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Diaminopimelic acid (DAP) analogs bearing isoxazoline moiety as selective inhibitors against *meso*-diaminopimelate dehydrogenase (*m*-Ddh) from *Porphyromonas gingivalis*

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Abstract

Two diastereomeric analogues (**1** and **2**) of diaminopimelic acid (DAP) bearing an isoxazoline moiety were synthesized and evaluated for their inhibitory activities against *meso*-diaminopimelate dehydrogenase (*m*-Ddh) from the periodontal pathogen, *Porphyromonas gingivalis*. Compound **2** showed promising inhibitory activity against *m*-Ddh with an IC₅₀ value of 14.9 μ M at pH 7.8. The two compounds were further tested for their antibacterial activities against a panel of periodontal pathogens, and compound **2** was shown to be selectively potent to *P. gingivalis* strains W83 and ATCC 33277 with minimum inhibitory concentration (MIC) values of 773 μ M and 1.875 mM, respectively. Molecular modeling studies revealed that the inversion of chirality at the C-5 position of these compounds was the primary reason for their different biological profiles. Based on these preliminary results, we believe that compound **2** has properties consistent with it being a lead compound for developing novel pathogen selective antibiotics to treat periodontal diseases.

Graphical abstract

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Keywords

diaminopimelic acid; antibacterial activity; *meso*-diaminopimelate dehydrogenase inhibitor; molecular modeling

Bacterial infections remain a major threat to human health with considerable treatment costs and an array of related clinical complications. With the mounting incidence and mortality rates of infectious diseases, the current pace for discovery and development of novel antibiotics is far from satisfactory.¹ A confounding factor is that, because maintenance of the human microbiome is vital for keeping in good physical health,^{2,3} the widespread use of broad-spectrum antibiotics in recent decades has perturbed the ecological balance of this microbiome. These drugs, with their unspecific effects on both pathogenic and non-pathogenic species, cause further unexpected clinical symptoms such as opportunistic infections, bacterial resistance and disorders of intestinal microbiota.^{4,5} Therefore, there is an urgent and continuing need to develop more efficient and less toxic antibiotics that could target a specific range of pathogenic species.

It is known that the diaminopimelic acid (DAP) biosynthesis pathway is the major source of *L*-lysine for most bacteria and some other related species,⁶ but this pathway is not present in mammalian cells. As a biosynthetic precursor in the DAP pathway, *meso*-diaminopimelic acid (*m*-DAP) plays a crucial role in the formation of peptidoglycan cell wall of Gram negative bacteria (Figure 1).⁷ Therefore, inhibitors that selectively suppress enzymes in this pathway may be able to act as novel antibiotics with selective anti-pathogenic action, satisfactory effectiveness and low toxicity. *Meso*-diaminopimelate dehydrogenase (*m*-Ddh), the only dehydrogenase present in the DAP biosynthetic pathway,⁸ acts as a critical enzyme, through which NADPH dependently catalyzes the conversion of *L*-tetrahydrodipicolinate (THDP) to *m*-DAP by utilizing an intermediate imine.^{9,10} One strategy to develop inhibitors against *m*-Ddh is to design and synthesize small molecules that are mimics of *m*-DAP. Consequently, a large number of *m*-DAP analogs have been previously reported;^{9,11,12} however, only a few of them showed notable activities. In particular, compound **1** (Figure 1) that contains an isoxazoline group had almost no activity against *m*-Ddh, while compound **2**, its diastereoisomer, showed pH-dependent inhibition against *m*-Ddh from *Bacillus sphaericus*. Additionally, it also showed moderate antibacterial activity against *B. sphaericus* in an *in vitro* study.¹³ The most highly studied DAP enzymes are derived from *B. sphaericus* and *Corynebacterium glutamicum*,^{14,15} but, because *Porphyromonas gingivalis* is crucial in

the onset and development of periodontal diseases that result from an unbalanced microbiome ecology in oral cavities,^{16,17} we are targeting *meso*-diaminopimelate dehydrogenase from that species in this study. In order to define a lead compound from DAP analogs to target *m*-Ddh, we synthesized compounds **1** and **2** in our laboratories, then evaluated their enzymatic inhibition against *m*-Ddh from *P. gingivalis*. Next, we tested their antibacterial activities against a panel of periodontal pathogens. Furthermore, molecular modeling studies were carried out to help understand interactions between these two compounds and the *m*-Ddh target protein.

The syntheses of target compounds were performed following the reported procedure¹³ with modifications (Scheme 1). Protection of the amino and carboxyl groups of commercially available *L*-allylglycine was carried out via its conversion to the *N*-Cbz methyl ester (**5**). The key step in generating the isoxazoline skeleton was the 1,3-dipolar cycloaddition of ethyl chlorooximidoacetate by treatment of **5** (with protected amino and carboxyl groups) in the presence of a weak base that, *in situ*, generated the expected diastereoisomers **7** and **8**.¹⁸ These two compounds were separated by flash column chromatography using Et₂O/hexane system. Then, compound **7** was subjected to hydrolysis in NaOH aqueous solution to afford the diacid **9**. Subsequent removal of Cbz group was carried out in the presence of TMSCl and NaI at room temperature in acetonitrile to generate compound **1**. Similarly, hydrolysis of compound **8** gave the diacid **10**, which was further deprotected to give compound **2**.

Both compounds were tested for their inhibition against *m*-Ddh according to the reported procedures.¹⁷ Since earlier work by Abbott *et al.* had shown the pH of the buffer solution was a crucial factor for inhibitory activity against *B. sphaericus m*-Ddh,^{9,13} we conducted the assay at pH 7.8 and pH 10.5, respectively (Figure 2). The results showed that compound **1** with a *R* configuration at C-5 position had no activity (up to 1 mM) at physiological condition (pH = 7.8), while compound **2** exhibited significant inhibitory activity against *m*-Ddh with an IC₅₀ value of 14.9 μM. At the more basic condition of pH = 10.5, compound **1** was still not active against *m*-Ddh up to 3 mM while compound **2** had a steep reduction in potency with an IC₅₀ value of 221 μM. These results were in line with previous reports, suggesting that the configuration inversion at the C-5 position in the isoxazoline ring diminished the binding interaction between compound **1** and *m*-Ddh. Additionally, compound **2** could exert its enzymatic inhibition effectively at physiological-like conditions, but was less effective at more basic conditions. A conformational change or ionization of the enzyme at high pH presumably accounts for the reduced enzymatic inhibition of compound **2**.¹³

Compound **2** was further evaluated for its potentially selective whole-cell activity against specific pathogens with respect to a panel of periodontal pathogens. In this assay, four strains were chosen, including *P. gingivalis* strains W83 and ATCC 33277, which possess the target *m*-Ddh, and *Fusobacterium nucleatum* strain ATCC 25586 and *Staphylococcus aureus* strain N315, which do not possess *m*-Ddh. Compound **2** was thus tested by a broth microdilution method.¹⁹ The assay results showed that compound **2** exhibited inhibitory activities against *P. gingivalis* strains W83 and ATCC 33277 with minimum inhibitory concentration (MIC) values of 773 μM and 1.875 mM,

respectively (Table 1). In contrast, there was no visual inhibition of cell growth by the *E. nucleatum* strain ATCC 25586 and the *S. aureus* strain N315 with concentrations of up to 3 mM. These preliminary screens suggest that compound **2** shows selective growth inhibition against *P. gingivalis*. A likely explanation for the significant enzymatic activity and relatively lower cellular activity of compound **2** is that the highly hydrophilic molecule does not easily penetrate through cell membranes. Thus, increasing its hydrophobicity by modifying of the DAP skeleton would appear to be a reasonable strategy for improving cellular activity.

The different biological assay results of these two enantiomers in their enzymatic inhibitions and antibacterial activities prompted us to look for the binding interactions with *m*-Ddh. *m*-DAP, compounds **1** and **2** were first sketched in Sybyl-X 2.0 and assigned Gasteiger-Hückel charges. Then, each was immersed in a square box of water molecules and these solvated systems were energy minimized (20,000 iterations) with the Tripos forcefield. The resulting 3D structures of *m*-DAP, compounds **1** and **2** are displayed in Figure 3. Overlaying these molecules showed that the conformations of portion A in compounds **1** and **2** were similar, while the conformations of portion B were different due to the effects of stereochemistry.

We are not aware of any report on the crystal structure of *m*-Ddh (oxidoreductase, Gfo/Idh/MocA family member) from *P. gingivalis* in complex with compounds **1** or **2**. However, the crystal structures of *m*-Ddh from *P. gingivalis* in complex with another compound, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (PDB ID: 3BIO) and compound **2** in complex with *C. glutamicum* diaminopimelate dehydrogenase have been reported (PDB ID: 3DAP).²⁰ Comparing these two crystal structures, we found that the binding site of compound **2** in *C. glutamicum* diaminopimelate dehydrogenase was very similar to that of 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid in *m*-Ddh from *P. gingivalis* (Figure 4). Thus, we adopted 3BIO as our template protein and the binding orientation of compound **2** in 3DAP for the target compounds in *m*-Ddh. The protein was built by adding hydrogen atoms, assigning Gasteiger-Hückel charges, and optimizing hydrogen coordinates by a 10,000 iteration minimization while holding all heavy atoms as fixed (an aggregate) under the Tripos forcefield (TAFF) in Sybyl-X 2.0.²¹

Compounds **1** and **2** were next docked into the *m*-Ddh binding site with GOLD 5.4.²² The docking models were analyzed based on two criteria: 1) the binding orientation of compounds **1** and **2** in *m*-Ddh being similar to that of compound **2** in the *C. glutamicum* protein and 2) high CHEM-PLP scores. We then ranked these docking models using the HINT (Hydrophobic INTERactions) free energy scoring tool.²³ The docking models with the highest HINT score of compounds **1** and **2** were chosen as the optimal docking models, respectively (Table 2 and Figure 5). Figure 5 shows that the hydrophobic interactions with Trp119, Phe144 and the electrostatic interactions with the side chains of Ser149 and Thr169 in the *m*-Ddh/compound **1** and *m*-Ddh/compound **2** complexes were almost the same, i.e., mainly with portion A of **1** and **2**. However, for portion B of compounds **1** and **2**, the interactions are very different: in the *m*-Ddh/compound **2** complex (Figure 5b), there is a hydrogen bond to Asp120, but in the *m*-Ddh/compound **1** complex (Figure 5a), the inversion of chirality leads to two consequences, a) that hydrogen bond to Asp120 is lost and 2) the carboxyl group of **1** moved closer to the side chain of Asp90. This produces an unfavorable

interaction between these two negative centers, which is detrimental to the binding of compound **1**. The two different configurations of **1** and **2**, particularly in portion B, produces variant binding affinities and ultimately, different enzymatic activities of the two compounds.

In conclusion, two diastereomers of DAP analogs bearing an isoxazoline moiety were synthesized and evaluated for their inhibition against *m*-Ddh from the periodontal pathogen *P. gingivalis*, and for their antibacterial activities. The results showed that only compound **2**, which has an *S* stereocenter at the C-5 position of the isoxazoline ring, displays significant inhibitory activity against *m*-Ddh under physiological conditions. Additionally, in our limited survey, compound **2** also shows selective inhibition against *P. gingivalis*. Molecular modeling studies suggested that the inversion of chirality at the C-5 position of isoxazoline ring between compounds **1** and **2** leads to their different binding affinities and that the hydrogen bond between compound **2** and *m*-Ddh plays a crucial role in its enzymatic inhibition. These preliminary results suggest that compound **2** may serve as a promising lead compound for the discovery of novel antibiotics to treat periodontal diseases with selective effectiveness and reduced toxicity. Further studies on rational design and synthesis of *m*-DAP analogs are underway.

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Figure 1.
The structures of *m*-DAP and its analogs.

(a) **(b)**

(c)

(d)

Figure 2.

Dose-dependent studies of compound 1 and 2 against *P. gingivalis m*-Ddh at different pH values. (a) Compound 1 and (b) Compound 2 inhibition against *P. gingivalis m*-Ddh at pH 7.8. (c) Compound 1 and (d) Compound 2 inhibition against *P. gingivalis m*-Ddh at pH 10.5.

Figure 3.

The 3D structures of *m*-DAP, compounds **1** and **2** after energy minimization. The parts in compounds **1** and **2** share similar conformation was defined as portion A and the rest as portion B. All oxygen atoms are in red and nitrogen atoms in blue; For carbon atoms, *m*-DAP is in yellow, compounds **1** in orange, compound **2** in green.

Figure 4.

The alignment of backbone atoms of proteins 3BIO (blue) and 3DAP (orange). The ligands in 3BIO and 3DAP are shown as sticks and colored in magentas and green, respectively.

Figure 5.

The binding modes of (a) compound **1** and (b) compound **2** in the binding pocket of *m*-Ddh from docking studies. Compounds **1** atoms: carbon (orange); compounds **2** atoms: carbon (green); amino acid residue atoms: carbon (yellow); oxygen (red); nitrogen (blue). The magenta dashed lines represent the hydrogen bond.

Scheme 1.

Synthesis route of *m*-DAP analogs **1** and **2** containing an isoxazoline ring system. Reagents and conditions: (a) TMSCl, MeOH, 97%; (b) CbzCl, Et₃N, DMAP, CH₂Cl₂, 49%; (c) NaHCO₃, AcOEt, 37% for **7** and 42% for **8**; (d) MeOH, 1 *N*NaOH, 90% for **9** and 95% for **10**; (e) TMSCl, NaI, MeCN, 35% for **1** and 43% for **2**.

Table 1

The minimum inhibitory concentrations (MIC) of compound **2** against periodontal pathogens.

Periodontal pathogens	MIC	<i>m</i> -Ddh homologs
<i>P. gingivaiis</i> strain W83	773 μ M	Yes
<i>P. gingivaiis</i> strain ATCC 33277	1.875 mM	Yes
<i>Fusobacterium nucieatum</i> strain ATCC 25586	No growth inhibition up to 3 mM	No
<i>Staphylococcus aureus</i> strain N315	No growth inhibition up to 3 mM	No

Table 2

The GOLD (CHEM-PLP) and HINT scores for the optimal binding poses of compounds **1** and **2**.

	Compound 1	Compound 2
CHEM-PLP	41	51
HINT	245	477