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Inhibiting dihydrodipicolinate synthase across species: Towards specificity for pathogens?

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Abstract—Dihydrodipicolinate synthase (DHDPS) is a key enzyme in lysine biosynthesis and an important antibiotic target. The specificity of a range of heterocyclic product analogues against DHDPS from three pathogenic species, *Bacillus anthracis*, *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus*, and the evolutionarily related *N*-acetylneuraminate lyase, has been determined. The results suggest that the development of species-specific inhibitors of DHDPS as potential antibacterials is achievable.

The development of narrow-spectrum antibacterials has several advantages, including limiting the development of drug-resistance[1] and minimising imbalances on important natural gut and intestinal flora.[2–4] While targeting genus-/species-specific proteins or membrane components has been one method used to achieve narrow-spectrum activity,[5] another is to target specific isoforms of essential bacterial enzymes.[6]

The bacterial diaminopimelate (DAP) pathway is responsible for the biosynthesis of the essential amino acid lysine and its immediate precursor *meso*-DAP, both of which are major constituents of the bacterial peptidoglycan cell wall.[7–9] Lysine is a constituent in Gram-positive bacteria (for example, the pathogenic bacterium *Staphylococcus aureus*) while the cell wall of Gram-negative bacteria, such as *Escherichia coli*, contains *meso*-DAP. Compounds that inhibit the DAP pathway may therefore represent a novel class of antibacterial agents. We have been engaged for some time in a study of the enzyme dihydrodipicolinate synthase (DHDPS, E.C. 4.2.1.52) which, as the first committed step of the biosynthetic pathway, is of particular interest as a drug target.[7,10–12] As part of this programme, we herein report on the potential for achieving species-specificity in the development of DHDPS inhibitors.

DHDPS is a homotetrameric enzyme that belongs to the *N*-acetylneuraminate lyase (NAL) sub-family of enzymes.[13] Each of the four monomeric units of DHDPS and NAL has a $(\beta/\alpha)_8$ barrel fold and these enzymes are believed to have evolved from a common ancestor.[14] The reactions catalyzed by DHDPS and NAL follow similar mechanisms, as shown in Figures 1 and 2.[13] Each enzyme has an active site lysine residue that condenses with pyruvate **1** to form a Schiff base. Tautomerization to the corresponding enamine is then followed by reaction with the relevant aldehyde-containing substrate (aspartate semi-aldehyde **2** or *N*-acetylmannosamine **6**) to generate a 4-hydroxy-2-iminoacid intermediate (**3** or **7**), which then proceeds to the heterocyclic product (HTPA **4** or sialic acid **8**). In the case of the DHDPS-catalyzed reaction, subsequent dehydration gives DHDP **5**.[15] Note that the NAL-catalyzed reaction is drawn in the reverse direction to the dominant physiological process, for comparison.[13]

Keywords: Dihydrodipicolinate synthase; DHDPS; Antibacterials; Species specificity; *N*-Acetylneuraminate lyase; NAL.
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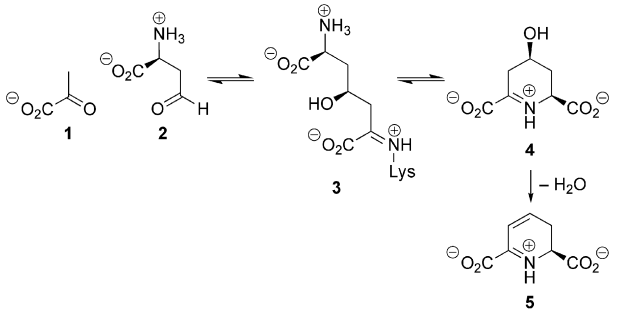

Figure 1. DHDPS-catalyzed reaction.

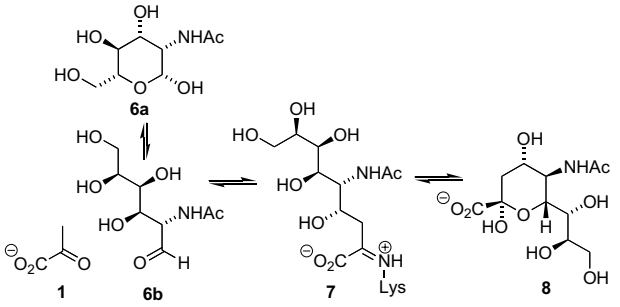

Figure 2. NAL-catalyzed reaction (reverse of physiological direction).

In earlier work, analogues of the DHDPS product 4-hydroxytetrahydrodipicolinate (HTPA **4**) were screened against *E. coli* DHDPS and shown to be moderate, non-competitive inhibitors.[10] Accordingly, we have investigated the efficacy of these compounds against DHDPS from a range of pathogenic bacterial species, in order to establish whether species selectivity of such compounds is feasible. The compounds were also screened against *E. coli* NAL, in order to establish whether the binding site for these inhibitors has been conserved across related enzyme families.

DHDPS from three pathogenic bacterial species, *Bacillus anthracis*,[16] *Mycobacterium tuberculosis*[17] and methicillin-resistant *S. aureus* (MRSA),[18] and *E. coli* NAL[13,18] were expressed in and purified from *E. coli*.

Compounds **9**–**18** (Fig. 3) were synthesised according to the reported methods[10] and were screened against all enzymes (Table 1). In the DHDPS inhibitor screen, our standard coupled assay was employed, in which the coupling enzyme was DHDPR.[12,19,20] For screening NAL, an analogous assay was used employing lactate dehydrogenase as a coupling enzyme[21,22].

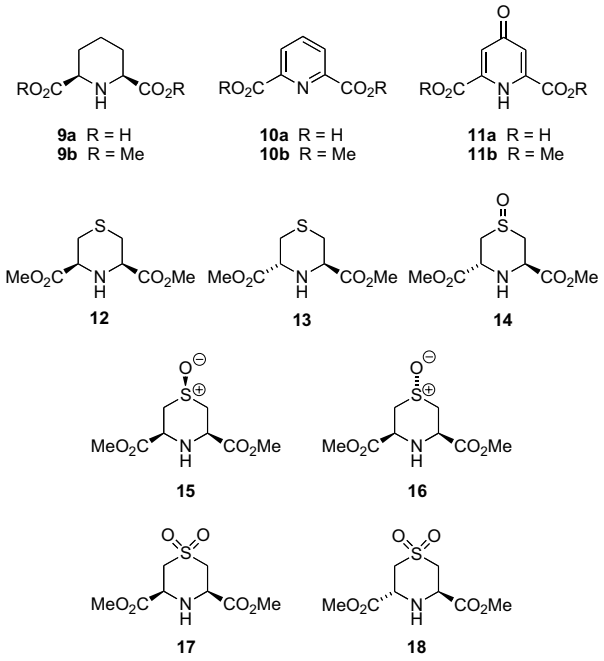

Figure 3. Heterocyclic compounds **9**–**18** tested for inhibition of DHDPS and NAL.

Table 1. Inhibition assays against bacterial DHDPS and NAL[a]

Compound	Inhibition of enzyme activity[b]				
	E. coli DHDPS	*B. anthracis* DHDPS	*M. tuberculosis* DHDPS	MRSA DHDPS	*E. coli* NAL
9a	49	73	43	51	21
9b	92	99	24	58	18
10a	76[c]	80	75	67	1
10b	5	13	0	0	0
11a	74[d]	0	73	83	27
11b	85[e]	0	84	88	64
12	35	23	0	0	1
13	12	19	0	0	0
14	0	7	0	7	7
15	20	22	14	12	38
16	8	8	1	10	0
17	0	19	6	0	3
18	14	6	7	0	0

[a] Assays were performed in duplicate and were typically within ±3%.
[b] % Inhibition in the presence of 20 mM **9**–**18**.
[c] K_i = 11 mM versus **1**, 18 mM versus **2** (Ref. 20).
[d] K_i = 22 mM versus **1**, 25 mM versus **2** (Ref. 10).
[e] K_i = 7 mM versus **1**, 14 mM versus **2** (Ref. 10).

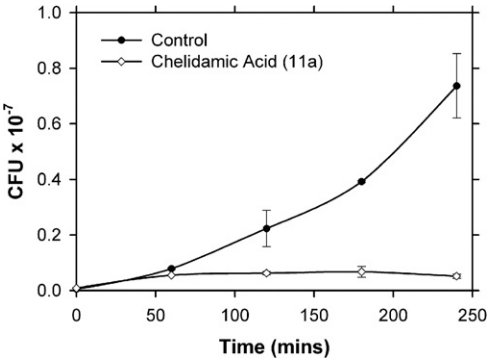

Figure 4. Inhibition of *E. coli* growth by chelidamic acid **11a**.

The DHDPS assays show that piperidine-2,6-dicarboxylate **9a** and dipicolinic acid **10a** are relatively potent inhibitors across all species with little selectivity.

In a dramatic example of species selectivity, chelidamic acid **11a**, and its diester **11b**, are potent inhibitors of DHDPS from *E. coli*, *M. tuberculosis* and MRSA, but in contrast display no inhibition at all of *B. anthracis* DHDPS. Similarly, several compounds, including piperidine diester **9b** and the thiazanes **12** and **13**, exhibit significantly greater inhibitory activity against *E. coli* and *B. anthracis* DHDPS than DHDPS from *M. tuberculosis* and MRSA.

Few of the compounds studied exhibited significant inhibition of *E. coli* NAL, with dimethyl chelidamate **11b** being the only one to show >50% inhibition at the concentration tested, and thiazane-*S*-oxide **15**, piperidine-diacid **9a** and chelidamic acid **11a** exhibiting lower activity. That *E. coli* NAL was inhibited to some degree by several of the compounds suggests that the inhibition site has been conserved across families and may therefore have some functional significance.

In order to validate the inhibitors as potential leads for the development of antibacterial agents, the antibacterial activity of selected compounds against *E. coli* was determined. Compounds **9b**, **11a** and **17** were chosen for analysis: piperidine diester **9b** as it exhibited the most potent inhibition of *E. coli* DHDPS, chelidamic acid **11a** as it shows significant species selectivity and sulfone **17** as a control as it displayed very low levels of DHDPS inhibition across all species tested. Chelidamic acid **11a** at a concentration of 20 mM displayed strong inhibition of bacterial growth (Fig. 4) relative to the control culture in the absence of **11a**. Piperidine ester **9b** also at a concentration of 20 mM displayed moderate inhibition of growth, whereas sulfone **17** displayed virtually no inhibition. These results suggest that DHDPS inhibitors are able to confer significant antimicrobial activity.

In conclusion, several compounds displayed clear differentiation in inhibition of DHDPS enzymes from different bacterial species, which suggests that latter generation compounds could be targeted to specific pathogens. Validation of the potential of these compounds as leads for the development of antibacterials was demonstrated; for example, chelidamic acid **11a** displayed high levels of inhibition of both DHDPS activity and bacterial growth, in contrast to sulfone **17**, which displayed no enzyme inhibition and no antibacterial activity.

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