Synthesis and testing of Cu(II)-binding peptoid monomers for use in design of amphipathic antimicrobial drugs

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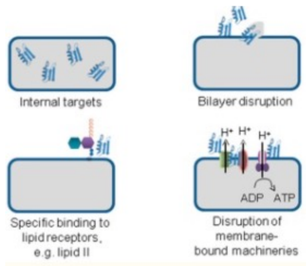
Antimicrobial Peptoids: Promising Drug Targets

Abstract

One solution to the problem of antibiotic resistance is to utilize peptoids as targets in drug development. These are a family of foldamers structurally related to peptides whose unique structure offers superior synthetic and clinical potential.

This research project investigated potential synthesis pathways for selected peptoid monomers using previously described methods. Novel substrates for synthesis were chosen to give the final structure the ability to form a strong multidentate coordinate bond with Cu(II) ions. This has been shown to enhance potency of peptoid drugs via selective oxidation of bacterial membrane lipids.

The success of the procedure was assayed at each step using a combination of TLC, GC-MS, IR, and HNMR analysis. Each monomer was also qualitatively assayed using UV-Vis Spectrophotometry, where increased absorption at 380 nm was correlated to enhanced Cu(II)-binding.



Research Focus: Mechanisms of action for noval antimicrobial peptoid (AMP) drugs. Focus on membrane disruption strategy.

- AMP's play in important role in the innate immune system of humans and other vertebrates.
- Advantages of Peptide Drugs: Difficult for bacteria to develop resistance to cytotoxic mechanisms.
- Disadvantages: In vivo toxicity, limited bioavailability, susceptibility to protease degradation, production costs
- Disadvantages overcome by substituting peptoids for peptides in conventional membrane disrupting amphipathic AMPs

Antimicrobial peptoids are promising target for antibiotic therapy, specifically for overcoming the limitations of antimicrobial peptides and avoiding development of resistant bacteria

AMP advantages and disadvantages: *J"Membrane-Active Small Molecules: Designs Inspired by Antimicrobial Peptides." Chem Med Chem. 2015, 10, 1606 – 1624.*

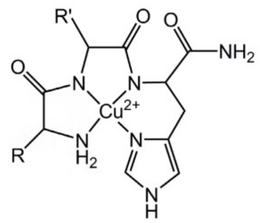
AMP mechanism of action figure: *Antimicrobial Peptides: Diversity, Mechanism of Action and Strategies to Improve the Activity and Biocompatibility In Vivo. Biomolecules. 2018;8(1):4*

Functionality of Cu(II) Binding Motif to Enhance Antimicrobial Properties

Points of consideration guiding synthesis targets:

- Peptoid analogs of conventional peptide antibiotics are clinically more effective but lack significant research
- Previous AMP drugs have been synthetically enhanced to improve cytotoxicity
- Importance of the ATCUN motif in previous clinical antibiotic drug targets: enhances membrane lysis via phospholipid oxidation by utilizing a stable, coordinately bonded Cu(II) ion

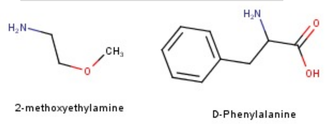
The ATCUN peptide oligomer motif



A generic ATCUN motif, showing coordinate binding to a Cu(II) ion. In this case, R is the side chain of the residue in position 1 of the motif. R and R' can be any amino acid (canonical or modified), giving immense synthetic flexibility in designing novel ATCUN-enhanced AMPs. The final nitrogen is provided by the imidazole ring of the C-terminal His residue. The C-terminal carboxyl group would usually be linked to the end of an AMP to form the peptide-motif complex.

Monomeric peptoid analog

Identified easily obtainable amines containing electronegative functional groups



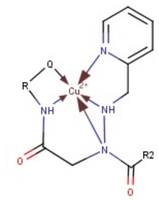
These two substrates were chosen to synthesize a peptoid monomer to serve a similar Cu(II) binding function as the ATCUN motif using the procedure previously described by Crapster, et al.

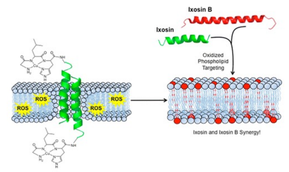
Previously Published Studies/Data on the ATCUN motif and Peptoid synthesis

- Amino Terminal Cu(II)- and Ni(II)-Binding (ATCUN) Motif of Proteins and Peptides: Metal Binding, DNA Cleavage, and Other Properties.1997. Acc. Chem. Res. 30, 123 – 130
- Antimicrobial and Antibiofilm Activities of Helical Antimicrobial Peptide Sequences Incorporating Metal-Binding Motifs. Journal of Biochemistry 2019, 58, 36, 3802-3812
- Design and conformational analysis of peptoids containing N-hydroxy amides reveals a unique sheet-like secondary structure. Biopolymers vol. 96,5 (2011): 604-16. doi:10.1002/bip.21599.

Proposed Structure/Function for Cu(II)-binding Peptoid monomers

- The methods described by Crapster, et al, were used with novel substrates to synthesize two versions of the generic peptoid monomer shown here. Q represents an electronegative heteroatom (usually N or O) which was variable between the various iterations tested. R was also variable based on the starting material used in the synthesis. R2 is the link to an amphipathic antimicrobial helical peptoid oligomer. This research simply used CH3 for ease of synthesis and simplicity of Cu(II) binding assays
- This research builds on the studies by Libardo, et al, in the antimicrobial properties of ixosin B antimicrobial peptides. We propose that similar cytotoxic functionality could be obtained from a synthetic helical peptoid molecule covalently bonded to the monomer shown here

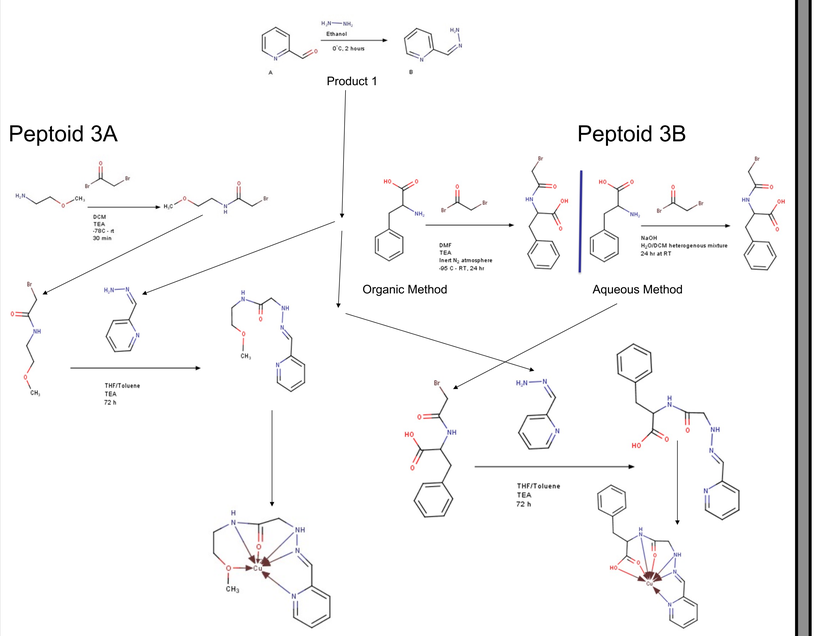




With proposed Cu(II) binding monomers attached to a helical amphipathic peptoid monomer with membrane-disrupting properties, similar antibiotic properties cold be observed as those studied by Libardo, et al, using the cytotoxic mechanism shown above.

Previous research in antimicrobial properties of Cu(II)-binding motifs:. Central Role of the Copper-Binding Motif in the Complex Mechanism of Action of Ixosin: Enhancing Oxidative Damage and Promoting Synergy with Ixosin B. 2016, ACS Infect. Dis. 2, 71−81..

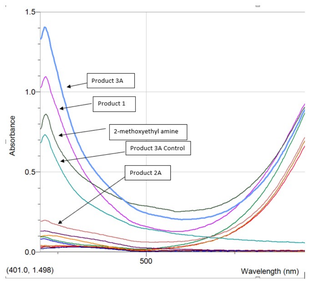
Synthesis of Peptoid Monomers using Selected Substrates



Cu(II)-Binding Data

- Assays
  - Control 1: Abs of Cu(II)Cl at 380 – 390 nm
  - Control 2: Abs of each ligand solution with no Cu(II)
  - Test: Compare abs of each ligand in Cu(II) solution to the controls
- Conclusions
  - Increased absorbance at 380-390 nm correlates to increased Cu(II) chelation
  - Peptoid 3A could successfully chelate Cu(II) better than the control compounds
  - Peptoid 3B was not successful in this regard
  - The reaction intermediates for Peptoid 3B were inefficient Cu(II) chelators in aqueous solution due to their poor solubility

Compound	Absorbance between 380-390 nm	actual location of peak (nm)
Cu Stock Control	0.034	385
2-Pyridinecarboxaldehyde control	0.083	385
2-Pyridinecarboxaldehyde test	0.095	384.1
Product 1 control	0.104	389.2
Product 1 test	1.095	385.8
2-methoxyethylamine control	0.034	385
2-methoxyethylamine test	0.862	386.7
bromoacetylbromide control	0.025	389.2
bromoacetylbromide test	0.04	385
Product 2a control	0.132	385
Product 2a test	0.198	385
Product 3a control	0.732	385.8
Product 3a Test	1.406	385



Compound	Absorbance between 380-390 nm	actual location of peak (nm)
Cu Stock Control	0.012	380.7
2-Pyridinecarboxaldehyde control	0.083	385
2-Pyridinecarboxaldehyde test	0.095	384.1
Product 1 control	0.104	389.2
Product 1 test	1.095	385.8
Phenylalanine control	0	n/a
Phenylalanine test	2.004	388.4
Diluted 2x	2.145	389.2
New phe stock soln	0.009	381.5
New Phe stock soln concentrated 2x	0.01	381.5
bromoacetylbromide control	0.025	389.2
bromoacetylbromide test	0.04	385
Product 2b (aqueous) control	0.002	383.2
Product 2b (aqueous) test	0.014	381.5
Product 2b (aqueous) new stock	0.034	385
Product 2b (aqueous) new stock concentrated	0.04	395.8
Product 2b (organic) control	0.703	385
Product 2b (organic) test 2	0.688	385
Product 2b (organic) test 1	0.505	385
Product 3B control	0.395	382.4
Product 3B test	1.836	388.4
Product 3B test diluted to 7.5 mL test	0.437	385
Product 3B test diluted to 7.5 mL control	0.194	382.4

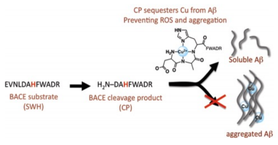
Table 1. Cu(II) binding data from the reaction intermediates from peptoids 3A (left table) and 3B (right table). Absorbance spectrum for Peptoid 3A and its synthetic intermediates is included but since the data was inconclusive for Peptoid 3B, only the data table is included

Future Directions

1) Identification of promising peptoid monomer candidates for Cu(II) chelation.
2) Perfection of synthesis methods.
3) Exploring possible linkages to existing peptide / peptoid drugs as an ATCUN motif analog
4) Ultimately, we hope these compounds will show antibiotic drug efficacy in animal models and clinical trials

Clinical Applications:

- Antibiotic Drug Targets
- Possible Alzheimer's Disease treatments
- Anticancer Applications



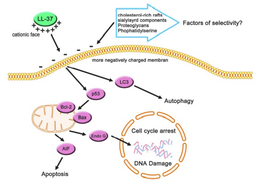


Image credits: Left: AMP disruption of amyloid beta plaque formation via ROS scavaging AMPs. Franz, K, et al. *A Prochelator Activated by Beta-Secretase Inhibits A-Beta Aggregation and Suppresses Copper-Induced Reactive Oxygen Species Formation.* J. AM. CHEM. SOC. 2010, 132, 4994–4995

Right: Potential for AMPs as anticancer agents. Isogi, E., et al. *The human cathelicidin antimicrobial peptide LL-37 and mimics are potential anticancer drugs. Front. Oncol., 30 June 2015 | https://doi.org/10.3389/fonc.2015.00144*

Acknowledgements

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