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Discovery of Sigma-2 Ligands and Prioritization of Marine Cyanobacteria Extracts for TNBC Drug Discovery

TIDGEWELL MARINE NATURAL PRODUCTS LAB

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Abstract

The role of natural products in drug development is well established. In recent years, marine cyanobacteria have been regarded as a major source of biologically active metabolites with chemical and pharmacological diversity. These cyanobacterial natural products serve as a promising source of drug leads for the discovery of therapeutic agents used in the treatment of many diseases of interest, such as CNS disorders, pain, and cancer. We have generated a library of 409 fractions from 37 field collected cyanobacterial samples and screened these fractions against a panel of CNS receptors using radiolabeled ligand competitive-binding assays. Upon analysis of the binding activity, we found that a significant amount of hits from our cyanobacterial samples were at the sigma 2 receptor. Sigma 2 has been known to be involved in CNS disorders and pain, as well as being upregulated in certain types of breast cancer, specifically, Triple Negative Breast Cancer (TNBC). For these reasons, certain cyanobacterial fractions with sigma 2 binding activity were prioritized and studied further using High-Performance Liquid Chromatography, Mass Spectrometry, and Nuclear Magnetic Resonance. Additionally, we found that fractions with a high affinity for sigma 2 had a significant cytotoxic effect on TNBC cell lines. The goal of this poster is to summarize our current analysis and results of cyanobacterial extracts with sigma 2 and TNBC cytotoxicity.

Background

Marine cyanobacteria produce pharmacologically active secondary metabolites

Figure 1.

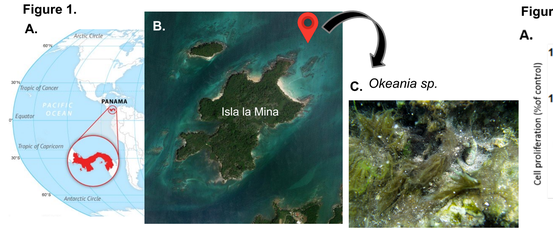


Figure 2.

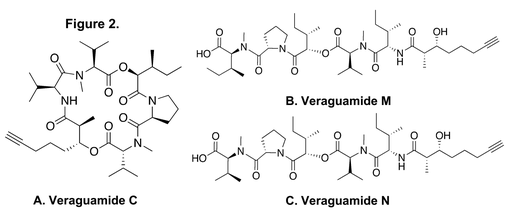


Figure 3.

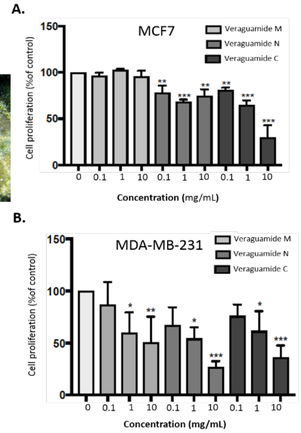


Figure 1. A. and B. Collection site of sample **C.** Cyanobacterial sample collected off the coast of Isla Mina in the Las Perlas Archipelago. **Figure 2.** One known **(A.)** and two new veraguamides. **(B. and C.)** were isolated from the extracts of this sample. **Figure 3.** Cell viability in ER+ MCF7 cells **(A.)** and MDA-MB-231 TNBC cells **(B.)** analyzed after treatment with DMSO, 0.1, 1 or 10 mg/mL of veraguamide M, N, or C for 72 hours using the MTT assay. **C.** IC_{50} values for veraguamides M and N in MDA-MB-231 TNBCs.

C.

Compound	TNBC IC_{50} (μM)
Veraguamide M	12.89 (+/- 4.20)
Veraguamide N	3.01 (+/- 1.57)

Analysis of Fraction Library

The sigma-2 receptor is a substantial target for further research

Figure 4.

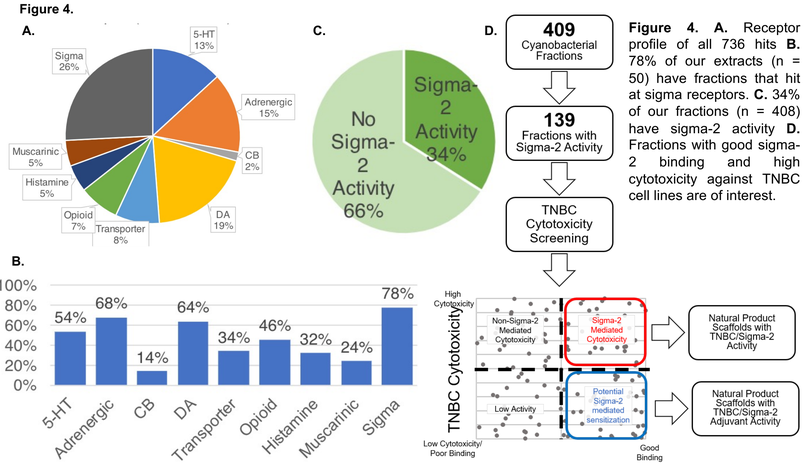


Figure 4. A. Receptor profile of all 736 hits **B.** 78% of our extracts (n = 50) have fractions that hit at sigma receptors. **C.** 34% of our fractions (n = 408) have sigma-2 activity **D.** Fractions with good sigma-2 binding and high cytotoxicity against TNBC cell lines are of interest.

Sigma-2 and TNBC Background

Sigma-2 receptors are upregulated in TNBC and sigma-2 ligands induce cancer cell death

Figure 5

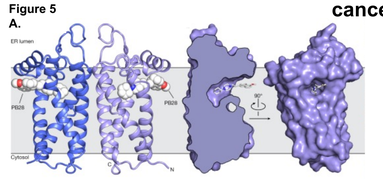


Figure 6

Figure 7.

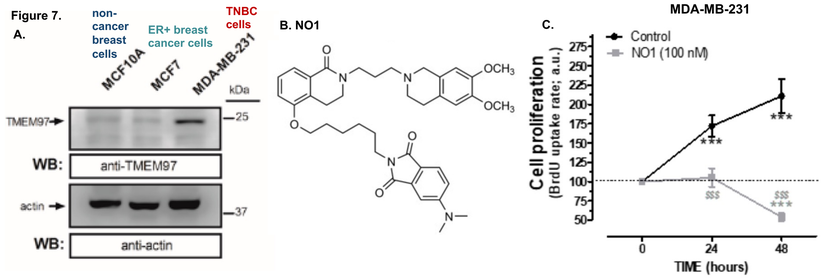
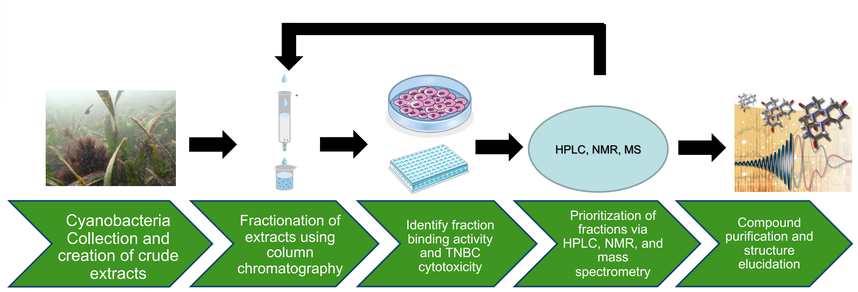


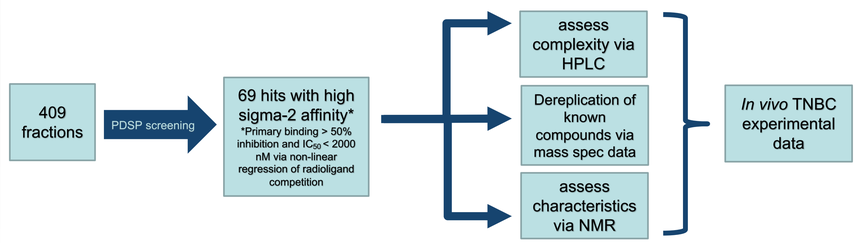
Figure 5. A. Sigma-2 receptor crystal structure. **Figure 6. A.** Breast cancer is classified based on the presence of estrogen, progesterone, and HER2 receptors, revealing four main subtypes. Triple negative breast cancers are typically more aggressive and have a worse patient prognosis. **Figure 7. A.** Western blot analysis of sigma-2/TMEM97 protein expression after cells were treated with anti-TMEM97 antibody. **B.** Structure of NO1, a known sigma-2/TMEM97 ligand. **C.** TNBC cell line MDA-MB-231 cells were grown for 48 h in the presence or absence of 100 nM of NO1 and analyzed using BrdU proliferation assay.

Methods



Sigma-2 Fraction Profiling

Creation of 50 fraction profiles to help prioritize fractions for dereplication



Results

DUQ0004F has good sigma-2 binding affinity and significantly inhibits MDA-MB-231 TNBC cell growth

Figure 8.

A.

	σ2R % inhibition	IC50
DUQ0004F	79.1	521

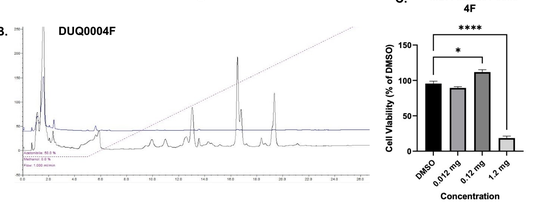
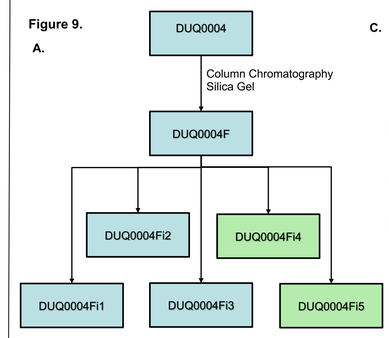
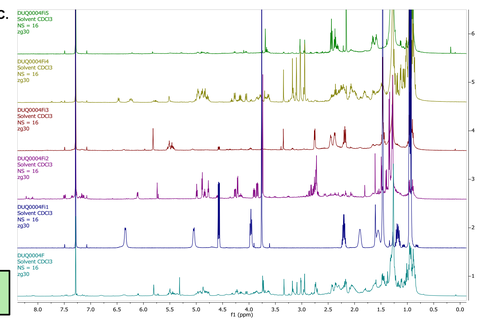


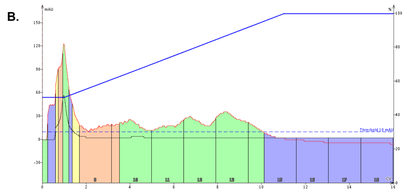
Figure 8. A. Primary binding assay (% inhibition) results for DUQ0004F at the sigma-2 receptor. Significant inhibition is seen at > 50% inhibition. **B.** HPLC reverse phase chromatography of DUQ0004F at 210 nm (black) and 254 nm (blue). **C.** MDA-MB-231 cell viability in the presence of DMSO, 0.012 mg, 0.12 mg, or 1.2 mg of DUQ0004F analyzed via MTT assay.

Fractionation and Analysis of DUQ0004F

Figure 9.







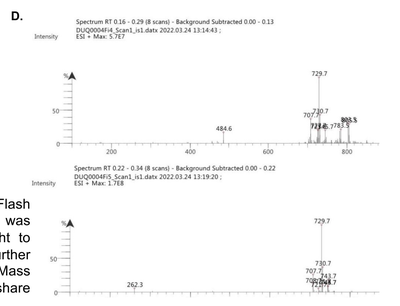


Figure 9. A. Fractionation scheme of DUQ0004. **B.** Flash chromatography of DUQ0004F and collection of all fractions was performed. Fractions DUQ004Fi4 and DUQ0004Fi5 were thought to share one major compound and were selected for further chromatography. **C.** ^{1}H-NMR spectra for each sub-fraction. **D.** Mass spectrum of sub-fractions DUQ0004Fi4 and DUQ0004Fi5, which share one major compound (mw = 729.7).

Conclusions and Future Research

- DUQ0004Fi4 and DUQ0004Fi5 may contain a novel or known veraguamide, which may be the main compound responsible for DUQ0004F's cytotoxicity against TNBC cells.
- These data suggest that DUQ0004F and other similar fractions may induce TNBC cell death through a mechanism involving the sigma-2 receptor.
- The ^{1}H-NMR spectra for all DUQ0004Fi4 and DUQ0004Fi5 sub-fractions will be obtained in order to identify the major compound from these fractions. Once isolated, the structure for this compound will be elucidated and will then be tested in a panel of biological assays. If it is active at the Sigma-2 receptor, it may be tested in multiple *in vivo* TNBC assays.
- The lab will utilize the sigma-2 fraction profile library to identify other fractions to study further

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