

Latent Inhibition of Dihydrofolate Reductase by a Spirocyclopropyl Pteridine

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2-Amino-7,8-dihydro-6-hydroxymethyl-7-spirocyclopropylpteridin-4(3*H*)-one is shown to be the first mechanism-based inhibitor of dihydrofolate reductase whereas 2,4-diamino-5-(4-chlorophenyl)-6-cyclopropylpyrimidine is a typical competitive inhibitor.

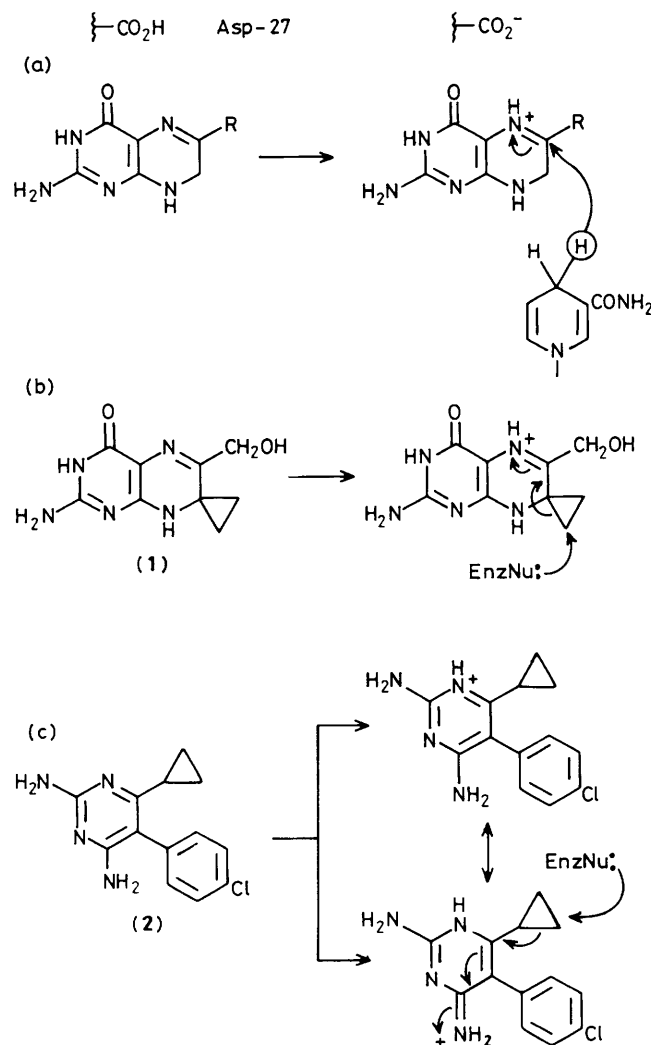
Dihydrofolate reductase (DHFR) is the target of many effective drugs for the treatment of bacterial and protozoal infections and also cancer.¹ Typical drugs are derivatives of 2,4-diamino pyrimidines or pteridines and the binding of such compounds to the enzyme has been studied in detail both by n.m.r. spectroscopy² and *X*-ray crystallography.³ There have, however, been no reports of pteridines with the natural 2-amino-4-oxo function having significant inhibitory properties. Such compounds might be expected to lack the toxicity of some of the 2,4-diamino series.

Our previous work has developed versatile syntheses of 7,7-dialkyl-7,8-dihydropteridines.⁴ We have also shown that cyclopropane-containing compounds can inhibit alcohol and lactate dehydrogenases by a mechanism involving the enhancement of positive charge at the carbon atom adjacent to the cyclopropane ring.⁵ Bearing in mind the likely mechanism of action of DHFR in which a proton is transferred *via* an aspartate residue to N-5 of the pteridine^{2,3} (Scheme 1a), we surmised that such a proton transfer could also activate a

cyclopropyl substituted pteridine [(1), Scheme 1b] or pyrimidine [(2), Scheme 1c] to nucleophilic attack at the enzyme's active site. The synthesis of compounds (1) and (2) was therefore undertaken.

The spirocyclopropyl pteridine (1) was synthesised following our established routes⁴ (Scheme 2) from the monophthalimide of 1-aminocyclopropanecarboxylic acid. The synthesis of the pyrimidine (2) was modelled on established routes for the synthesis of 2,4-diamino-5-arylpyrimidines⁶ (Scheme 3) starting from ethyl cyclopropanecarboxylate. Both (1) and (2) were thoroughly characterised by spectroscopy (i.r., u.v., 250 MHz n.m.r., high resolution mass), and microanalysis.

The properties of (1) and (2) as inhibitors of DHFR were assessed using the enzyme from *Escherichia coli* in a spectrophotometric assay monitoring the decrease in absorbance of the cofactor NADPH. We found that the pyrimidine (2) behaved unexceptionally as a good competitive inhibitor of the enzyme, $K_i = 10^{-7}$ M, with no suggestion of any time-dependent properties. In contrast, the pteridine was a

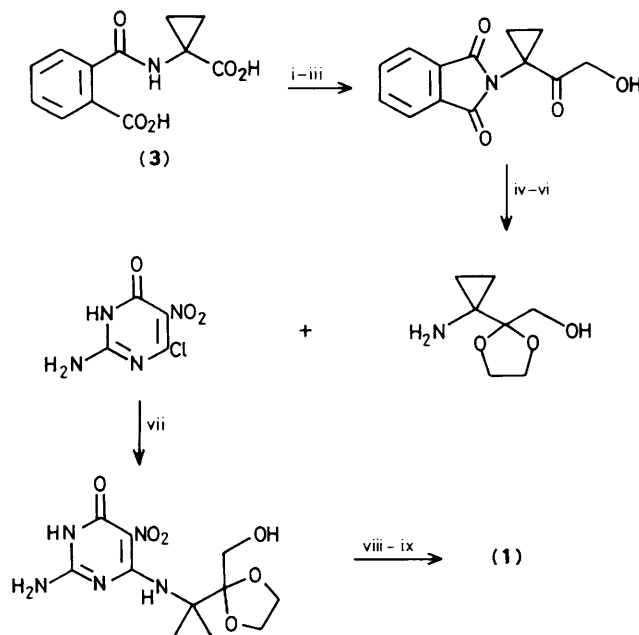


Scheme 1. Mechanistic hypothesis for inhibitor design.

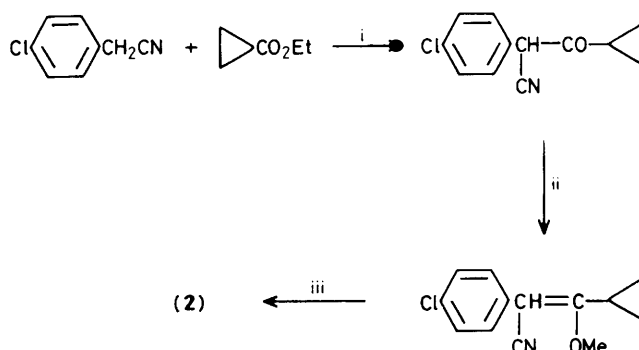
time-dependent inhibitor of the enzyme showing good first order kinetics of inactivation. The rate constant for inhibition was determined as $1.4 \times 10^{-3} \text{ s}^{-1}$ at 25°C . The kinetics of binding to DHFR were complex.⁷

To our knowledge, the pteridine (1) is the first compound with the natural 2-amino-4-oxo substitution pattern to be an inhibitor of DHFR and is also the first mechanism-based inhibitor of this enzyme.⁸

The kinetic studies thus showed that the pteridine (1) behaved in a manner consistent with its design whereas the pyrimidine (2), although it bound tightly, showed no evidence of reaction at the active site. A possible explanation of this paradox can be derived from a computer graphics analysis of the binding of the two inhibitors to the active site of *E. coli* DHFR. The crystal structure of a binary complex of this enzyme with the inhibitor methotrexate has been described³ and we used the co-ordinates of this study to build a model of the active site through the INTERCHEM molecular graphics system.⁹ Figure 1 shows the likely binding of the pyrimidine (2) to DHFR; the orientation of (2) at the active site is determined by the established protonation of such diamino pyrimidines at N-1 by aspartate-27^{2,10} and the location of the 4-chlorophenyl group and hence the cyclopropane ring in a hydrophobic pocket of the enzyme.¹¹ In this binding orienta-



Scheme 2. Synthesis of (1). Reagents: i, SOCl_2 ; ii, CH_2N_2 ; iii, aq. H_2SO_4 ; iv, $\text{HO}(\text{CH}_2)_2\text{OH}$, $p\text{-MeC}_6\text{H}_4\text{SO}_3\text{H}$; v, MeNH_2 ; vi, aq. KOH ; vii, Et_3N ; viii, H_3O^+ ; ix, aq. $\text{Na}_2\text{S}_2\text{O}_4$.



Scheme 3. Synthesis of (2). Reagents: i, NaOEt ; ii, CH_2N_2 ; iii, guanidine.

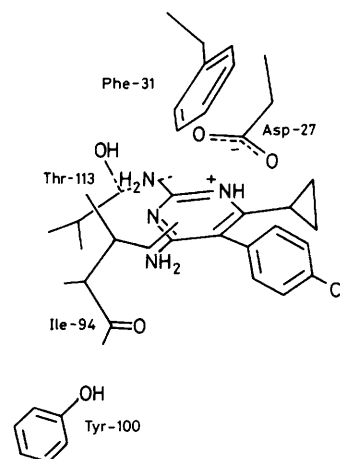


Figure 1. Schematic representation of binding of (2) to DHFR showing the proton donor (Asp-27) and amino acids around the binding site.

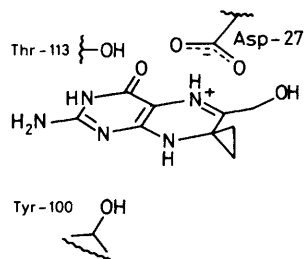


Figure 2. Schematic representation of a possible binding orientation of (1) to DHFR.

tion, we were unable to identify any nucleophiles at the active site within a reasonable range of the cyclopropane ring. Our previous studies have suggested that likely nucleophiles must approach within 2.5 Å of the target cyclopropane for reaction to occur.

In contrast to this probable orientation of the pyrimidine (2), the binding mode of the pteridine (1) is ambiguous. Inhibitor (1) has the natural 2-amino-4-oxo substitution pattern in the pyrimidine ring and would therefore be expected to bind in an inverted orientation from (2) so that its 5-nitrogen atom comes within range of the proton donating aspartate-27. In such a position, once again, no nucleophiles appear to be close enough to attack the cyclopropane (Figure 2). However the pteridine inhibitor (1) does not possess the *p*-aminobenzoylglutamate side chain that is important in the binding of the substrate³ and accordingly is likely to have much greater freedom of binding to the active site of DHFR. An alternative orientation may permit nucleophilic groups such as threonine-113 or tyrosine-100 to attack (1). Further work will establish whether the model proposed is correct.

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