

# Targeting Dihydro-orotate Dehydrogenase in *C. difficile*

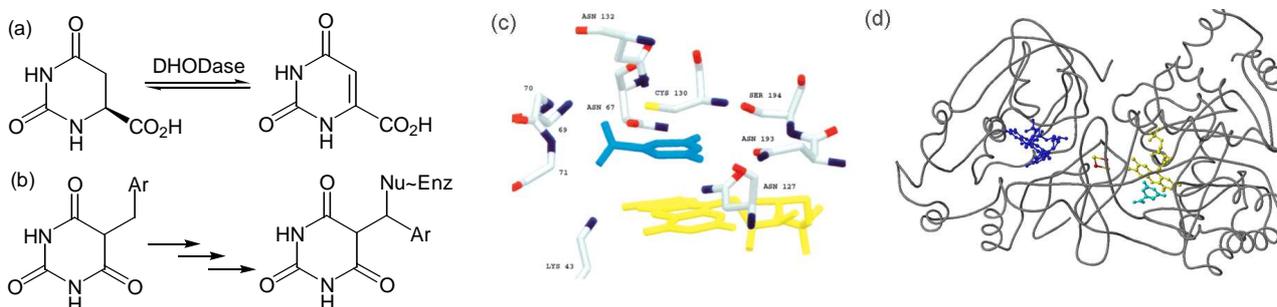
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*Clostridium difficile* (CD) is the leading hospital-associated infection in the UK with many strains possessing multiple-resistance to common antibiotics such as the fluoroquinolones, fusidic acid, erythromycin, clarithromycin and clindamycin (Cosello et al 2008). We have identified dihydroorotate dehydrogenase (DHODase) in *C. difficile* as a potential target for the development of pro-drug inhibitors of this enzyme. DHODase has been the target for a number of antiproliferative agents (Baumgartner et al 2006); it catalyses the fourth step in *de novo* pyrimidine biosynthesis ((a) below left). Human DHODase is well characterised as are the *E. coli*, *L. lactis*, and *Clostridium oroticum* DHODases, among others. The structural and functional differences between human and bacterial enzymes should allow for potential, selective inhibition of bacterial DHODase. In *C. oroticum* ((c) and (d) below), Cys130 is thought to remove the C<sub>5</sub>-*proS* hydrogen with concomitant transfer of hydride to flavine (FMN) to create the C=C bond in a concerted conversion of dihydroorotic acid to orotic acid (Argyrou et al 2000).

We have achieved irreversible inhibition of DHODase from *C. oroticum* using arylidene barbituric acid derivatives with the rate of enzyme inactivation being strongly dependant upon the electronic properties of the aryl substituents (Fraser et al 1990). Arylidene barbiturates are electrophilic Michael-type acceptors and would make very poor drug candidates due to their reactivity towards nucleophiles. We discovered C5-monosubstituted barbituric acids to have growth inhibitory properties against *C. difficile* that were designed as novel pro-drug inhibitors of DHODase that exploit the enzyme's ability to create C=C bonds. Once bound at the DHODase active site, the benzylic C-C bond may be oxidised to give an exocyclic C=C bond, generating the reactive arylidene *in situ*. Attachment of an active site nucleophile (Nu~Enz), followed by deactivating protonation, should lead to irreversible enzyme inhibition ((b) below left).

We propose the preparation of recombinant *C. difficile* and human DHODase enzymes for assay of our existing compounds and to inform the design of second generation inhibitors with improved potency and target selectivity. Although DHODase from *C. difficile* remains structurally uncharacterised, its amino acid sequence is known. Homology modelling will be undertaken to construct a representation of the *C. difficile* DHODase to aid in inhibitor design. The new DHODase inhibitors will be prepared by chemical synthesis for assay against the recombinant DHODase enzymes and will be entered into screening against *C. difficile* and various Gram-positive bacteria using methods currently employed in-house (Costello et al 2008).



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.

Baumgartner et al (2006) Dual binding of a novel series of DHODH inhibitors. *J. Med. Chem.* **49**:1239-1247.

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.