

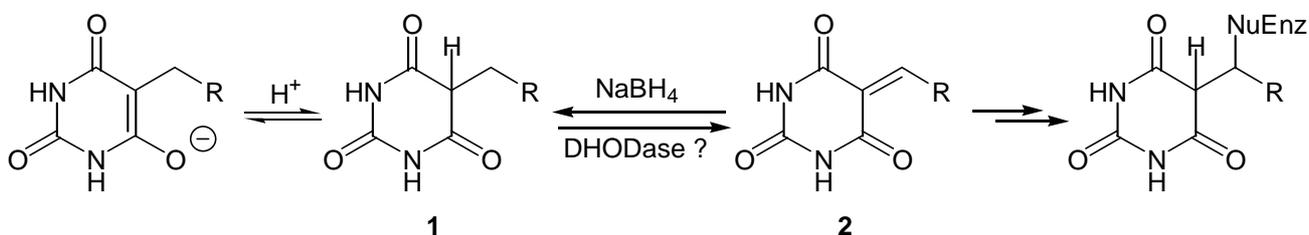
Pro-drug Inhibitors of *Clostridium difficile*

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The project aims to address the pressing clinical need for effective new agents against *Clostridium difficile*, the UK's leading hospital-associated infection (Costello et al 2008). Deaths attributable to the organism have doubled in two years with strains of *C. difficile* having emerged that are resistant to many of the commonly used antibiotics. We have prepared prototype aralkylpyrimidinetrienes (APTs) **1**, some of which are active against *C. difficile* but inactive against *E. coli* and *S. aureus*. Selective targeting of *C. difficile* would provide a significant advantage over current antibiotic treatments that also destroy various gut-colonising bacteria and exacerbate life-threatening *C. difficile* associated diarrhoea (CDAD).

Dihydroorotate dehydrogenase (DHODase) catalyses the reversible and rate-determining fourth step of *de novo* pyrimidine biosynthesis (Argyrou et al 2000). Arylidene barbiturates **2** are known to inhibit purified DHODase from the organism *Clostridium oroticum* with the rate of inhibition being strongly dependant upon the electronic properties of the aryl substituents. Indiscriminate reactivity towards nucleophiles, poor aqueous solubility and the risk of unwanted anxiolytic, hypnotic and sedative properties associated with uncharged CNS-active barbiturate drugs, render arylidene barbiturates **2** poor candidates as potential anti-infective agents.

The APT compounds **1** are considered as having the potential to act as novel pro-drug inhibitors of DHODase that exploit the enzyme's ability to create C=C bonds (Fraser et al 1990). Once bound at the DHODase active site, the benzylic C-C bond in **1** (R = Ar) may be oxidised to give an exocyclic C=C bond, generating the reactive arylidene **2** *in situ*. Attachment of an active site nucleophile (NuEnz), followed by deactivating protonation, should lead to irreversible inhibition of the enzyme (Scheme). Better aqueous solubility and closer resemblance to the enzyme's natural substrate is predicted for the APT derivatives **1**, were ionization to generally occur at C5 (1 R = CH₃; pK_a = 3.9) under physiological conditions.



Knoevenagel condensation between barbituric acid and various aromatic aldehydes will be carried out to give arylidene barbiturates **2**. From these a series of APT derivatives **1** will be prepared by reduction of the respective exocyclic C=C double bonds using sodium borohydride. The arylidenes **2** and the APT derivatives **1** will be tested for activity against *C. difficile*, *E. coli* and *S. aureus*. In an initial screen, compounds active against *C. difficile* but inactive against the other organisms included **1** (R = anthracenyl) and **1** (R = pyrenyl). These preliminary data will be used in parallel with the synthesis work to initiate QSAR studies and computer-aided molecular modelling to predict better patterns of substitution to design and develop more potent inhibitors of *Clostridium difficile*.

Argyrou et al (2000) Dihydroorotate dehydrogenase from *Clostridium oroticum* is a class 1B enzyme and utilizes a concerted mechanism of catalysis. *Biochemistry* **39**:10373-10384.

Costello et al (2008) Thiosemicarbazones active against *Clostridium difficile*. *Bioorg. Med. Chem. Lett.* **18**:1708-1711.

Fraser et al (1990) Latent inhibitors 7. Inhibition of dihydro-orotate dehydrogenase by spirocyclopropanobarbiturates. *J. Chem. Soc. Perkin Trans. 1* (11):3137-3144.