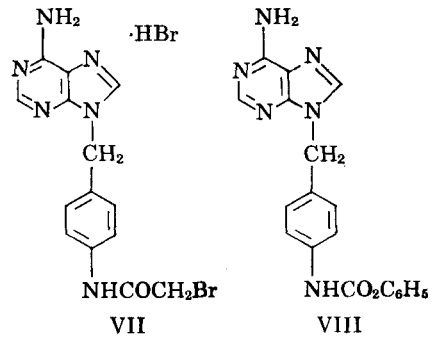
Enzyme Inhibitors XII. Synthesis of an Active-Site-Directed Irreversible Inhibitor of Adenosine Deaminase

Sir:

The formation of a complex between an enzyme and its substrate appears to be an essential process for enzymic reactions. Similarly, the reversible inhibition of an enzymic reaction involves the formation of an enzyme–inhibitor complex. Since many reversible inhibitors are quite specific as to the enzyme or class of enzymes which they will inhibit, it appears that in the design of an irreversible inhibitor, it would be desirable to incorporate as part of the structure of the inhibitor the requisite binding groups for reversible complex formation. Such an irreversible inhibitor could reversibly complex with an enzyme and only then become irreversibly bound to the enzyme. In this way, it should be possible to synthesize irreversible inhibitors which are quite specific for a given enzyme because of their initial reversible complex formation. This general concept has been described by Baker (1), who in a recent review coined the term "active-site-directed irreversible inhibitor" for this type of irreversible inhibitor (2). Other investigators have described similar concepts (3, 4).

The authors have been interested in determining which atoms and functional groups of adenosine are important for binding to the enzyme, adenosine deaminase. As a result of some of these studies, it has been found that the enzyme has relatively little bulk tolerance for either branched or unbranched groups on the 6-amino group of the purine nucleus (5). Consequently, it does not appear likely that the 6-position of the purine nucleus is a good position to modify for the attempted preparation of an irreversible inhibitor. However, it has been shown that the enzyme can tolerate bulky groups which are located at the 9-position of the purine nucleus. Recently, it was found that hydrophobic bonds are important in the formation of an enzyme–inhibitor complex between adenosine deaminase and the alkyl group of some 9-alkyladenines. This nonpolar region of adenosine deaminase appears to extend in the complex from about three to seven carbon atoms from the 9-position of the adenine nucleus (6). In an attempt to take advantage of this nonpolar region of adenosine deaminase for the preparation of an irreversible inhibitor, we decided to prepare some 9-*p*-aminobenzylpurines. The *p*-amino group of the benzene nucleus could be modified with certain alkylating or acylating groups so that they could bridge to a more polar region of the enzyme to allow covalent bond formation. The key compound for this study, therefore, was 9-*p*-aminobenzyladenine since the amino group on the *para* position of the benzene nucleus could be caused to undergo reaction with bromoacetyl bromide or phenyl chloroformate resulting in the formation of an alkylating or acylating group required for a potential irreversible inhibitor.

The synthetic method which was used is a modification of the procedure previously employed. Condensation of 6-chloropurine (I) with *p*-nitrobenzyl bromide (II) gave a mixture of the 9- and 7-substituted-6-chloropurines (III and IV) which were separated by chromatography on alumina. When III was allowed to react with methanolic ammonia, a good yield of the corresponding 6-amino derivative (V) was obtained which on hydrogenation with a palladium-on-charcoal catalyst gave the desired 9-*p*-aminobenzyladenine (VI). Treatment of VI with either bromoacetyl bromide or phenyl chloroformate gave the desired bromoacetamido analog (VII) or the phenoxycarbonylamino derivative (VIII). (Table I.) Enzymic evaluation of VII and VIII revealed that they were both reversible inhibitors of adenosine deaminase.[1] The K_i of VII is 1.2×10^{-5} M, and the K_i of VIII is 2.7×10^{-5} M. When VII and VIII were tested as irreversible inhibitors by incubation with adenosine deaminase, it was found that VII was an irreversible inhibitor, whereas VIII was not active. Figure 1 shows the effect of VII at two different concentrations on adenosine



VII VIII

[1] Adenosine deaminase (type I) was purchased from the Sigma Chemical Co.

TABLE I.—PHYSICAL CONSTANTS OF THE 9-(*p*-SUBSTITUTED BENZYL)ADENINES

Compd.	m.p.,°C.	Anal. Calcd.	Found
III	196–198	C, 49.75	C, 49.48
		H, 2.78	H, 2.59
		N, 24.18	N, 24.40
IV	194–195	C, 49.75	C, 50.00
		H, 2.78	H, 2.89
		N, 24.18	N, 24.42
V	256–257	C, 53.33	C, 53.42
		H, 3.73	H, 3.68
		N, 31.10	N, 31.29
VI	273–275 dec.	C, 59.98	C, 60.08
		H, 5.03	H, 5.10
		N, 34.98	N, 34.72
VII[a]	225–230 dec.[b]	C, 38.03	C, 37.82
		H, 3.19	H, 3.04
		N, 19.01	N, 18.74
VIII	318–322 dec.	C, 63.32	C, 63.16
		H, 4.48	H, 4.38
		N, 23.32	N,2 3.14

[a] Isolated as the hydrobromide salt. [b] This compound decomposes from 225–230° without liquefaction.

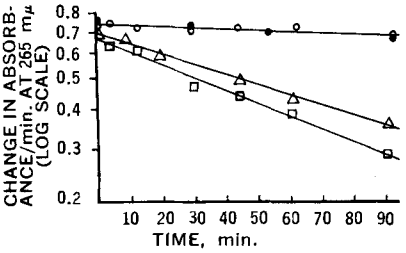


Fig. 1.—Comparison of the irreversible inhibition of adenosine deaminase by 9-*p*-bromoacetamidobenzyladenine (VII) and iodoacetamide. Key: ○, enzyme control; △, 0.015 m*M* VII; □, 0.030 m*M* VII; ●, 0.18 m*M* iodoacetamide.

deaminase as well as the lack of effect of iodoacetamide.

These data indicate that the irreversible inhibition of adenosine deaminase by VII proceeds through an initial reversible enzyme–inhibitor (E–I) complex in which a covalent bond is formed between the inhibitor and the enzyme resulting in irreversible inhibition. Random alkylation of the enzyme by VII as a significant pathway for irreversible inhibition can be excluded by the fact that iodoacetamide did not cause inactivation of adenosine deaminase, even when the concentration of iodoacetamide employed was 12 times greater than the concentration of VII. Comparative chemical reactivities (7, 8) of VII and iodoacetamide using 4-(*p*-nitrobenzyl)pyridine as the nucleophilic reagent revealed that VII is approximately four times more reactive than iodoacetamide. Consequently, the lack of irreversible inhibition of the enzyme by a concentration of iodoacetamide 12 times greater than VII eliminates the possibility of a random alkylation of the enzyme by VII.

In order to determine if a conformational change of the enzyme occurred during the formation of an E–I complex causing exposure of a functional group which could then be alkylated, an equimolar mixture of 9-benzyladenine, a reversible inhibitor, and iodoacetamide was incubated with adenosine deaminase. However, this mixture was not capable of producing irreversible inhibition since it exhibited only the normal thermal loss of enzyme as did the control. Thus, the irreversible inactivation of the enzyme by VII appears to be specifically related to the E–I complex in which covalent bond formation occurs.

When the enzyme was incubated with a mixture of VII and a reversible inhibitor, 9-(3-hydroxypropyl)adenine, the rate of irreversible inactivation of the enzyme was lower. Such protection by a reversible inhibitor has been taken as evidence that the irreversible inhibitor involves the active site of the enzyme (9). Finally, it has been found that 9-(*p*-acetamidobenzyl)-adenine is a reversible, but not an irreversible inhibitor of adenosine deaminase. Thus, the irreversible inhibition caused by VII is dependent on its alkylating ability and not upon some nonspecific poisoning effect of its gross structure.

Consequently, the authors believe that these data indicate that the irreversible inhibition of adenosine deaminase by VII proceeds through a reversible E–I complex which is dependent on the gross structure of the inhibitor. The enzyme is then irreversibly inhibited by alkylation within the E–I complex by the bromoacetamido moiety of VII with the resultant formation of a relatively stable covalent bond between the enzyme and VII.

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HOWARD J. SCHAEFFER
EUGENE ODIN

Department of Medicinal Chemistry
School of Pharmacy
State University of New York at Buffalo

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