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Final Report

**Development and testing of the in vivo efficacy of a synthetic Coibamide A
formulation**

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Objective

The primary goal of this study was to test the anti-cancer efficacy of a promising drug candidate that was discovered at OSU. Coibamide A is a unique cyanobacterial peptide that shows nano-molar potency as a growth inhibitory and toxic agent against invasive and metastatic cancer cells grown in culture. Several investigators at OSU now collaborate to work on the chemistry and biology of coibamide A

Summary of Activities

1. In Vitro Testing of Synthetic Coibamide A

A library of synthetic coibamide analogs was generated as a consequence of pursuing the total synthesis of coibamide A. Elucidation of molecular structure, by LC-MS/MS, NMR and degradative Marfey's analysis (for stereochemical assignment), of a closely-related synthetic coibamide product revealed that the latter comprised a single diastereomer in which the only difference to the natural product was the presence of D- instead of L-N-methylalanine. Extensive testing of this D-N-MeAla coibamide in four human cancer cell lines represented in the National Cancer Institute 60 cell line (NCI60) panel (PC-3 prostate, MDA-MB-231 breast, H292 lung and SF-295 glioblastoma) showed that the synthetic molecule retains nanomolar activity but is approximately 4-8 fold less potent (IC_{50} 420-820 nM) than the natural compound (IC_{50} 66-220 nM) (Figure 1).

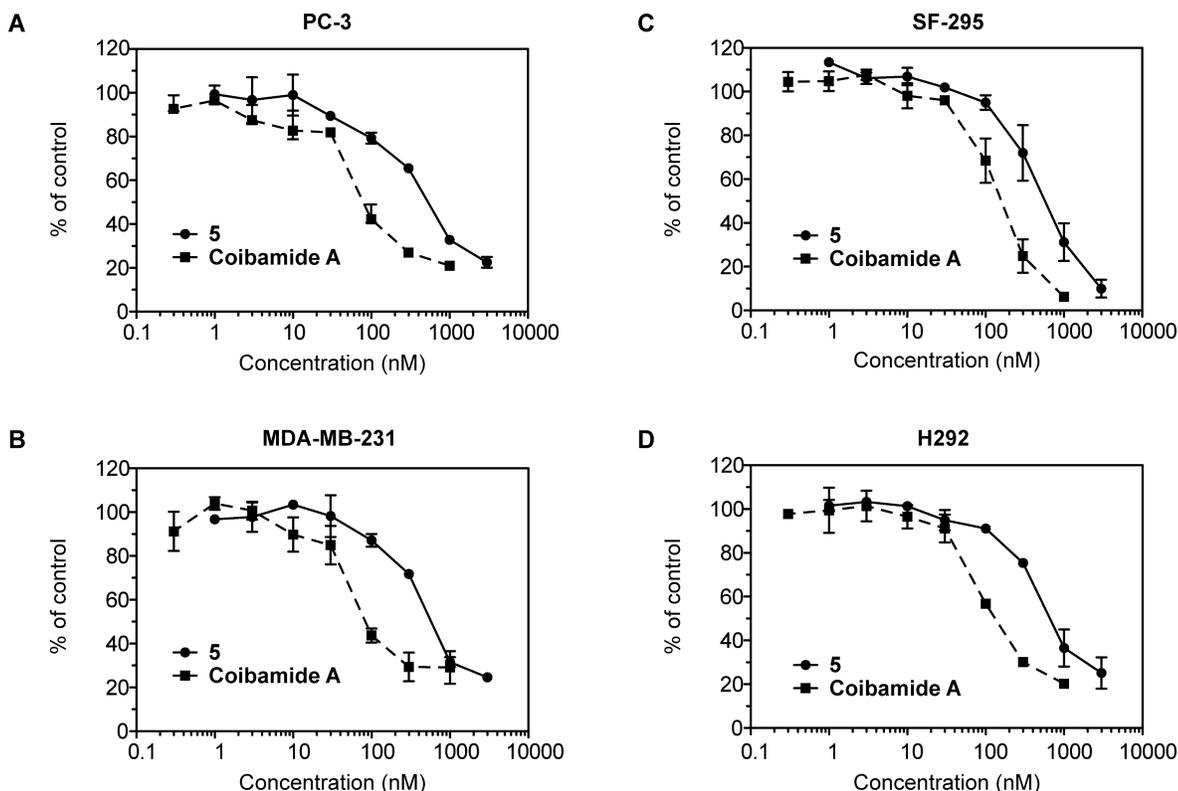


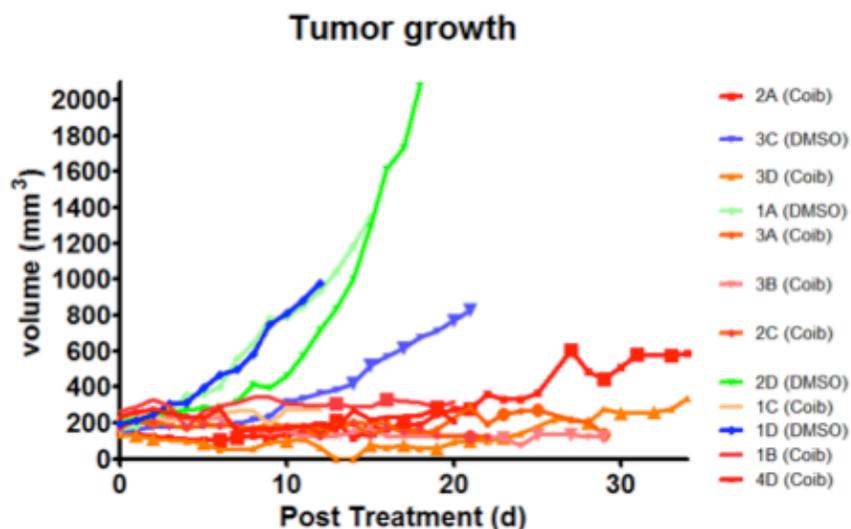
Figure 1. Comparison of cell viability in four human cancer cells following exposure to a synthetic D-N-MeAla coibamide analog or natural coibamide A. Concentration-response profiles for coibamide A-induced cytotoxicity in human PC-3, MDA-MB-231, SF-295 and H292 cells treated 3 days. Cytotoxicity was determined by MTT assay with the viability of control cells defined as 100%.

2. Analysis of In Vivo Efficacy

During this funding period we have also been able to further evaluate the in vivo efficacy and safety of naturally-derived coibamide A (Figure. 2A.). A mouse xenograft model was established in six-week old immunodeficient nude mice by subcutaneous injection of human U87-MG glioblastoma cells into the right flank. Tumors were allowed to reach a comparable size (~200-300 mm³) and the mice then received a schedule of intra-tumor injections of 300 µg/Kg (n=8 animals) or vehicle (n=4 animals). We observed a consistent delay in the progression of tumor volume in response to administration of 300 µg/Kg coibamide A, relative to those animals that had received vehicle.

Figure 2. Anti-tumor efficacy of the marine cyanobacterial metabolite Coibamide A

Tumor volume relative to coibamide A treatment in human glioblastoma xenograft model.



The animals tolerated this procedure, although two treated animals showed decreased body weight to the point where they had to be removed from the study (80% of starting weight). We are presently analyzing the excised tumors for expression of Ki-76, a biomarker of cellular proliferation. Blood samples were also submitted to the Veterinary Diagnostic Laboratory at OSU for analysis of common markers of liver and kidney damage.

External Funding Requests

Data shown in Figures 1 and 2 was generated as a direct result of funding from the GRF. These data were included in the following applications:

- 1 **St. Baldrick's Foundation – Childhood Cancer Research Grant** (March, 2013)

Mechanism of action and anti-cancer efficacy of Coibamide A

- 2 **National Brain Tumor Society** – letter of Intent/Mini-proposal (March, 2013 – still pending)

Mechanism of action and in vivo efficacy of Coibamide A

Planned Activity

These data will be used to support NIH applications (R15 and R01) planned for the next academic year and will be submitted in a manuscript planned for submission to *Investigational New Drugs*

I would like to thank the University for funding this proposal.

Yours sincerely,

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