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PYRIDINE AND PIPERIDINE DERIVATIVES AS INHIBITORS OF DIHYDRODIPICOLINIC ACID SYNTHASE, A KEY ENZYME IN THE DIAMINOPIMELATE PATHWAY TO L-LYSINE

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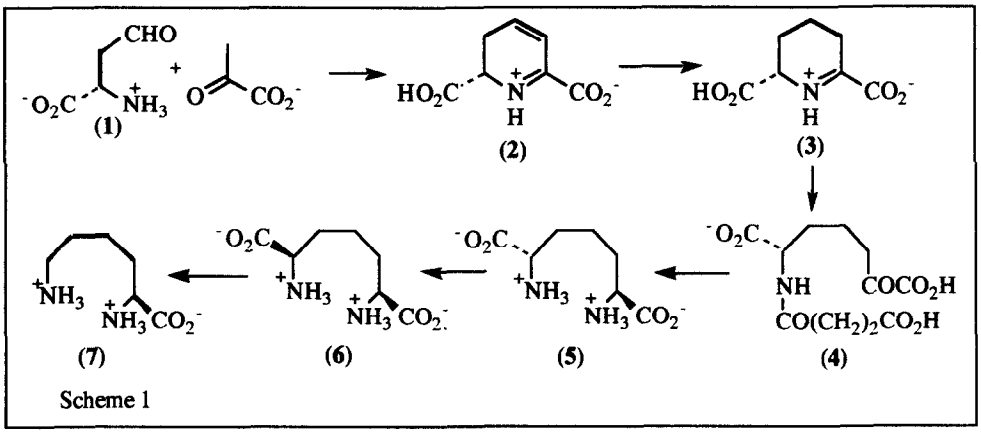
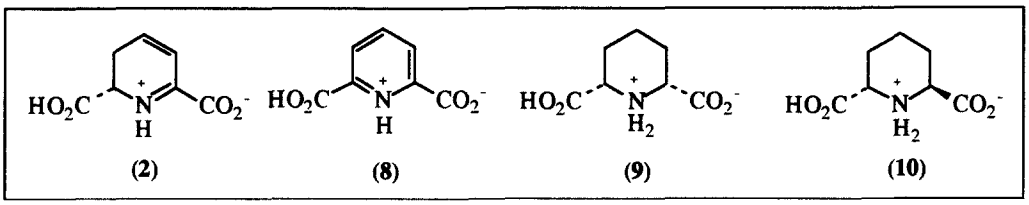
Abstract. A key step in the diaminopimelate (DAP) pathway to L-lysine (**7**) involves condensation of pyruvate with aspartic acid β-semialdehyde (**1**) to yield L-2,3-dihydrodipicolinic acid (**2**) (DHDPA) catalyzed by DHDPA synthase. The best inhibitors of DHDPA synthase of the thirty pyridine and piperidine derivatives prepared were the *N*-oxide (**15**) of dipicolinic acid and the di-imidate (**13**) of dimethyl pyridine-2,6-dicarboxylate each with an IC_{50} value of 0.2 mM. The *N*-oxide (**15**) and dinitrile (**12**) are non-competitive inhibitors with K_i values of 0.29 and 1.25 mM against aspartate semialdehyde and 0.06 and 0.34 mM against pyruvate, respectively.

The enzyme dihydrodipicolinic acid (DHDPA) synthase catalyzes the reaction of L-aspartic acid β-semialdehyde (aspartate semialdehyde) (**1**) with pyruvate to form L-2,3-dihydrodipicolinic acid (**2**). This is a key step in the diaminopimelate (DAP) pathway to the essential amino acid L-lysine (**7**) (Scheme 1). This pathway is present in bacteria and higher plants,[1] whereas an alternative route via α-aminoadipate is characteristic of yeast and fungi.[2] In the DAP pathway the action of a reductase then generates L-2,3,4,5-tetrahydrodipicolinic acid (THDPA) (**3**). DHDPA synthase and the reductase enzymes have been characterised from *E. coli*[3] and from plants.[4] Further enzyme catalysed reaction of THDPA leads to the succinyl derivative (**4**) *en route* to LL-DAP (**5**), DL-DAP (**6**), and L-lysine (**7**). A plasmid containing the dapA gene of *E. coli* has been used to overexpress DHDPA synthase. This enzyme has been purified to homogeneity.[5,6] A preliminary X-ray structure for DHDPA synthase has recently been published.[5] L-Aspartate semialdehyde (**1**), one of the substrates of DHDPA synthase, has been synthesized and characterized spectroscopically as the trifluoroacetate salt.[7] The availability of pure DHDPA synthase, and the two substrates pyruvate and L-aspartate semialdehyde (**1**), has enabled us to begin the first systematic study for inhibitors of this enzyme. We have prepared and examined a number of compounds with the common structural feature of a 5- or 6-membered heterocyclic system containing nitrogen substituted with acid mimics at the α- or α,α'-positions. These compounds were designed as product or reaction intermediate mimics of DHDPA. Previously Yamada *et al.*[8] showed that the wheat enzyme is subject to strong feedback inhibition by low concentrations of L-lysine (**7**) in an allosteric manner. Bromopyruvic acid was found to inhibit DHDPA synthase with a K_i value of 1.8 mM and sodium dipicolinate had an IC_{50} value of 1.2 mM against the synthase.[5] Since the DAP pathway is found in bacteria and higher plants, and DL-DAP (**6**) is a

building block for the cell wall cross-linking material of most bacteria, enzyme inhibitors of this enzyme might possess antibacterial or herbicidal activity without mammalian toxicity.



Scheme 1

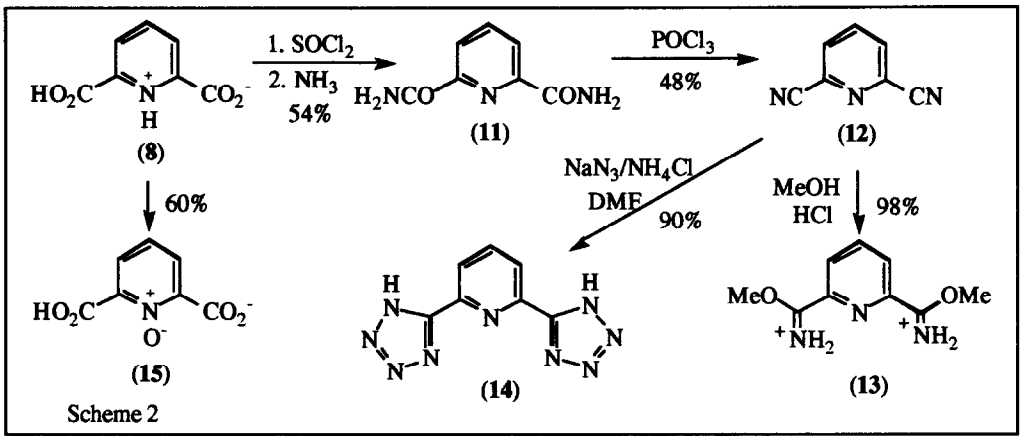


In the reaction of pyruvate and L-aspartate semialdehyde (**1**) to form DHDPA (**2**), the catalytic sequence has been shown to involve binding of pyruvate first to form an imine with the terminal amino group of a lysine residue on the enzyme.[5,6] Then reaction with L-aspartate semialdehyde (**1**) occurs to give DHDPA (**2**). The K_m values for pyruvate and L-aspartate semialdehyde are reported to be 0.52 and 0.55 mM, respectively.[5] The latter value has now been revised by us to 0.23 mM using a sample of pure L-aspartate semialdehyde not previously available.[7] Three main types of structural analogue of **2** were initially considered for assessment as inhibitors based on dipicolinic acid (**8**), and *cis*- (**9**) and *trans*-piperidine-2,6-dicarboxylic acid (**10**). These three compounds have different dispositions of the carboxylate groups with respect to the six-membered ring. Dipicolinic acid is a competitive inhibitor of DHDPA reductase with a K_i value of 0.9 mM.[9]

Dipicolinic acid (**8**) was converted into the diamide (**11**) via the acid chloride, and dehydration of the amide gave the dinitrile (**12**).[10] The di-imidate (**13**) and the ditetrazole (**14**) were prepared in good yields as mimics for the carboxylates (Scheme 2).[11] The *N*-oxide (**15**) and the dimethyl ester (**16**) were also made.

The production, isolation and purification of DHDPA synthase was carried out as described.[6] Enzyme assays were performed with the enzyme (16 absorbance units[6]) using pyruvate (1 mmol) and aspartate semialdehyde (**1**) (1 mmol).[8] The product of the enzymatic reaction, DHDPA (**2**), is oxidised spontaneously in air to give dipicolinic acid which is estimated spectrophometrically by measuring its absorbance at 270 nm.

Potential inhibitors were added to the assay mixture at a range of concentrations and the level of inhibition was measured as a percentage of the initial rate. IC_{50} values were estimated from a plot of the percentage inhibition against the concentration of inhibitor. The results are an average of three determinations and are subject to an error of ±20%. Where IC_{50} values could not be measured, the % inhibition at the highest concentration tested is given (mmol). All of the dipicolinate derivatives tested (Table 1) were good inhibitors (IC_{50} values < 0.7 mM) with the best being the di-imidate (**13**) and dipicolinic acid *N*-oxide (**15**) each with an IC_{50} value of 0.2 mM. For comparison, the methyl ester (**18**) and *N*-oxide (**19**) of picolinic acid (**17**) were prepared and tested as inhibitors of the synthase (Table 2). The IC_{50} values were considerably higher than the corresponding dipicolinate derivatives although the acid (**17**) and the *N*-oxide (**19**) were reasonable inhibitors. The indication is that substituents at both the 2- and 6-positions are preferred for good binding to the enzyme.



Scheme 2

Table 1. Dipicolinic acid derivatives as inhibitors of DHDPA synthase.

Compound	8	12	13	14	15	16
IC_{50} (mM)	0.4	0.3	0.2	0.25	0.2	0.7

Table 2. Picolinic acid derivatives as inhibitors of DHDPA synthase.

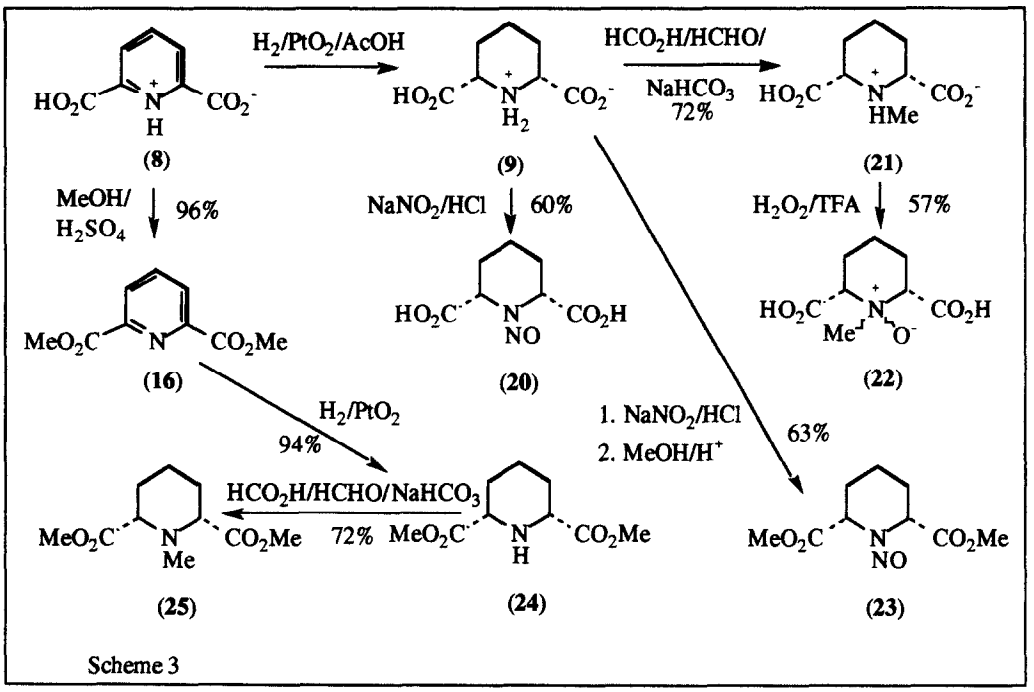
Compound	17	18	19
IC_{50} (mM)	1.0	0% at 10*	0.8

* % Inhibition at highest concentration tested (mmol)

In order to prepare *cis*-piperidine-2,6-dicarboxylate derivatives, dipicolinic acid was hydrogenated to give the *cis*-diacid (**9**), which was converted into the *N*-nitroso derivative (**20**) by the method of Lijinsky *et al.*[12] (Scheme 3). Reductive methylation of the diacid (**9**) afforded the *N*-methyl derivative (**21**) and formation of the

N-oxide (**22**) as one diastereoisomer was achieved using hydrogen peroxide in TFA. Diester derivatives (**23**) - (**25**) were also prepared. Cyclization of diethyl αα'-dibromopimelate with liquid ammonia[13] yielded a mixture of *cis*- and *trans*-diamides (**26**) and (**27**) in a 3:1 ratio. The *cis*-isomer was separated by careful crystallization from water. Treatment of glutaraldehyde with sodium cyanide and ammonium chloride afforded a mixture of the *cis*- and *trans*-dinitriles (**28**) and (**29**),[14] which were separated by trituration with ether in 30 and 23% yields, respectively. The di-imidate (**30**) of the *cis*-dinitrile (**28**) was prepared in 98% yield. The best inhibitor of the synthase from the *cis*-isomers tested was surprisingly the diester (**23**) with an IC_{50} of 0.7 mM where the negatively charged carboxylates have been replaced by ester groups.

Hydrolysis of the *trans*-diamide (**27**) with barium hydroxide gave the *trans*-diacid (**10**) in 96% yield and the dimethyl ester (**31**) and *N*-methylated diacid (**32**) and its dimethyl ester (**33**) were prepared. None of these



Scheme 3

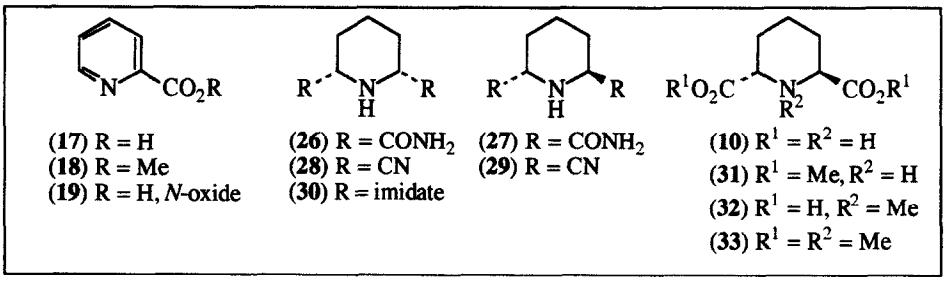


Table 3. *cis*-Piperidine-2,6-dicarboxylic acid derivatives as inhibitors of DHDPA synthase.

Compound	**9**	**20**	**21**	**22**	**23**
IC_{50} (mM)	0% at 10*	2.3	0% at 5*	0% at 10*	6.2
Compound	**24**	**25**	**26**	**28**	**30**
IC_{50} (mM)	0.7	0% at 5*	16% at 10*	7.4	7.2

trans-compounds showed any significant inhibition again surprisingly except the diester (**33**) (Table 4). It should be noted that the *trans*-derivatives will probably have the two substituents the furthest distance apart of the three types of diacid derivative tested (one group axial and one equatorial).

Table 4. *trans*-Piperidine-2,6-dicarboxylic acid derivatives as inhibitors of DHDPA synthase.

Compound	**10**	**27**	**29**	**31**	**32**	**33**
IC_{50} (mM)	0% at 1*	23% at 10*	6.3	5.2	0% at 1*	0.7

For comparison with the saturated *cis*- and *trans*-diacid derivatives some derivatives of DL-pipecolinic acid (**34**) were prepared, including the methyl ester (**35**), the *N*-methylated aminoacid (**36**), the *N*-nitroso-aminoacid (**37**), and the 1,2-didehydro-aminoacid (**38**) (made by treatment of the *N*-tosyl derivative of the methyl ester with potassium *t*-butoxide). All of these compounds were poor inhibitors of the DHDPA synthase except for *N*-nitroso-DL-pipecolinic acid (**37**) (Table 5). Finally, to study the effect of five-membered rings, L-proline (**39**), the methyl ester (**40**), the *N*-nitroso-aminoacid (**41**), and the 1,2-didehydro-aminoacid (**42**) were assessed as inhibitors of DHDPA synthase. Again only *N*-nitroso-L-proline (**41**) showed any inhibition.

Two of the inhibitors, dipicolinic acid *N*-oxide (**15**) and the dinitrile (**12**) were shown to be non-competitive inhibitors (Lineweaver Burk plots) with K_i values of 0.29 and 1.25 mM against aspartate semialdehyde and 0.06 and 0.34 mM against pyruvate, respectively. The apparently stronger inhibition against pyruvate may be due to an indirect blocking effect on the formation of the imine between pyruvate and the enzyme. The fact that **12** and **15** are good inhibitors but have different charge distribution is difficult to explain, but may indicate that there is more than one binding site for these non-competitive inhibitors. Further work is

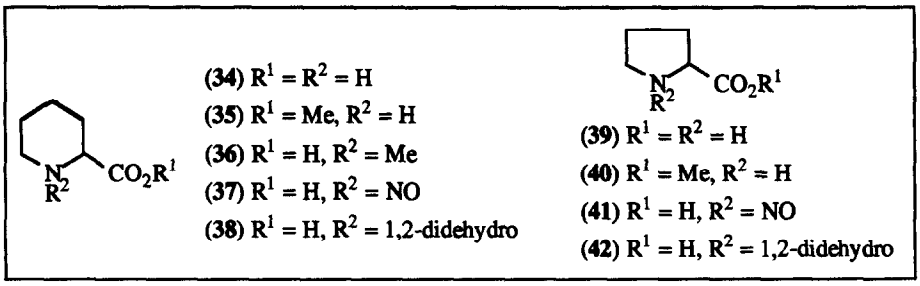


Table 5. Pipecolic acid derivatives as inhibitors of DHDPA synthase.

Compound	**34**	**35**	**36**	**37**	**38**
IC_{50} (mM)	0% at 10*	0% at 10*	0% at 10*	3.5	0% at 5*

Table 6. Proline derivatives as inhibitors of DHDPA synthase.

Compound	**39**	**40**	**41**	**42**
IC_{50} (mM)	0% at 5*	0% at 5*	7.5	43% at 10*

required to determine the mode of inhibition of the other inhibitors. From examination of a variety of dipicolinic acid derivatives and saturated *cis*- and *trans*-analogues as inhibitors of DHDPA synthase, it seems that two substituents adjacent to the nitrogen are required, which can be acid mimics or esters in place of the carboxylates, and that near planarity of these two groups is preferred for good inhibitory action.

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