Synthesis and Biological Evaluation of New Ceramide Analogs

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Abstract

Breast cancer is the most diagnosed form of cancer in women in the United States. It is estimated that on average, every two minutes a woman is diagnosed with breast cancer; and one woman will die of the disease every thirteen minutes. Many anti-cancer drugs used to clinically treat breast cancer mediate tumor cell death through the initiation of apoptosis. Multidrug-resistance is a major cause of cancer chemotherapy failure in clinical treatment. As a result, molecular pathways involved in tumor cell proliferation, including the ceramide signaling pathway, have become potential targets for pharmacological intervention.

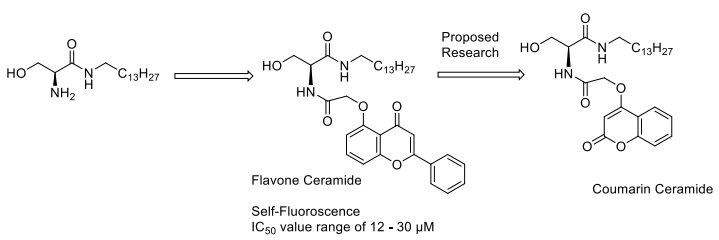
Ceramides have been shown to potentiate signaling events that drive apoptosis, autophagic responses, and cell cycle arrest. Ceramide analogs can be designed to inhibit ceramide-metabolizing enzymes in order to increase intra-cellular ceramide levels in cancer cells, leading to increased cell death. Our approach is to design and synthesize such ceramide analogs. Some of our synthesized analogs have been shown to have greater efficacy and specificity than endogenous ceramides. Evidence shows that multidrug-resistant cancer cells are as sensitive as corresponding regular cancer cells under the exposure to some of our anti-cancer ceramide analogs.

Previously, a number of ceramide analogs with a flavone moiety on the backbone were synthesized. Initial docking studies showed that flavone moieties are too big for the binding pocket of human ceramidase. Coumarin-containing ceramide analogs, however, are smaller in size, and are expected to have increased efficiency along with self-fluorescence. For this project, new ceramide analogs containing a coumarin moiety on the sidechain were synthesized in order to study their biological activities.

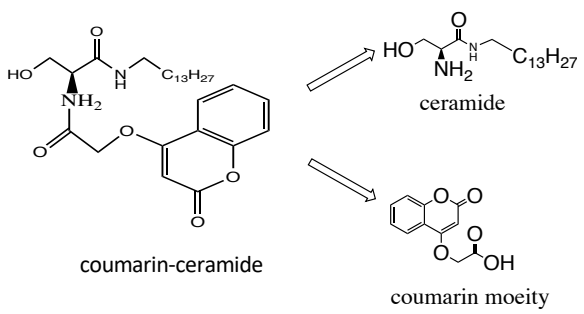
Introduction

- Each year approximately 252,710 women and 2,470 men in the United States alone are diagnosed with breast cancer, and 40,500 women and 460 men are estimated to die from the disease.
- Even though Caucasian women have higher diagnosis rates, African Americans and other minorities have higher mortality rates.
- Current treatments for breast cancer include mastectomy, radiation therapy, chemotherapy, and hormonal therapy. However; there is a great need for more effective and less toxic treatments.
- Previously, ceramide analogs with flavone on the N-sidechain were synthesized.
- New ceramide analogs containing a coumarin moiety on the N-sidechain were synthesized for this project.

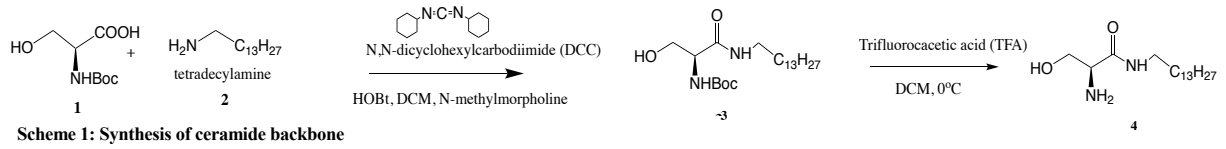
Rational Drug Design



Synthetic Strategy



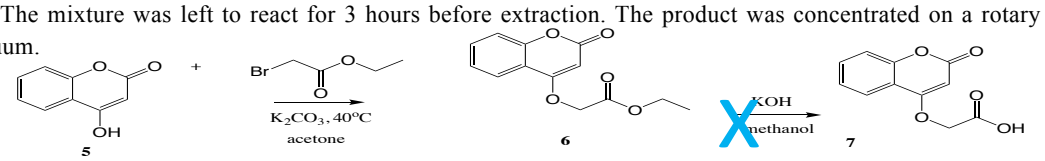
Synthetic Scheme



Scheme 1: Synthesis of ceramide backbone

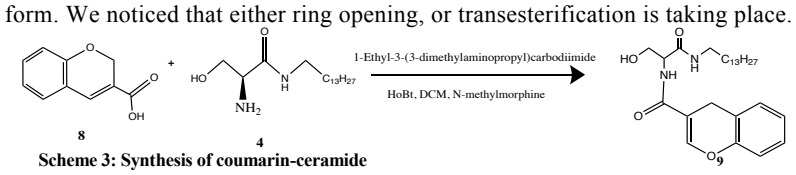
The amide bond formation reaction was performed in a 100 mL round bottom flask under nitrogen atmosphere. L-Boc-serine **1** (0.500 g, 2.436 mmol) was dissolved in 30 mL of dichloromethane. *N*-Methylmorpholine (0.8035 mL, 7.308 mmol, 3.0 eq.), the base, was then added using a syringe. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.5138 g, 2.680 mmol, 1.1 eq.) and hydroxybenzotriazole (0.3621 g, 2.680 mmol, 1.1 eq.) were then added as coupling reagents. Corresponding tetradecyl amine (0.6358 g, 2.680 mmol, 1.1 eq.) was then added to allow the product to form. The reaction was left overnight before concentration followed by purification.

Boc-deprotection of the intermediate was then performed in a round bottom flask under nitrogen atmosphere. Boc-ceramide (2.0 g) was dissolved in 50 mL of dichloromethane. The flask was then lowered into an ice bath to allow cooling before adding trifluoroacetic acid (4.0 mL) dropwise. The mixture was left to react for 3 hours before extraction. The product was concentrated on a rotary evaporator and dried under high vacuum.



Scheme 2: Synthesis of N-terminal side chain utilizing N-hydroxycoumarin

The first part of this reaction is performed in a round bottom flask in an oil bath. The starting material, 4-hydroxy-coumarin was dissolved in acetone. Potassium carbonate (2.557 g, 18.501 mmol) was then added. After five minutes of stirring, ethyl bromoacetate was added to the reaction mixture. The reaction was left overnight. The ester intermediate (6) was dissolved in methanol in a round bottom flask. Aqueous potassium hydroxide was then added to the reaction, which initiated the hydrolysis. The reaction was left overnight. TLC determined that the desired product did not form. Tetrahydrofuran was then used as the solvent for the reaction in place of methanol. TLC determined that again the desired product did not form. We noticed that either ring opening, or transesterification is taking place.



Scheme 3: Synthesis of coumarin-ceramide

Commercially available coumarin containing carboxylic acids were used to achieve the targeted final compound. The amide formation reaction was conducted in a 100 mL round bottom flask under Argon gas. 2H-Chromene-3-carboxylic acid **8** (0.200 g, 1.135 mmol) was dissolved in 30 mL of dichloromethane. *N*-Methylmorpholine (0.6239 mL, 5.675 mmol, 5.0 eq.), the base, was then added using a syringe. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.2394 g, 1.249 mmol, 1.1 eq.) and hydroxybenzotriazole (0.1688 g, 1.249 mmol, 1.1 eq.) were then added as coupling reagents. Corresponding ceramide backbone **4** (0.3753 g, 1.249 mmol, 1.1 eq.) was then added to allow the product to form. The reaction was left overnight before extraction and purification by recrystallization. Other commercially available coumarins used were coumarin-3-carboxylic acid, 7-methoxy-coumarin carboxylic acid, 7 methoxycoumarin-4-acetic acid, (7 methyl 2 oxo 2 2h chromen-4-yl)acetic acid, and 7 hydroxycoumarin 3 carboxylic acid . The same reaction steps were performed with those compounds.

Results

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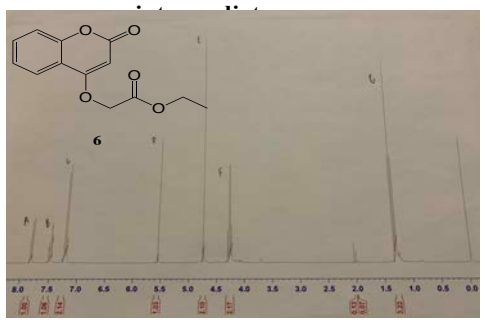
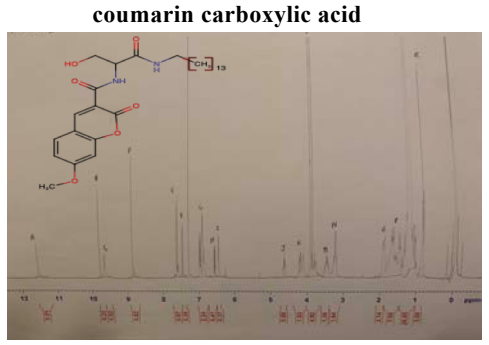
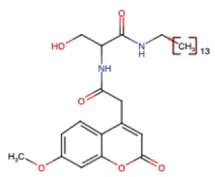


Targeted molecule using 7-methoxy-coumarin carboxylic acid

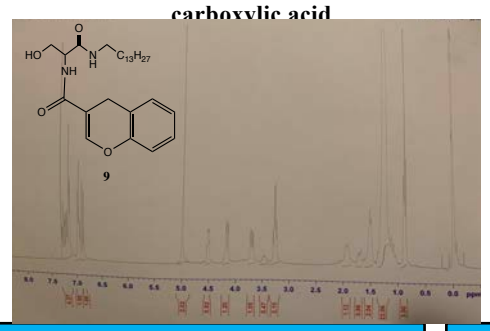


Targeted compound using 7 methoxycoumarin-4-acetic acid

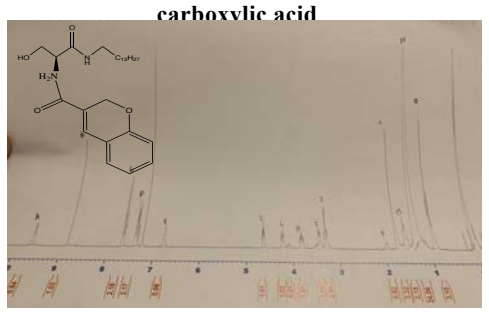


Analysis In Progress

Targeted molecule using 2H-chromene-3-carboxylic acid



Targeted molecule using coumarin 3-carboxylic acid



Future Works

- Biological Analysis of coumarin-ceramide products
- Using different substituted coumarins in making new ceramide analogs

References

1.“3-Ketone-4,6-diene Ceramide Analogs Exclusively Induce Apoptosis in Chemo-resistant Cancer Cells”, A. Ponnapakkam, J. Liu, B.A. Drew, T.L. Wang, J.W. Antoon, T.T. Nguyen, P.S. Dupart, M. Foroozesh, and B.S. Beckman, *Bioorganic and Medicinal Chem.*, Vol. 22, No. 4, pp. 1412-1420, 2014.

2.“Understanding Breast Cancer.” *Breastcancer.org,* http://www.breastcancer.org/>.

Acknowledgements

Research reported in this publication was supported by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Numbers TL4GM118968 and 5RL5GM118966, the NIMHD-RCMI grant number 5G12MD007595, and the Louisiana Cancer Research Consortium at Xavier University of Louisiana. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.