Distributed Drug Discovery (D3) Synthesis and Testing of Multiple Unnatural Dipeptides

Identifies a Subset with Potent Antimicrobial Activity against Pseudomonas aeruginosa (Pa)


Indiana University Purdue University Indianapolis (IUPUI), Colorado College, Santa Clara University; Universidad Nacional Autónoma de México, University of Puerto Rico, Innovenxty, Goshen College, University of Indianapolis

Abstract

Through Distributed Drug Discovery (D3) the unnatural amino acid (S)-4-fluorophenylalanine (Fmoc-4-FPhe) was rediscovered as a potent inhibitor of Pseudomonas aeruginosa (Pa) growth. Three close analogs (Fmoc-4-FPhe, Fmoc-3-FPhe, 2-Fmoc-4-FPhe) were also made and found to be active. Subsequently it was shown that unnatural dipeptides of generic structure 2 and 3 were active, presumably as produgs of 4. The D3 program, which teaches solid-phase organic and peptide synthesis to students at global schools, targeted an 80 compound subset of 2 for distributed synthesis. In this subset, the C-terminal residue is one of 20 natural amino acids and the N-terminal residue is either (S)-4F, 3F, 2F, or 3,4-difluorophenylalanine. A complementary biology lab was developed at IUPUI to explore the activity of these compounds against Pa. We report the multi-school distributed synthesis and testing of members of this targeted 80 compound subset and the resulting discovery of many unnatural dipeptides active against Pa.

Identification of FPhe Pa Inhibitors

Possible drug relevant: D-peptide synthase 5 modulate immune function in Pseudomonas aeruginosa

D-Tyrosine analogs: Candidates for D-Pseudomonas aeruginosa (Pa) antibiotic research?

Target: Analogs of Unnatural enantiomer D-Tyrosine

D-Amino Acids Target Biofilm Assembly/Maturation - D-Tyrosine: D-Peptide synthetase 5 modulate immune function in Pseudomonas aeruginosa

Possible drug relevant: D-peptide synthase 5 modulate immune function in Pseudomonas aeruginosa

D-Tyrosine

Possible drug relevant: D-peptide synthase 5 modulate immune function in Pseudomonas aeruginosa

Synthesis/Test Results

At 10 µg/mL, % biofilm formation relative to bacteria control: Active = <10%.

Other literature noted mammalian toxicity: How to minimize – Prodrugs?

D3 Prodrugs of FPhe’s

Unnatural Dipeptide Prodrugs 2 or 3: Possible Points of Selective Toxicity for Microbes versus Mammalian Cells

4-FPhe Hit Follow Up

Head-to-Head Comparison of 4-Fluorophenylalanine with Tobramycin

D3 Unnatural Dipeptide Lab 2

80 Compound 2a Target Quadrants with Test Results

Summary and Conclusions

- D3 Synthesis and testing of 80 target prodrugs identifies many potent Pa inhibitors.
- Activity likely a function of documented transport of dipeptides into Pa with subsequent enzymatic cleavage.
- Most active quadrant has N-terminal 4-FPhe, consistent with activity of 4-FPhe itself.

Important Qualifications:

- Only 11-30% active.
- Most active quadrant has N-terminal 4-FPhe, consistent with activity of 4-FPhe itself.
- Early testing at 10 µg/mL, later down to 0.5 µg/mL.
- Made but not yet tested.

At each stage of the discovery process D3 breaks large challenges into smaller pieces and distributes them globally for both education and problem solving.

Equipment Used: Simple Solid-Phase Synthesis Apparatus

Simple, inexpensive solid-phase synthesis Bill-Good equipment

Best So Far

- Emphasize Replication
- Teach Biochemistry
- Teach Drug Discovery

Other D3 Goals

- Small organic libraries
- Innovative, under-researched diseases
- Biopolitical activity

References and Acknowledgements

Funding

Contact email: wscott@iupui.edu

Student Researchers: Multiple chemistry and biology students too numerous to (2020) to list individually

Other D3 Goals

- Emphasize Replication
- Teach Biochemistry
- Teach Drug Discovery

Other D3 Goals

- Small organic libraries
- Innovative, under-researched diseases
- Biopolitical activity

References and Acknowledgements

Funding

Contact email: wscott@iupui.edu

Student Researchers: Multiple chemistry and biology students too numerous to (2020) to list individually