure of murine ADA with 1-deazaadenosine (DAA) (PDB code: 1ADD),[13] the hydrogen bond between ADA and the N3 of DAA is not considered important, because the bonding distance for this atom pair is longer than those of other hydrogen bonds. Also, the environment occupied by the ribose moiety is rather hydrophobic, whereas the 2′- and 3′-hydroxyl groups are facing to a solvent accessible region, forming hydrogen bonds with water molecules. On the other hand, the 5′-hydroxyl group of the ribose moiety locates at the bottom of the active site forming tight hydrogen bonds with Asp-19 and His-17. These hydrogen bonds are considered to be very important for recognition of ligands and stabilization of complexes. According to such a binding mode analysis, we designed **3**, as shown in Scheme 2.[14] Thus the 1-deazaadenine moiety of DAA is converted to imidazole 4-carboxamide, the ribose ring to a benzyl and the 5′-hydroxyl group to a hydroxymethyl group. The binding mode of DAA and the predicted binding mode of **3** to ADA are shown in Figure 1 (left) and (center), respectively. The N3 atom and the hydroxyl group of **3** form hydrogen bonds to ADA as expected, while the phenyl ring locates at the entrance portion of the active site. This compound was shown to inhibit human ADA with a $K_i$ value of 54 μM (Table 1).[15]



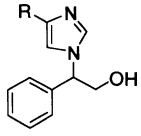

**Scheme 2.** Synthesis of **3** and FR221647. Reagents and conditions: (i) (a) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C, 1 h; (b) 4-imidazolecarboxamide, NaH, DMF, 60 °C, 4 h (38–42%); (ii) $NaBH_4$, MeOH, rt, 30 min (82–100%).

Three derivatives of the carboxamide group of **3**, which can easily be synthesized (methods not shown) and have substituents which may form hydrogen bonds with ADA, did not show inhibitory activities (Table 1). The calculated interaction energies[12] of these compounds with ADA are also shown in Table 1. Generally speaking, calculated interaction energies consider only non-bonded energy terms, and do not reflect the affinities of ligands because they do not take into account desolvation energies and other entropic factors. In this study, however, we adopted the interaction energy to evaluate unfavorable intermolecular interactions such as large inter-atomic clashes or electrostatic repulsions. From this point of view, a negatively charged moiety is unsuitable as a substituent in place of the carboxamide group, because the *E* value for the deprotonated state of **4** is much higher than that for the protonated state.

As for a reason why only **3** has inhibitory activity, hydrogen bonding mediated by water molecules can be considered. According to the predicted binding mode of **3**, Figure 1 (center), there is still a vacant space between the R group (carboxamide in this case) and ADA. Several water molecules should exist in this space, and the carboxamide group may form hydrogen bonds with ADA in a better manner than other compounds.

A detailed inspection of the binding mode of **3** indicates that the entrance of the active site, the hydrophobic space where the phenyl ring exists, is not fully buried by

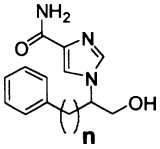
**Table 1.** Simulated interaction energies and inhibition constants for designed compounds with ADA




| Compd | R | *E*(kcal/mol)[a] | $K_i$(μM)[b] |
|---|---|---|---|
| **3** | $CONH_2$ | −54.4 | 54 |
| **4**[c] | COOH | −51.7 | >100 |
| | $COO^-$ | −38.3 | |
| **5** | COOMe | −54.5 | >100 |
| **6** | $CH_2OH$ | −52.4 | >100 |

[a]Calculated interaction energies.
[b]$K_i$ values were measured with human ADA.
[c]Energies of **4** were calculated for both protonated and deprotonated states of the carboxylate group.

**Table 2.** Simulated interaction energies for methylene-inserted analogues of **3**




| *n* | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| *E* (kcal/mol)[a] | −54.4 | −55.6 | −63.9 | −61.6 | −66.6 |

[a]Calculated interaction energies.

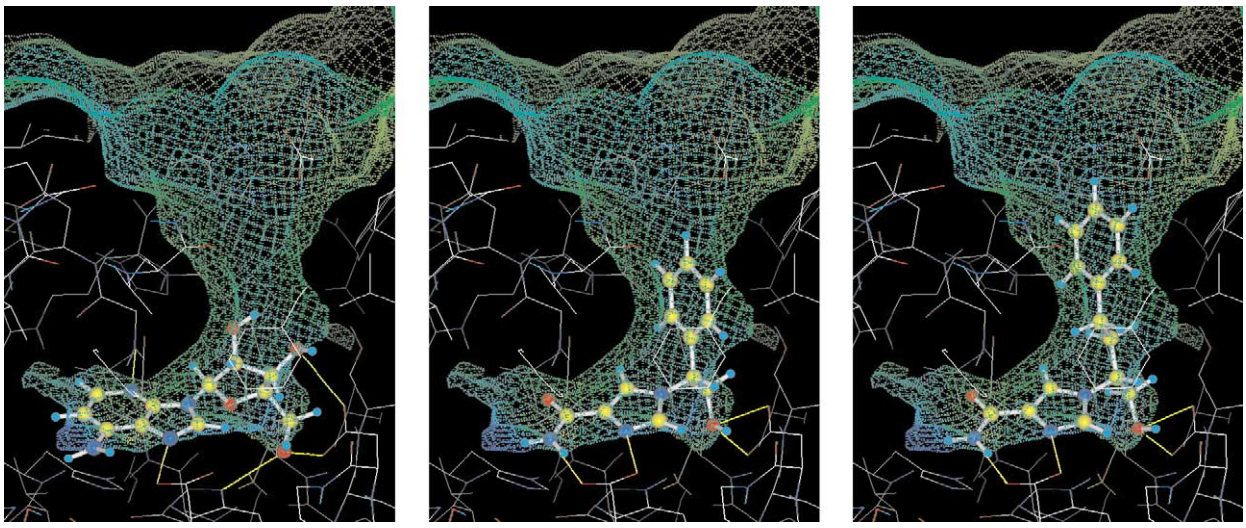


**Figure 1.** Binding modes of 1-deazaadenosine (left), **3** (center) and FR221647 (right). The surface of the active site and the ligands are drawn by mesh and ball-and-stick model, respectively. The proposed hydrogen bonds between ADA and the ligands are indicated by yellow lines. The front part of protein atoms is omitted for clarify.

the inhibitor molecule. Moreover, the fit of **3** to the active site is not so good, hence it was considered that a flexible spacer introduced between the imidazole moiety and the phenyl ring would improve the fit to the active site. Compounds possessing methylene chains of varying lengths were thus designed. Table 2 shows the simulated interaction energies of the designed compounds. Clearly, a chain of two or more methylenes results in lower energies, however, when the chain length is longer than three, the phenyl ring would locate outside the active site, i.e., in the solvent region. Computational simulation can not determine the exact position of the phenyl ring of these compounds, because conformational freedom derived from the flexibility of the methylene chain is largely increased and there are many local energy minima for these compounds. In the case of a chain length of one methylene, it was impossible to locate the phenyl ring in the hydrophobic region while retaining the imidazole moiety in a position where N3 of the imidazole ring and hydroxyl group can form hydrogen bonds. This appears to be reflected in the lower simulated interaction energy. Therefore, we selected a chain length of two and only FR221647 was synthesized. The ADA inhibitory activity of this compound was 5.9 μM. Thus, by introducing a suitable number of spacer atoms, a 10-fold increase of potency has been achieved relative to **3**. The predicted binding mode of FR221647 is shown in Figure 1 (right). This compound fits well to the active site from the entrance through to the bottom of the active site. The pharmacokinetic parameters[16] of FR221647 are shown in Figure 2 and Table 3, in comparison to those of pentostatin and EHNA. Both the oral absorption and the AUC in plasma of FR221647 are drastically improved. Furthermore FR221647 was not cell-toxic to the concentration of 100 μM.

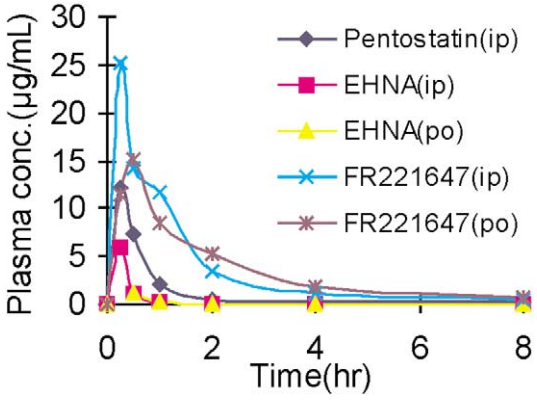



**Figure 2.** Pharmacokinetic parameters of pentostatin, EHNA and FR221647 after ip or po administration to mice (32 mg/kg, $n = 2$–3).

**Table 3.** Pharmacokinetic parameters of pentostatin, EHNA and FR221647 after ip or po administration to mice (32 mg/kg, $n = 2$–3)

| Compd | $K_i$[a] | $C_{max}$ (μg/mL) | $AUC_{0-8\,h}$ (μg h/mL) |
|---|---|---|---|
| Pentostatin (ip) | 33 pM | 12.2 | 9.5 |
| EHNA (ip) | 3.7 nM | 5.9 | 2.2 |
| EHNA (po) | | 1.1 | 1.0 |
| FR221647 (ip) | 5.9 μM | 25.2 | 29.6 |
| FR221647 (po) | | 15.2 | 29.9 |

[a] $K_i$ values were measured with human ADA.

In conclusion, we have succeeded in the de novo design of a non-nucleoside type of ADA inhibitor using the three dimensional structure of ADA complexed with known nucleoside inhibitors. Simultaneously, the undesirable properties of the nucleoside-type inhibitors were also improved. We believe that FR221647 is a good lead compound for further modification studies in respect to both inhibitory activity and pharmacokinetic properties. For efficient design of superior compounds, crystallographic analyses are now in progress.

## Acknowledgements

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12. In this study, docking simulation of the designed compounds was performed prior to synthesis. The enzyme portion of the crystal structure of bovine ADA complexed with 6(*R*)-hydroxy-1,6-dihydropurine riboside (PDB code: 1KRM)[17] was used as the human ADA model, because the sequence identity between human and bovine ADA is 93% and the putative active site amino acid residues are completely conserved. Modeling of the designed compounds used SYBYL.[18] The docking simulation of the modeled compounds at the active site of ADA was performed using GREEN[19] in the following way: Each compound was placed at the active site manually, and the energetically stable positions were explored using the Monte Carlo simulation mode considering the flexibility of the rotatable bonds. From all the minima found, the best ten were further minimized, and the interaction energy of each compound was calculated based on the most stable complex structure. These interaction energies were calculated considering only non-bonded energy terms, i.e., van der Waals and coulombic terms.
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14. Data for **3** and **FR221647**: **3**: mp 147–149 °C; MS: 232 $(M+H)^+$; $^1$H NMR (200 MHz, $CDCl_3$) δ: 4.26 (2H, d, $J=5.4$ Hz), 5.35 (2H, br), 7.05 (1H, br), 7.10–7.50 (5H, m), 7.64 (1H, s), 7.75 (1H, s); IR (KBr, $cm^{-1}$) : 3324, 3187, 1668. Anal. calcd for $C_{12}H_{13}N_3O_2{\cdot}0.2H_2O$: C, 61.37; H, 5.75; N, 17.89. Found: C, 61.51; H, 5.60; N, 17.62. **FR221647**: mp 127.0–129.5 °C; MS: 260 $(M+H)^+$; $^1$H NMR (200 MHz, DMSO-$d_6$) δ: 2.06 (2H, q, $J=7.6$ Hz), 2.39 (2H, t, $J=7.6$ Hz), 3.63 (2H, t, $J=5.5$ Hz), 4.10 (1H, qui, $J=6.4$ Hz), 5.01 (1H, t, $J=5.3$ Hz), 7.04 (1H, brs), 7.10–7.33 (6H, m), 7.70 (1H, s), 7.75 (1H, s); IR (KBr, $cm^{-1}$) : 3500–2700, 1664. Anal. calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.2. Found: C, 65.06; H, 6.59; N, 16.36.
15. Human recombinant ADA was expressed and purified from an ADA-deficient bacterial strain.[20] The reaction velocity ($v$) is measured by change in absorbance at 265 nm (A265) resulting from the deamination of adenosine. Reaction mixtures in a total volume of 200 μL contained 25 mU/mL of ADA and varying concentrations of adenosine and test compounds in 10 mM phosphate buffer saline (pH 7.4). The reaction was started by addition of ADA to a mixture of adenosine and test compound. The reaction was followed at room temperature by recording the decrease in A265 for 5 min in SPECTRAmax 250 (Molecular Devices, USA) to automatically calculate $V_{max}$. The inhibition constant ($K_i$) values of test compounds were determined by Dixon plot.
16. Pharmacokinetic parameters were evaluated after ip or po administration (32 mg/kg) to BDF1 mice ($n=2$–3).
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