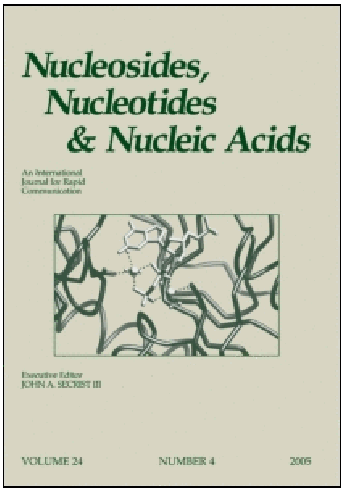

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Irreversible, Tight-Binding Inhibition of Adenosine Deaminase by Coformycins: Inhibitor Structural Features That Contribute to the Mode of Enzyme Inhibition

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IRREVERSIBLE, TIGHT-BINDING INHIBITION OF ADENOSINE DEAMINASE BY COFORMYCINS: INHIBITOR STRUCTURAL FEATURES THAT CONTRIBUTE TO THE MODE OF ENZYME INHIBITION

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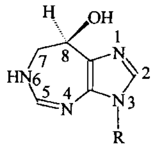
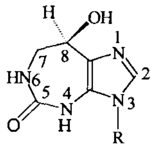
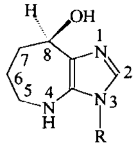
ABSTRACT: *Coformycin analogues 1-6 were synthesized and biochemically screened against adenosine deaminase in order to assess the relative contributions of N-4, N-6, and the N-3 sugar moiety to the mode of enzyme inhibition. Our results indicate that N-4 plays a relatively greater role than N-6 in enzyme tight-binding, and that a benzyl group can substitute for the sugar moiety at N-3. The absence of a sugar or benzyl group at N-3, however, leads to loss of activity. The hydroxyl group at C-8, while crucial for activity, does not alone confer the tight-binding characteristics to coformycins.*



Coformycins
R = Ribosyl or 2'-Deoxyribosyl



1; R = Ribosyl
3; R = CH_2Ph
5; R = H



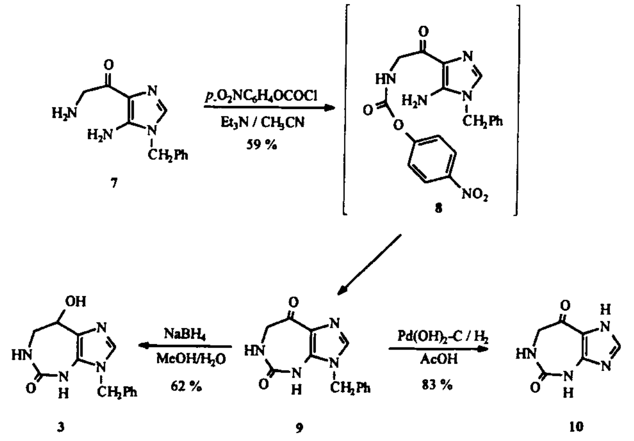
2; R = Ribosyl
4; R = CH_2Ph
6; R = H

Coformycin[1] and 2'-deoxycoformycin (pentostatin)[2] are naturally occurring antitumor antibiotics. Their antitumor activity is known to arise from two different pathways, both of which have the origin in their ability to strongly inhibit the enzyme adenosine deaminase (ADA) ($K_i < 10^{-11}$ M).[3] In the first pathway, coformycins exert synergistic effects upon co-administration with potent antitumor adenosine analogues such as Ara-A,[4a] formycin A,[4b] or cordycepin,[4c] which would otherwise be hydrolyzed by ADA into their inactive inosine counterparts. In the second pathway, coformycins act as immunosuppressants,[5] by mimicing ADA deficiency, to control several lymphoproliferative disorders, including lymphomas, leukemias, and other diseases associated with the hyperimmune system. The ADA deficiency is known to be the principal cause of the severe combined immunodeficiency (SCID) syndrome in children.[6] However, despite their promising therapeutic efficacy, the severe toxicities of coformycins have greatly limited their clinical use to-date.[7] The toxicities have now been traced to the nearly irreversible, prolonged inhibition of intracellular ADA, thus requiring synthesis of a new enzyme molecule each time for recovery from toxic effects.[8] Therefore, the search must continue for a reversible, less tight-binding, potent inhibitor of ADA, which could permit shorter duration of action with faster enzyme recovery, and therefore, minimum toxicity. To this end, it would be necessary to explore the structural parameters of coformycin that are crucial for rendering its tight-binding characteristics during interaction with ADA. Reported herein are our preliminary findings on some of the structural features of coformycin that contribute to its tight-binding, irreversible inhibition of ADA.

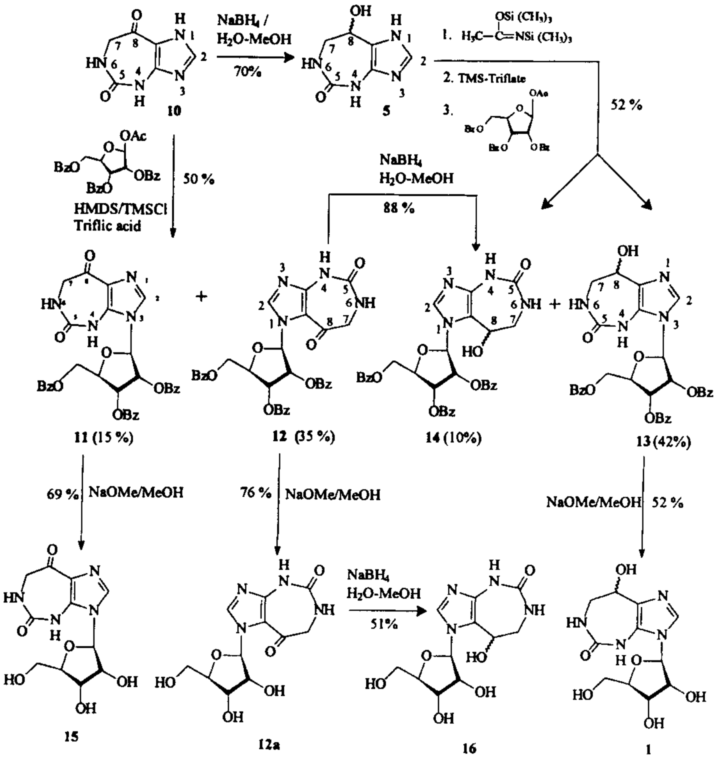
Based on the recently solved crystal structure of a complex of ADA with another ADA inhibitor HDPR ((6R)-hydroxy-1,6-dihydropurine ribonucleoside),[9]coupled with our molecular modeling studies on ADA-coformycin complex,[10] we set out to initially address the following questions related to the enzyme-inhibitor tight-binding: (a) What is the effect of deleting a possible hydrogen bonding interaction of N-4 of coformycin with the protein on the extent and mode of inhibition of ADA?, (b) what would be the outcome of deleting such interactions from both position 4 and 6?, (c) what would be the effect of replacing the hydrophilic sugar moiety with a hydrophobic group?, and (d) what is the consequence of completely eliminating hydrophobic or hydrophilic functionality from position 3? Compounds **1**-**6** are the probes to address these questions. In structure **1**, the amide NH replaces an imine N at position 4. This, in effect, is the conversion of a hydrogen bond acceptor to a hydrogen bond donor, resulting in the elimination of the possible stabilizing interaction of N-4 with the protein. In compound **2**, the NH at position 6 has been replaced by an isosteric CH_2 group, thus eliminating any H-bond interaction of NH-6 with ADA. Analogues **3** and **4** are intended to probe the effects of replacement of the N-3 sugar moiety with a hydrophobic benzyl group, while aglycones **5** and **6** are to reveal the effects of total elimination of hydrophilic or hydrophobic interaction stemming from N-3.

The synthesis of target compounds are outlined in Schemes 1-4. All products were characterized by ^1H NMR, elemental microanalyses and/or high resolution mass spectral analyses. The structures of compounds **10** and **23**, the two key heterocycles in the diazepine and azepine series, respectively, were also confirmed by single-crystal X-ray diffraction analyses. Compounds **1**-**6**, along with **10**, **15**, **16**, **23** and **25** were screened *in vitro* against ADA in a 50 mM phosphate buffer (pH 7) at 25 °C, by spectrophotometrically monitoring the rate of hydrolysis of substrate adenosine at 265 nm. A total of seven different concentrations of the substrate, ranging 10-40 μM, was employed for each inhibitor concentration that ranged 20-25 μM, while the amount of enzyme in each assay was 0.022 unit. The K_i's were computed from Lineweaver-Burk plots, and are collected in Table 1. Compounds **1**-**4** were all found to be competitive inhibitors of ADA with K_i's in the range of 10^{-5} M, whereas **5**, **6**, **10**, **16**, **23**, and **25** were inactive.

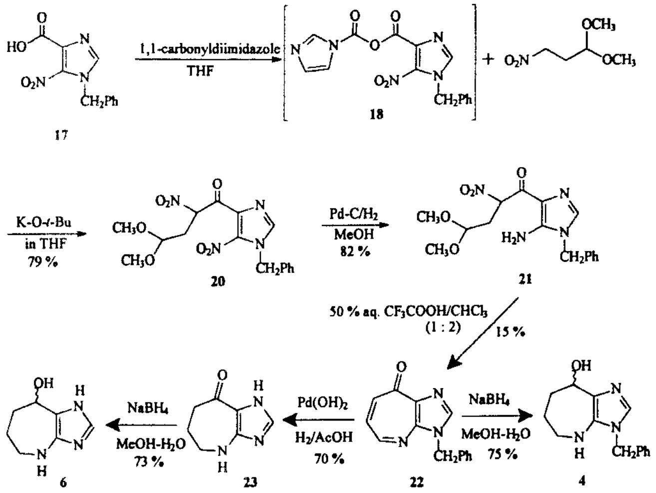
The above results lead to the following conclusions: (a) Both N-4 and N-6 play important roles in the mode and extent of ADA inhibition. However, the deletion of N-6 has minimal effect upon binding as there was no significant change in K_i's of **2** and **4** as compared with those of **1** and **3**, respectively, (b) a benzyl group can replace the N-3 sugar moiety since K_i's of **1** and **2** were not much lower than those of **3** and **4**. This is somewhat surprising since the normal substrates for ADA are nucleosides, and not heterocycles. Nevertheless, it appears that the presence of a benzyl or sugar group at position 3 is necessary for activity as the unsubstituted **5** and **6** were completely inactive, and (c) the hydroxyl group at C-8 seems to be crucial for activity since **15** and **25** which



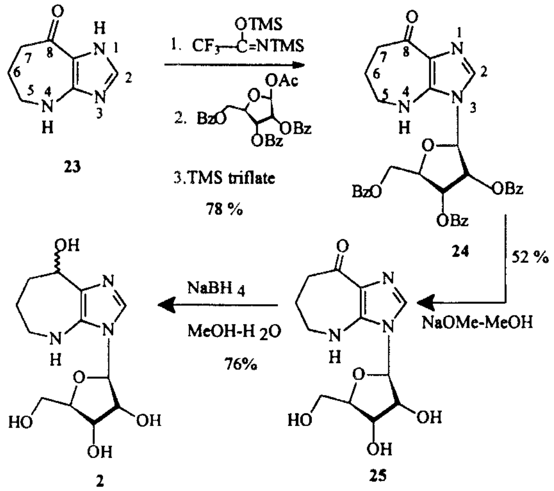
Scheme 1



Scheme 2



Scheme 3



Scheme 4

Table 1. K_i's for target compounds 1-6

Compd	K_i x $10^{-5}M$	Compd	K_i x $10^{-5}M$
1	2.02±0.5	2	3.10±0.42
3	3.79±0.3	4	4.86±0.31
5	~ 0	6	~ 0

possess a C=O group at that position were devoid of activity. Furthermore, the movement of this hydroxyl to a different site in the same molecule, as in **16**, also results in loss of activity. However, the OH group at C-8 alone does not seem to confer the tight-binding characteristics to coformycin since compounds **1-4**, which all contain such a functionality, were found to be reversible, competitive inhibitors of ADA.

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REFERENCES AND NOTES

(1) (a) Nakamura, H; Koyama, G.; Iitaka, Y.; Ohno, M.; Yagisawa, N.; Kondo, S.; Maeda, K.; Umezawa, H. *J. Am. Chem. Soc.* **1974**, *96*, 4327-4328. (b) Ohno, M.; Yagisawa, N.; Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H. *J. Am. Chem. Soc.* **1974**, *96*, 4326-4327. (c) Acevedo, O. L.; Krawczyk, S. H.; Townsend, L. B. *J. Org. Chem.* **1986**, *51*, 1050, and the references cited therein.

(2) (a) Woo, P. W. K.; Dion, H. W.; Lange, S. M.; Dahl, L. F.; Durham, L. J. *J. Heterocyclic Chem.* **1974**, *11*, 641-643. (b) Baker, D. C.; Putt, S. R. *J. Am. Chem. Soc.* **1979**, *101*, 6127-6128. (c) Baker, D. C.; Hawkins, L. *J. Org. Chem.* **1982**, *47*, 2179-2184. (d) Truong, T. V.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 6090-6096.

(3) (a) Agarwal, R. P.; Cha, S.; Crabtree, G. W.; Parks, R. E., Jr., "Coformycin and Deoxycoformycin: Tight-binding Inhibitors of Adenosine Deaminase," in "Chemistry and Biology of Nucleosides and Nucleotides," Harmon, R. E.; Robins, R. K.; Townsend, L. B., Ed.: Academic Press, New York, 1978; pp. 159-197. (b) Agarwal, R. P.; Spector, T.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1977**, *26*, 359-367. (c) Wolfenden, R.; Wentworth, D. F.; Mitchell, G. N. *Biochemistry* **1977**, *16*, 5071-5077. (d) Frick, L.; Wolfenden, R.; Amal, D.; Baker, D. C. *Biochemistry* **1986**, *25*, 1616-1621. (e) Kati, W. M.; Wolfenden, R. *Biochemistry* **1989**, *28*, 7919-7927. (f) Kutz, L. C.; Moix, L.; Riley, M. C.; Frieden, C. *Biochemistry* **1992**, *31*, 39-48.

(4) (a) LePage, G. A.; Worth, L. S.; Kimball, A. P. *Cancer Res.* **1976**, *36*, 1481. (b) Umezawa, H.; Tsutomu, S.; Yasuo, F.; Ikuyo, H.; Masaaki, I.; Tomio, T. *J. Antibiot. (Tokyo), Ser. A.* **1967**, *20*, 308. (c) Johns, D. G.; Adamson, R. H. *Biochem. Pharmacol.* **1976**, *25*, 1441.

(5) (a) Lum, C. T.; Sutherland, D. E. R.; Najarian, J. R. *New Engl. J. Med.* **1977**, *296*, 819. (b) Chassin, M. M.; Chirigos, M. A.; Johns, D. G.; Adamson, R. H. *ibid.*, 1232.

(6) (a) Giblett, E. R.; Anderson, J. E.; Cohen, F.; Pollara, B.; Meuwissen, H. J. *Lancet* **1972**, *2*, 1067. (b) Graver, M. R.; Siaw, M. F. E.; Jacob, W. F.; Niedhart, J. A.; Miser, J. S.; Coleman, M. S.; Hutton, J. J.; Balcerzak, S. P. *Blood* **1981**, *57*, 406.

(7) (a) Major, P. P.; Agarwal, R. P.; Kufe, D. W. *Cancer Chemother. Pharmacol.* **1981**, *5*, 193. (b) *ibid.*, *Blood* **1981**, *58*, 91. (c) Grever, M.; Miser, J.; Balcerzak, S.; Neidhart, J. *Pro. Am. Ass. Cancer Res.* **1980**, *21*, 335.

(8) Lambe, C.; Bugge, C. J. L.; Lafon, S. W.; Nelson, D. J.; Elion, G. B. *Fed. Proc. Am. Soc. Exp. Biol.* **1979**, *38*, 670.

(9) Wilson, D. K.; Rudolph, F. B.; Quiocho, F. A. *Science*, **1991**, *252*, 1278.

(10) Molecular modeling studies were performed using Insight II/Discover (Biosym) software, available from Molecular Simulations, Inc., San Diego, CA.