Latent Inhibition of Dihydrofolate Reductase by a Spirocyclopropyl Pteridine

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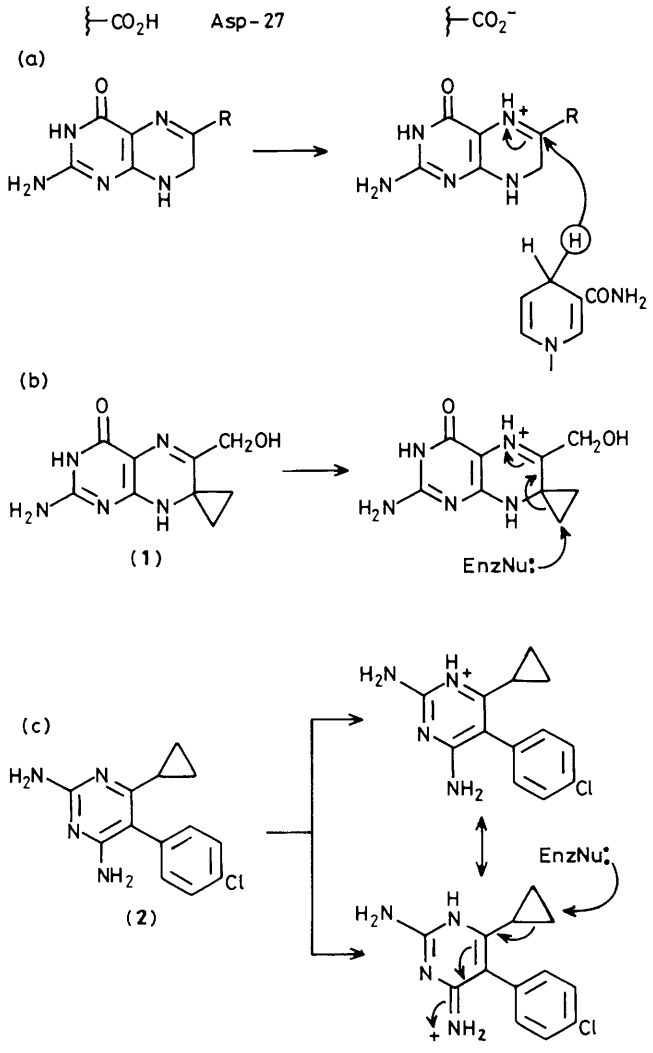
2-Amino-7,8-dihydro-6-hydroxymethyl-7-spirocyclopropylpteridin-4(3*H*)-one is shown to be the first mechanism-based inhibitor of dihydrofolate reductase whereas 2,4-diamino-5-(4-chlorophenyl)-6-cyclopropylpyrimidine is a typical competitive inhibitor.

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. spectroscopy[2] 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.

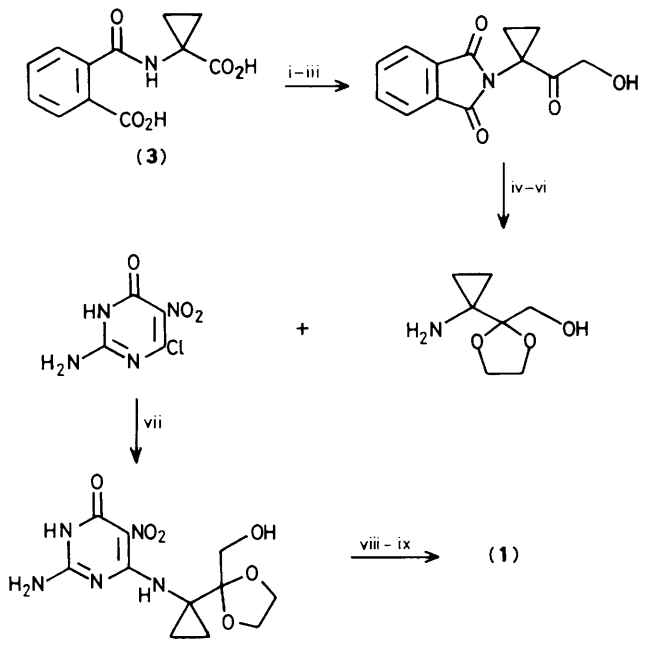
Our previous work has developed versatile syntheses of 7,7-dialkyl-7,8-dihydropteridines.[4] We have also shown that cyclopropane-containing compounds can inhibit alcohol and lactate dehydrogenases by a mechanism involving the enhancement of positive charge at the carbon atom adjacent to the cyclopropane 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 routes[4] (Scheme 2) from the monophthalamide of 1-aminocyclopropanecarboxylic acid. The synthesis of the pyrimidine (**2**) was modelled on established routes for the synthesis of 2,4-diamino-5-arylpyrimidines[6] (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.

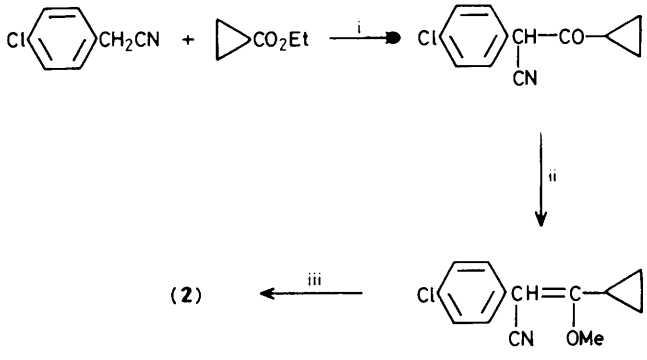
The properties of (**1**) and (**2**) as inhibitors of DHFR were assessed using the enzyme from *Escherichia coli* in a spectrophotometric assay monitoring the decrease in absorbance of the cofactor NADPH. We found that the pyrimidine (**2**) behaved unexceptionally as a good competitive inhibitor of 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.



Scheme 2. Synthesis of (**1**). *Reagents:* i, $SOCl_2$; ii, CH_2N_2; iii, aq. H_2SO_4; iv, $HO(CH_2)_2OH$, *p*-$MeC_6H_4SO_3H$; v, $MeNH_2$; vi, aq. KOH; vii, Et_3N; viii, H_3O^+; ix, aq. $Na_2S_2O_4$.



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

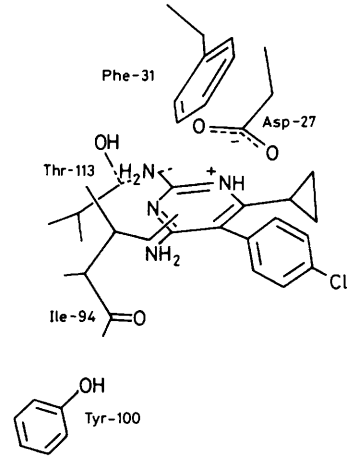


Figure 1. Schematic representation of binding of (**2**) to DHFR showing the proton donor (Asp-27) and amino acids around the binding site.

time-dependent inhibitor of the enzyme showing good first order kinetics of inactivation. The rate constant for inhibition was determined as 1.4×10^{-3} 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 described[3] 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-

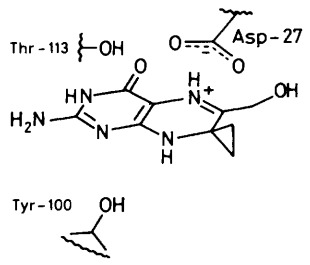


Figure 2. Schematic representation of a possible binding orientation of (**1**) to DHFR.

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 substrate[3] 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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References

1 'Inhibition of Folate Metabolism in Chemotherapy,' ed. G. H. Hitchings, Springer Verlag, Berlin, 1953.
2 G. C. K. Roberts in 'Chemistry and Biology of Pteridines,' ed. J. Blair, de Gruyter, Berlin, 1983, p. 197.
3 D. A. Mathews, R. A. Alden, S. T. Freer, N. Xuong, and J. Kraut, *J. Biol. Chem.*, 1979, **254**, 4144; J. T. Bolin, D. J. Filman, D. A. Mathews, and R. C. Hamlin, *ibid.*, 1982, **257**, 13650; D. J. Filman, J. T. Bolin, D. A. Mathews, and J. Kraut, *ibid.*, 1982, **257**, 13663.
4 S. S. Al Hassan, R. Cameron, A. W. C. Curran, W. J. S. Lyall, S. H. Nicholson, D. H. Robinson, A. Stuart, C. J. Suckling, I. Stirling, and H. C. S. Wood, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1645; R. Cameron, S. H. Nicholson, D. H. Robinson, C. J. Suckling, and H. C. S. Wood, *ibid.*, 1985, 2133.
5 I. MacInnes, D. C. Nonhebel, S. T. Orszulik, C. J. Suckling, and R. Wrigglesworth, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2771; R. J. Breckenridge and C. J. Suckling, *Tetrahedron*, 1986, **42**, 5665.
6 P. B. Russell and G. H. Hitchings, *J. Am. Chem. Soc.*, 1951, **77**, 3763.
7 R. Eisenthal and A. Cornish-Bowden, *Biochem. J.*, 1974, **139**, 715.
8 U.K. Pat. Appl. No. 8619103/1986.
9 P. Bladon and R. Breckenridge, INTERCHEM, Interprobe Chemical Services Ltd., University of Strathclyde.
10 S. R. Stone and J. F. Morrison, *Biochemistry*, 1984, **23**, 2753.
11 V. Cody, *J. Mol. Graphics*, 1986, **4**, 69; C. D. Selassie, Z.-X. Fang, R. L. Li, C. Hansch, T. Klein, R. Langridge, and B. T. Kaufmann, *J. Med. Chem.*, 1986, **29**, 621.