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Inhibitors of Bacterial N-Succinyl-L,L-diaminopimelic Acid Desuccinylase (DapE) and Demonstration of in vitro Antimicrobial Activity

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Abstract—The dapE-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE) is a critical bacterial enzyme for the construction of the bacterial cell wall. A screen biased toward compounds containing zinc-binding groups (ZBG's) including thiols, carboxylic acids, boronic acids, phosphonates and hydroxamates has delivered a number of micromolar inhibitors of DapE from *H. influenzae*, including the low micromolar inhibitor L-captopril (IC_{50} = 3.3 μM, Ki = 1.8 μM). In vitro antimicrobial activity was demonstrated for L-captopril against *E. coli*.

Bacterial infections are a significant and growing medical problem in both the United States and around the world.[1] The CDC recently reported that there are now several strains of *Staphylococcus aureus* that are resistant to all known antibiotics including vancomycin.[2] These cases underscore the fact that limited numbers of drugs are available to prevent a simple *Staph* infections from becoming deadly.[3] At least four other strains of common bacterial species capable of causing life-threatening illnesses (*Enterococcus faecalis*, *Mycobacterium tuberculosis*, *Escherichia coli* O157:H7, and *Pseudomonas aeruginosa*) are already resistant to nearly all drugs in clinicians arsenal comprised of more than 100 drugs.[4, 5] Thus, the search for new antibiotics that target enzymes in unexplored bacterial biosynthetic pathways is critically important, as confirmed in Supuran's excellent review of bacterial protease inhibitors.[6]

The dapE-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE) enzyme is a member of the *meso*-diaminopimelate (mDAP)/lysine biosynthetic pathway.[7] The amino acids mDAP and/or lysine are essential components of the peptidoglycan cell wall for Gram-negative and most Gram-positive bacteria, providing a link between polysaccharide strands.[7] Therefore, enzymes involved in the mDAP/lysine biosynthetic pathway, including DapE, are potential antibiotic targets.

DapE's are small, dimeric enzymes (41.6 kDa/subunit) that require two Zn(II) ions per mole of polypeptide for full enzymatic activity.[8,9] On the basis of sequence alignments with other aminopeptidases[10] and several DapE gene sequences, all of the residues that function as ligands in the dinuclear active site of those enzymes are strictly conserved in DapE from *Haemophilus influenzae*. Studies on the E134A- and E134D- altered DapE revealed that E134 acts as the general acid/base in the hydrolysis of the substrate and is absolutely required for catalytic activity.[11] Investigations on H67A- and H349A-altered enzymes together with construction of a three-dimensional homology structure of DapE from *H. influenzae* (generated using the X-ray crystal structure of the Apo-DapE from *Neisseria meningitidis* as a template with superposition on the structure of the aminopeptidase from *Aeromonas proteolytica* (AAP)) confirmed the identification of active-site histidine zinc ligands.[12] Based on this homology model, the active site of DapE contains two Zn(II) ions at a distance of ~3.30 Å (Figure 1).

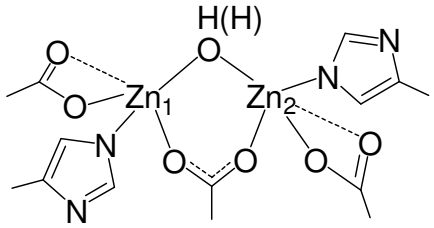


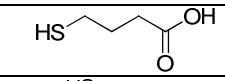
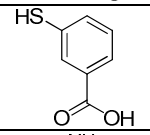
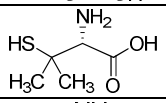
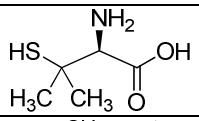
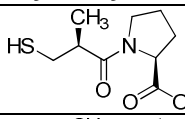
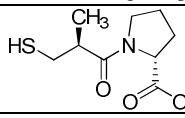
Figure 1. Proposed active site of DapE enzymes.

Each of the Zn(II) ions adopts a distorted tetrahedral geometry and is coordinated by one imidazole group (H67 for Zn1 and H349 for Zn2) and one carboxylate group (E163 for Zn1 and E135 for Zn2). Both Zn(II) ions are bridged by an additional carboxylate groups (D 100) on one side and water/hydroxide on the opposite side, forming a (μ-aquo)(μ-carboxylato)dizinc(II) core with one terminal carboxylate and one histidine residue at each metal site.

In order to identify appropriate lead molecules for the inhibition of DapE, we have screened[13] over thirty molecules representing various structural classes and containing different zinc-binding groups (ZBG's) using N-succinyl L,L-diaminopimelic acid (L,L-SDAP) as the substrate.[14] These ZBG's include thiols, hydroxamates, carboxylic acids, boronic acids, and phosphates, and this fruitful initial screen has led to the identification of a number of low micromolar inhibitors (Table 1). We biased our initial screen to include bifunctional molecules that contained, in addition to the ZBG, a carboxylate moiety that could interact with the positively-charged lysine and/or arginine side chains that purportedly reside near the active site. Table 1 shows carboxylic acid-containing thiols that were found to inhibit DapE fairly potently. Even delta-mercaptobutyric acid has an IC_{50} of 43 μM versus DapE, and meta-mercaptobenzoic acid has a measured IC_{50} of 34 μM. L-penicillamine gave an IC_{50} of 13.7 μM, and a measured K_i of 4.6 μM. DapE is stereoselective with respect to recognition of inhibitors, as D-penicillamine gave an IC_{50} of 50 μM. Given the success with these carboxylic acid-containing thiols, we turned our attention to captopril, which contains the requisite ZBG and carboxylate functionalities. L-Captopril exhibited an IC_{50} of 3.3 μM and a measured Ki of 1.8 μM (competitive). Again, the binding is stereoselective, as D-captopril[15] was an order of magnitude less potent, with an IC_{50} of 42.0 μM.

Table 2 shows boronic acid derivatives that were tested as inhibitors of DapE. Phenylboronic acid itself was encouraging with an IC_{50} of 107 μM and a measured K_i of 56.9 μM (competitive). Incorporation of a carboxylic acid along with the boronic acid was not productive, as both 4-carboxyphenylboronic acid and 3-carboxyphenylboronic acid did not inhibit DapE. It is hypothesized that geometric constraints may have

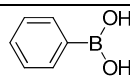
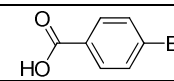
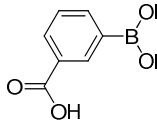
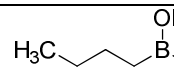
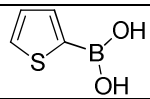
Table 1. Thiols tested as inhibitors of DapE

Compound	Structure	IC_{50} (μM)
delta-mercapto-butyric acid		43±4
3-mercapto-benzoic acid		34
L- penicillamine		13.7 *Ki =4.6±0.6 μM (competitive)*
D-penicillamine		50
L-Captopril		3.3 *Ki =1.82±0.09μM (competitive)*
D-Captopril		42.0±2.5

[a]Values are means of three experiments, standard deviation is given in parentheses.

precluded a productive Coulombic interaction of the carboxylate with positively charged residues in the active site. Butylboronic acid was a weak inhibitor of DapE (IC_{50} ~10 mM). 2-Thiopheneboronic acid was comparable to phenylboronic acid, with an IC_{50} of 92 μM and a measured K_i of 67 μM, but the inhibitor was noncompetitive. Surprisingly, 1-butaneboronic is only a very weak inhibitor of DapE (IC_{50} ~ 10,000 μM) even though it is a potent inhibitor of AAP (K_i = 10 μM).[16, 17]

Table 2. Boronic acid tested as inhibitors of DapE

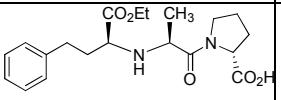
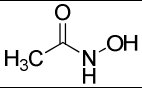
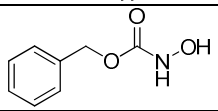
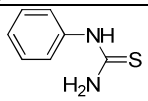
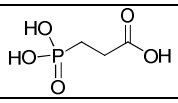
Compound	Structure	IC_{50} (μM)
phenylboronic acid		107/154 μM *Ki=56.9±3.6 μM (competitive)*
4-carboxyphenyl-boronic acid		>10,000
3-carboxyphenyl-boronic acid		>10,000
butylboronic acid		~10,000 uM
2-thiopheneboronic acid		92 μM *Ki=67±3.8 μM (non-competitive)*

[a]Values are means of three experiments, standard deviation is given in parentheses.

Table 3 shows several other compounds that were screened versus the DapE enzyme. Given the very good potency of the ACE inhibitor L-captopril noted above (IC_{50} = 3.3 μM), the ACE inhibitor enalapril was

screened, but did not show any potency versus DapE. Two simple hydroxamate compounds, acetohydroxamic acid and N-(benzyloxycarbonyl)hydroxylamine (actually an N-hydroxy carbamate) were also screened but were too weak to measure the inhibitory potency. N-Phenylthiourea could potentially function as a zinc-binding compound and showed some inhibition of DapE, but the IC_{50} is greater than 100 μM. Phosphonic acids can also inhibit metalloproteases, and (2-carboxyethyl)phosphonic acid was shown to have a very weak IC_{50} of 1,620 μM.

Table 3. Other potential inhibitors explored for the DapE enzyme

Compound	Structure	IC_{50} (μM)
Enalapril (maleate salt)		>1000
Aceto-hydroxamic acid		>1000
N-(Benzyloxy-carbonyl)hydro xylamine		>1000
N-phenyl-thiourea		> 100
(2-carboxyethyl)-phosphonic acid		1620 Ki = 800 μM

[a]Values are means of three experiments, standard deviation is given in parentheses.

Several alkyl and aryl phosphates have also been tested in addition to diaminopimelic acid amides and ornithine amides (structures not shown). In all cases, these molecules exhibited little or no inhibitory potency (IC_{50} >1000 μM - >10,000 μM). Finally, (D,L)-(2-phosphonomethyl)-pentanedioic acid did not inhibit DapE at concentrations up to 10 mM. 2-Carboxyethyl phosphonic acid is a significantly better inhibitor (K_i = 800 μM) than phosphonoacetic acid, 3-phospho-propanoic acid, or N-(phosphonomethyl)glycine, thus presenting an optimal chain length for interactions with DapE. We expect an optimal distance between thiol and carboxylate in the thiol series as well, depending on conformational mobility of the series.

We have confirmed antibiotic activity with the DapE inhibitor, L-captopril, in an antibiotic plate assay as illustrated in the following photos (Figure 2). Application of L-captopril directly to plates cultured with *E. coli* showed a dose-responsive antibiotic activity for this DapE inhibitor. Very little inhibition is observed for 1 mg of L-captopril, but 5 mg demonstrates a clear positive antibiotic result, and the zone of inhibition is even greater for 20 mg of L-captopril. Furthermore, this confirms that the enzyme inhibitor is crossing the bacterial cell membrane and reaching the desired target. The positive antibiotic control ampicillin is shown as well showing its zone of inhibition.

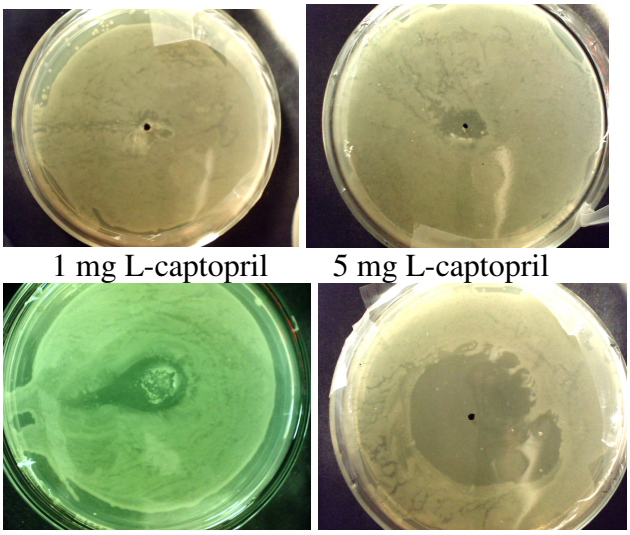


Figure 2. Antibiotic Activity of L-Captopril Against *E. coli.*

In summary, a screen of compounds containing ZBG's against DapE from *Haemophilus influenzae* has delivered a number of micromolar inhibitors including captopril, functioning as a competitive, reversible inhibitor with an IC_{50} of 3.3 μM (K_i = 1.8 μM). Furthermore, antibiotic activity has now been demonstrated for the DapE inhibitor L-captopril. Captopril is an excellent lead for medicinal chemistry optimization in consideration of its low molecular weight (217) and low measured logP of 0.34[18,19] following the rules of Lipinski[20] and containing few rotatable bonds,[21] consistent with its known oral bioavailability.

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