The On-resin Synthesis and Pharmacokinetic Study of Wewakazole B: An Anticancer Peptide

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Introduction

Natural products contain biologically active functional groups, allowing them to be important lead compounds in drug design. Peptides show significant potential as therapeutics due to their selectivity, specificity, and pharmacological properties, but face challenges in bioavailability, permeability, and stability. Common ways to improve these limitations include incorporation of of unnatural amino acids and N-methylation. More modern ways include incorporation of heterocycles. Heterocycles, such as imidazoles, thiazoles and oxazoles, are found in many naturally occurring peptides. Notably, oxazoles display a wide range of biological activities: antiviral, anti-inflammatory, antibacterial and anticancer. Methods for synthesizing oxazoles include the traditional Robison-Gabriel synthesis and Fischer synthesis and more recently oxazole synthesis from β-hydroxy amide cyclization. These methods prove efficient, producing moderate yields, but are not compatible with peptide starting materials.

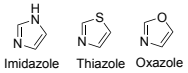

Figure 1. Common heterocycles found in natural occurring peptides.

Cyanobactins, a class of small cyclic peptides, are well known for containing heterocycles. Wewakazole B (Wewa B), isolated from cyanobacterium in the Red Sea in 2016 by the Okino group, contains 3 oxazole rings and is cytotoxic against MCF7 breast cancer and H460 lung cancer cell lines. Compared to Wewakazole A (Wewa A), isolated in 2003, Wewa B displays more potent activity against H460 lung cancer cells.

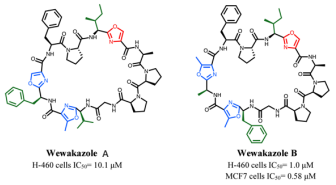

Figure 2. Wewakazole A and Wewakazole B.

Common ways to synthesize peptides include classical solution phase peptide synthesis (CSPS) and solid phase peptide synthesis (SPPS). Previously established CSPS of Wewa B requires tedious and time-consuming reaction and purification steps resulting in low yields and therefore improving methods for the synthesis of Wewa B is beneficial as its mode of action is still unknown. Hence, exploration of the synthesis of Wewa B using SPPS has sparked our interest with an aim to improve yield for thorough pharmacokinetic evaluation.

Objective

- This work aims to develop a novel approach for Wewakazole B synthesis using oxazole-containing amino acids and SPPS and investigate the pharmacokinetic properties of Wewakazole B and its analogues.
- We focus on developing methods for synthesizing oxazole-containing peptides and conducting a structure activity relationship (SAR) study of first-generation analogues to improve cytotoxicity of peptide structure against drug-resistant cancer cell lines.

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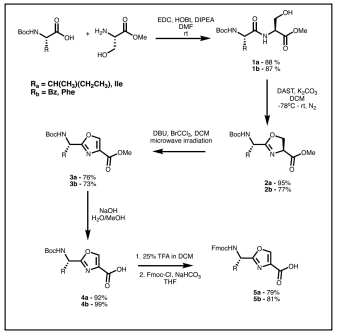
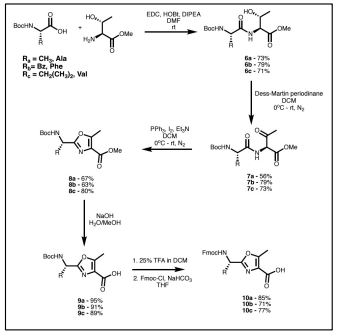
Methods & Results

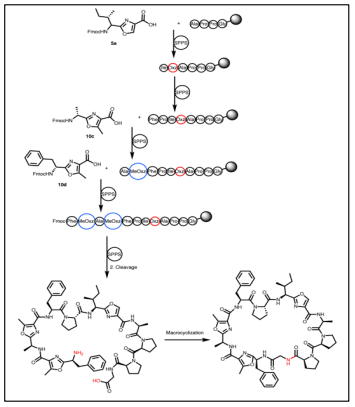
- The solution phase synthesis of Fmoc-oxazole and Fmoc-methyloxazole building blocks produced moderate to good yields for each synthetic step. Scheme 1 was adapted from the β-hydroxy amide cyclization method and Scheme 2 was adapted from the Robison-Gabriel Method



Scheme 1. Synthesis of Fmoc-oxazole building blocks.



Scheme 2. Synthesis of Fmoc-methyloxazole building blocks.



Scheme 3. SPPS of Wewakazole B using Fmoc-amino acids, Fmoc-oxazole, and Fmoc-methyloxazole building blocks.

- Wewa B and analogues were synthesized via SPPS strategy in Scheme 3 and the number of oxazoles present were varied to determine which were important in terms of cytotoxicity. This would determine if any could be left out of the mainstream synthesis of Wewa B.
- Synthesized Wewa A, Wewa B and analogues were analyzed for metabolic activity of MDA-MB-231 using an MTT Assay. MDA-MB-231 cells were seeded at 5,000 cells/well density in 96-well plates and incubated overnight in a 5% CO_2 incubator at 37 ℃ to support cell adhesion. Then the cells were treated with varying concentrations of Wewa A, Wewa B and Wewa B analogues for 72 hours.

Compound	Analogue	Peptide Sequence
Wewa B	---	c [Phe-MeOxz-Ala-MeOxz-Phe-Pro-Ile-Oxz-Ala-Pro-Pro-Gly]
CCL-0002	1	c [Phe-Thr-Ala-Thr-Phe-Pro-Ile-Ser-Ala-Pro-Pro-Gly]
CCL-0003	2	c [Phe-MeOxz-Ala-Thr-Phe-Pro-Ile-Ser-Ala-Pro-Pro-Gly]
CCL-0004	3	c [Phe-Thr-Ala-MeOxz-Phe-Pro-Ile-Ser-Ala-Pro-Pro-Gly]
CCL-0005	4	c [Phe-MeOxz-Ala-MeOxz-Phe-Pro-Ile-Ser-Ala-Pro-Pro-Gly]
CCL-0006	5	c [Phe-Thr-Ala-Thr-Phe-Pro-Ile-Oxz-Ala-Pro-Pro-Gly]
Wewa	---	c [Val-MeOxz-Phe-Oxz-Phe-Pro-Ile-Oxz-Ala-Pro-Pro-Gly]

Table 1. Peptide Sequences of synthesized Wewa B analogues used in oxazole focused SAR study

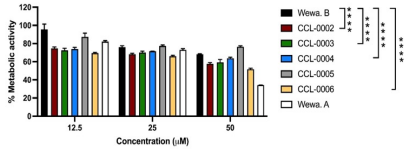


Figure 3. Oxazole focused SAR study displaying metabolic activity of MDA-MB-231 compared across varying concentrations of Wewa A, Wewa B and Wewa B analogues. The results are shown as mean ±SEM of three replicates. **** *p<0.0001.*

- Wewa B analogues significantly inhibit the metabolic activity of triple-negative breast cancer cells.

Discussion & Conclusion

This work developed a novel approach for the synthesis of Wewakazole B and its analogues using SPPS. Further work will be conducted to develop more accessible methodology but until then, the use of SPPS in combination with oxazole incorporation offers an effective approach for synthesizing Wewakazole B, with improved yields compared to classical solution-phase methods. The results from our oxazole focused structure activity relationship study demonstrated that our analogues exhibit promising cytoxicity against drug resistant cancer cell lines like MDA-MB-231. The significant inhibition of cancer cell growth highlights the therapeutic potential of these peptides, particularly in targeting drug-resistant cancers. Further SAR studies, including an alanine scan and D-amino acid scan, will optimize the potency and pharmacokinetic profiles of these compounds and pave the way for developing more potential compounds for viable therapies against therapy-resistant cancers.

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