

Synthesis and Characterization of Potential Inhibitors of dapE and argE Enzymes as New Antimicrobial Agents

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Introduction

Microbial enzymes, especially those catalyzing metabolic processes exclusive and essential for bacteria and not for humans, might be very promising targets for potent and selective enzyme inhibitors, potential antimicrobial drugs

We paid our attention to two enzymatic systems: The first system involves a bacterial enzyme N^α-acetyl-L-ornithine deacetylase (argE [1,2]), which catalyzes conversion of N^α-acetyl- ornithine to ornithine in the fifth step of the biosynthetic pathway for arginine that serves as a source of both carbon and nitrogen in microorganisms (Fig. 1). The second system includes N-succinyl-L-diaminopimelic acid desuccinylase (dapE [3,4]), a member of *meso*-diaminopimelate (mDAP)/lysine biosynthetic pathway catalyzing the hydrolysis of N-succinyl-L,L-diaminopimelic acid to succinate and L,L-diaminopimelic acid that is turned to corresponding *meso* form – essential component of peptidoglycan based bacterial cell walls providing a link between polysaccharide strands (Fig. 2).

In our study, we firstly focused on the synthesis and characterization of compounds **1a-1k** and **2a-2d**, designed to be N^α-protected derivatives of ornithine and diaminopimelic acid. These compounds might exert a function of potential inhibitors of both the enzymes argE and dapE with the respect to stability of corresponding amide bond against degradation. The elimination of such degradation could possibly interrupt the pathways leading to development of bacteria. In our preliminary assay, the compounds prepared were incubated with bacterial stems *Escherichia coli* and *Bacillus subtilis*.

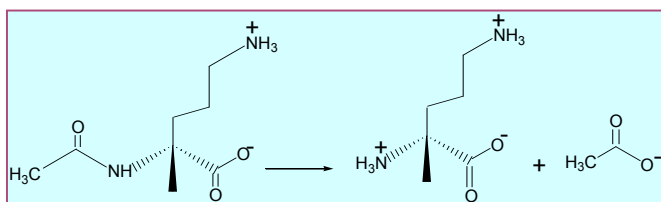


Fig. 1. Conversion of N^α-acetyl- ornithine to ornithine

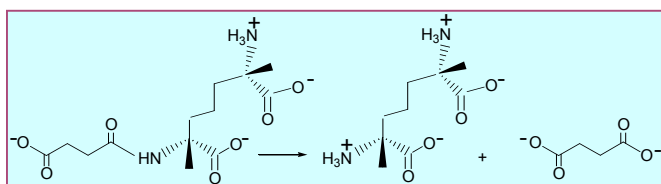


Fig. 2. Hydrolysis of N-succinyl-L,L- diaminopimelic acid to succinate and L,L-diaminopimelic acid

Results and Discussion

Syntheses of ornithine R-CO-Orn(H)-OH (**1a-1k**) and diaminopimelic acid HO₂C-CH(NH-R)-(CH₂)₃-CH(NH₂)-CO₂H (**2a-2d**) derivatives are described in Figures 3-5. Analytical data are in Table 1.

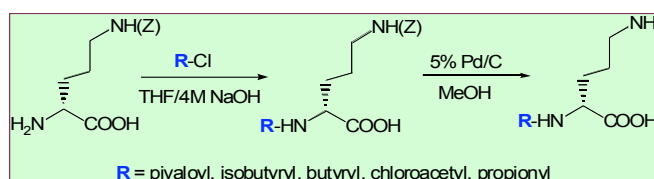


Fig. 3. Synthesis of Orn derivatives **1a-1e**

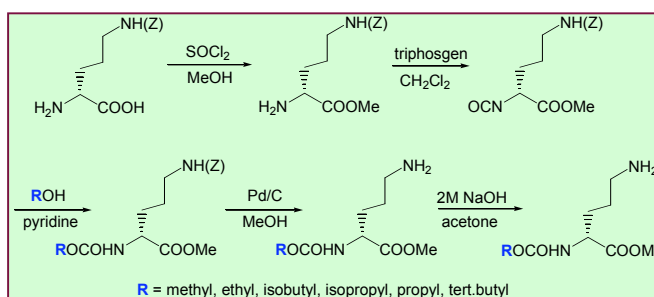


Fig. 4. Synthesis of Orn derivatives **1f-1k**

Antimicrobial test

Antimicrobial activity was tested quantitatively by the drop diffusion method carried on LB agar (SIGMA). Minimal inhibitory concentration (MIC) was established by growth in multiwell plates in LB broth. As the test organisms we used *Bacillus subtilis*168 and *Escherichia coli*. Bacteria were grown first in 5 ml of LB broth and in mid exponential phase added to a solution of compound tested and incubated at 37°C in final volume of 0.2 ml for 20h. The absorbance was measured at 540 nm till 20h.

In our preliminary study, the compounds prepared were assayed against bacterial stems *Escherichia coli* and *Bacillus subtilis*. A weak inhibitory activity measured as the minimal inhibitory concentration (MIC) was found in ornithine derivatives with regard to structure of N^α-substituent. The largest inhibitory potency exhibited N^α-butyryl-ornithine with MIC₅₀ 10 μM on *Bacillus subtilis*.

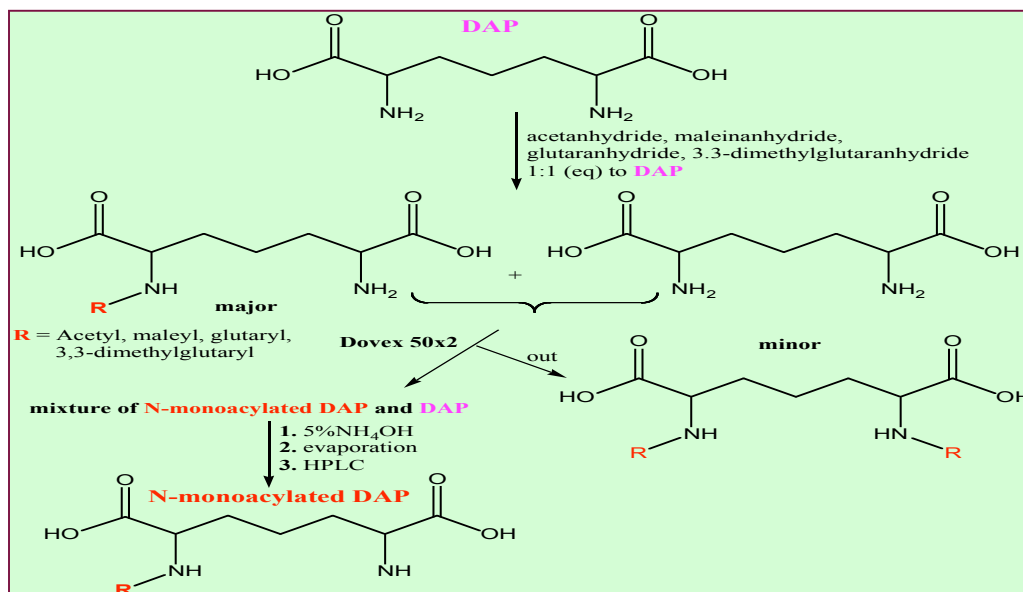


Table 1. Analytical data of Orn and DAP derivatives

Compound	Formula ^a Calc./found(m/z)	HPLC elution time (min) ^b
1a, R = (CH ₃) ₃ CO	C ₁₀ H ₂₀ N ₂ O ₃ 216.28/203.2	11.02
1b, R = (CH ₃) ₂ CHCO	C ₉ H ₁₈ N ₂ O ₃ 202.26/203.2	11.22
1c, R = CH ₃ (CH ₂) ₂ CO	C ₉ H ₁₈ N ₂ O ₃ 202.26/203.2	11.12
1d, R = ClCH ₂ CO	C ₇ H ₁₃ N ₂ O ₃ Cl 208.65/209.5	7.32
1e, R = CH ₃ CH ₂ CO	C ₈ H ₁₆ N ₂ O ₃ 188.23/189.1	7.05
1f, R = CH ₃	C ₇ H ₁₄ N ₂ O ₄ 190.10/191.0	4.12
1g, R = C ₂ H ₅	C ₈ H ₁₆ N ₂ O ₄ 204.23/205.1	5.54
1h, R = (CH ₃) ₂ CHCH ₂	C ₁₀ H ₂₀ N ₂ O ₄ 232.28/233.3	9.51
1i, R = (CH ₃) ₂ CH	C ₉ H ₁₈ N ₂ O ₄ 218.25/219.2	7.56
1j, R = CH ₃ (CH ₂) ₂	C ₉ H ₁₈ N ₂ O ₄ 218.25/218.2	7.58
1k, R = (CH ₃) ₃ C	C ₁₀ H ₂₀ N ₂ O ₄ 232.28/233.3	9.17
2a, R = CH ₃ CO	C ₉ H ₁₆ N ₂ O ₅ 232.32/233.3	2.70
2b, R = HO ₂ CCH=CHCO	C ₁₁ H ₁₆ N ₂ O ₆ 272.25/273.2	9.38
2c, R = HO ₂ C(CH ₂) ₃ CO	C ₁₂ H ₂₀ N ₂ O ₆ 288.28/229.2	3.77
2d, R = HO ₂ CCH ₂ C(CH ₃) ₂ CH ₂ CO	C ₁₄ H ₂₄ N ₂ O ₆ 316.37/317.3	4.02

^aDetermined with ESI MS technique, ^bFor HPLC a TSP instrument with an SP 8800 pump, an SP 4290 integrator, TSP Spectra 100 UV detector and 5µm Supelco 15x0.4 cm column were used. For compounds 1a-1k a gradient 0-50% ACN in 0.05% TFA, 20 min and for compounds 2a-2d an isocratic analysis with 0.05% TFA. were used.

Acknowledgments

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References

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Fig. 5. Synthesis of DAP acid derivatives 2a-2d