ENZYME INHIBITION STUDIES WITH DICHLOROBENZOPRIM: A NOVEL LIPOPHILIC ANTIFOLATE

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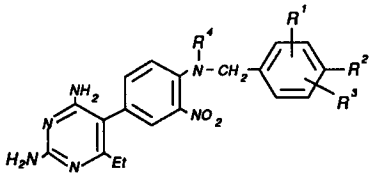
The potential chemotherapeutic utility of lipophilic antifolates as agents for the treatment of infective and proliferative disease is well established, a notable recent indication being for the therapy of Pneumocystis carinii infections in the AIDS patient (Allegra et al 1987). However, clinical development has been overshadowed by toxicity problems possibly attributable to the long biological half-life of these drugs, and the absence of selectivity with respect to inhibition of the target enzyme dihydrofolate reductase (DHFR).

Dichlorobenzoprim (I) has been selected as the most promising of a series of 2,4-diaminopyrimidine DHFR inhibitors on the basis of significant antitumour activity against a methotrexate-resistant tumour cell-line in vivo (Griffin et al 1989). In order to assess the additional possible therapeutic utility of (I) as an antibacterial agent, DHFR-inhibitory activity was determined in comparison with a related series of compounds against enzyme from both bacterial and mammalian sources, such that any diaminopyrimidines exhibiting selectivity for bacterial DHFR could be identified.

Bacterial and mammalian DHFR were isolated from E. coli cells and rat liver homogenate respectively, and partially purified by ammonium sulphate precipitation. Inhibitory activity was determined spectrophotometrically (Bertino and Fischer 1964) by monitoring the consumption of NADPH at 340nm, and the results are summarised in Table 1.

Table 1. Inhibition of E. coli and Rat liver DHFR by 2,4-Diaminopyrimidines

Compound	R^1	R^2	R^3	R^4	IC_{50} (μM) [a]		Selectivity Ratio (Ec/RL)
					Rat Liver DHFR	E. coli DHFR	
I	3-Cl	Cl	H	H	0.008	2.00	250.0
II	H	Cl	H	H	0.018	0.74	41.1
III	H	F	H	H	0.0096	0.038	3.96
IV	H	CF_3	H	H	0.004	0.90	225.0
V	H	Me	H	H	0.0009	0.20	222.2
VI	3-OMe	OMe	H	H	0.008	0.12	15.0
VII	2-Cl	H	H	H	0.0064	0.38	59.4
VIII	2-OMe	OMe	6-OMe	H	0.030	3.00	100.0
IX	H	H	H	Me	0.009	0.90	100.0



[a]Defined as the concentration of inhibitor required to reduce enzyme activity by 50%

All of the diaminopyrimidines evaluated proved to be potent inhibitors of the rat liver enzyme with IC_{50} values ranging from approximately 0.9-30nM. In contrast activity against E. coli DHFR was markedly lower with IC_{50} values ranging from 0.04-3.0μM, only the 4-fluoro analogue (III) exhibiting activity against the bacterial enzyme approaching that observed against mammalian DHFR. The lead compound dichlorobenzoprim (I) proved some 250 times more potent against rat liver enzyme than against that from E. coli suggesting that it would be unsuitable as an anti-infective agent. However, the potential utility of (I) as an antitumour agent prompted further studies regarding inhibition of rat liver DHFR, and a kinetic analysis showed that inhibition is competitive with respect to both dihydrofolate and NADPH, with inhibition constants (K_i) of 0.6 and 0.5nM respectively.

Allegra, C.J. et al (1987) N. Engl. J. Med. 317: 978-985
Griffin, R.J. et al (1989) J. Med. Chem. 32: 2468-2474
Bertino, J.R. and Fischer G.A. (1964) Methods Med. Res. 10: 297-306