Discovery of Novel Natural Products for Treatment of Drug Resistant Microbial Pathogens

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Introduction

The field of natural products chemistry utilizes nature for medicinal purposes. The term natural product represents compounds derived from living organisms such as microorganisms, plants, invertebrates and vertebrates[1]. Natural products are almost exclusively secondary metabolites, compounds that play ecologically important roles in how the living organism deals with their surroundings[1]. Historically, natural products have played a vital role in medicine, providing many anticancer, antiviral, and antibacterial remedies. Many revolutionary and novel drugs have been developed from natural products including morphine, aspirin, quinine, paclitaxel, and, most noteworthy, penicillin[2] .

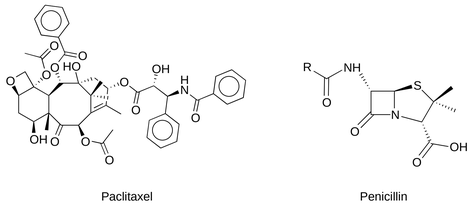

Figure 1. Examples of drugs derived from natural products

Presently, over 87% of all classified human diseases are being treated with drugs associated with natural products[3]. *Streptomyces* is a diverse genus of gram positive, filamentous bacteria found primarily in soils and associated with plants. Known colloquially as “streptomycetes,” they became of interest in 1943 after streptomycin, an effective treatment for tuberculosis was discovered[4]. Many *Streptomyces* antibiotics were soon discovered, establishing the importance of streptomycetes[4]. These bacteria produce a wide variety of antibiotics, including treatments to fight most bacterial diseases[4]. With these thoughts in mind, this project aimed to isolate a *Streptomyces* strain from a marine bryozoan (**Figure 2**), strain CES 088. This strain was shown to be specifically active against the human pathogen *Acinetobacter baumannii*. *A. baumannii* is a gram negative and highly drug-resistant bacterium that is responsible for a growing number of hospital acquired infections[5]. We hypothesize that the strain produced a novel natural product with specific inhibitory activity against *A. baumannii*. Accordingly, this project aimed to determine isolate and identify the active components produced by strain CES088. This will be accomplished by iterative fractionation (**Figure 5.** and **6**.) and bioassay steps (bioassay guided fractionation, **Figure 4**). Following purification of the active compounds, advanced spectroscopy techniques will be used to elucidate their structures.

Figure 2. Marine Bryozoan

Goals

To successfully isolate and elucidate the structure of the active components from strain CES088. If a new compound with unique biological activity is discovered, it could be studied and potentially used to develop novel antibiotics for important microbial pathogens.

Methods and Materials

The *Streptomyces* strain CES088 was cultured in 250mL of liquid medium and incubated at 30 ºC for seven days. The cultures were centrifuged to separate the mycelia (cells) from the supernatant and both samples were extracted with methanol and ethyl acetate EtOAc, respectively. The extracts were dried using a rotovap, reconstituted in dimethylsulfoxide at 10mg/mL and tested against *A. baumannii* using a standard broth dilution assay[6].

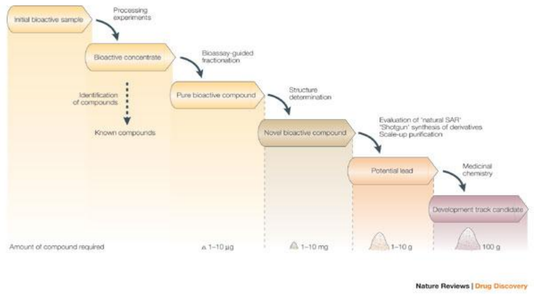

Figure 3. Representation of a customary process for natural products discovery. Image from Koehn and Carter.[7]

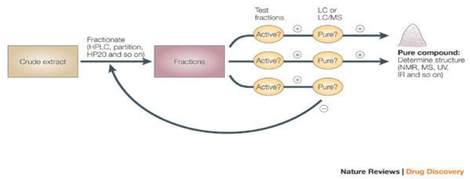

Figure 4. bioassay-guided fractionation process. Image from Koehn and Carter.[7]

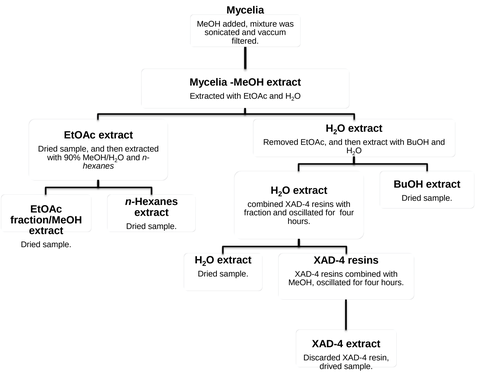

Figure 5. Mycelia extraction sequence.

Methods and Materials

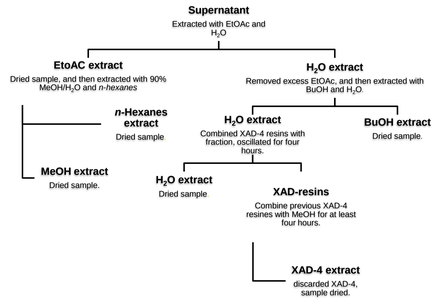

Figure 6. Supernatant extraction sequence.

The supernatant EtOAc extract was found to be active and subjected to a series of liquid/liquid partition fractionations (**Figure 5** and **6**) to purify the active components further[8]. It was found that several fractions were all able to be combined, based on NMR analysis, to form fraction 1011301 from strain CES088. Advanced spectroscopic techniques were then used to elucidate the structure of fraction 1011301.

Results

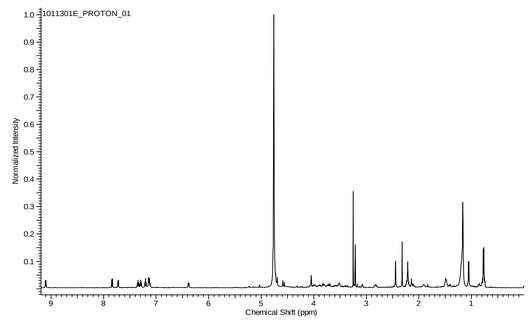

Figure 7. ^{1}H NMR of fraction 1011301.

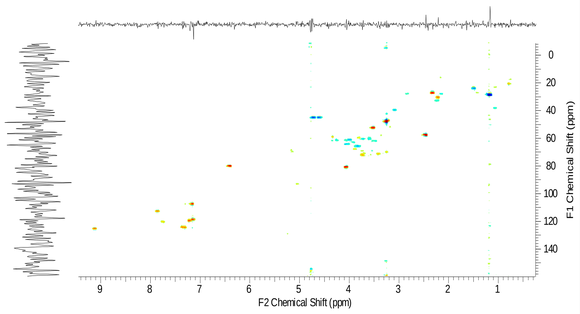

Figure 8. HSQC of fraction 1011301.

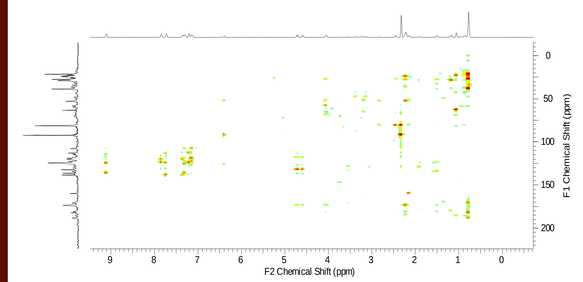

Figure 9. HMBC of fraction 1011301.

Conclusions and Future Directions

Mass spectrometry was employed to obtain a mass of fraction 1011301. The fraction was found to fly in APCI-Positive mode, and an internal standard was utilized to find an exact mass of 466.2154. Three possible formulas were found to be within 5 ppm of the exact mass found; they are as follows $C_{32}H_{26}N_4$, $C_{31}H_{30}O_4$, $C_{34}H_{28}NO$. However, through NMR analysis, no formula seemed logically correlated to the fraction. Further purification and structure elucidation will be necessary to determine the structure. If it is a novel natural product, it could be studied and potentially used to develop novel antibiotics for important microbial pathogens. However, If the active compound is a previously identified natural product, the project may evolve into studies of the unique mechanism of action against *A. baumannii*.

References

[1] Bhat, S.V.; Nagasampagi, B.A.; Sivakumar, M. *Chemistry of Natural Products*; Springer: India, **2005.** 1-20
[2] Bulter, M.S. Journal of Natural Product, **2004**, 67, 2141-2153.
[3] Chin, Y.; Baluanas, M.J.; Chai, H.B.; Kinghorn, A.D. *AAPS J.* **2006**, 8, E239-E253.
[4] Hopwood, D.A. *Streptomyces in Nature and Medicine;* Oxford University Press: New York, **2007**. 1-5
[5] Cisneros,M. Jose, Reyes, J. Maria. Bacteremia Due to Acinetobacter baumannii: Epidemiology, Clinical findings, and Prognostic Features. *Clinical Infectious Diseases* **1996**; 22:1026-32.
[6] Clinical and Laboratory Standards Institute. **1999**. Methods for determining bactericidal activity of antimicrobial agents; Approved guideline; CLSI Document M26-A.
[7] Koehn, F.E.; Carter, G.T. *Nat. Rev. Drug Discovery* **2005**, 4, 206-220.
[8] Kupchan, S.M., Britton, R.W., Ziegler, M.F. and Sigel, C.W. **1973**. Isolation and structural elucidation of bruceantin and bruceantinol, new potent antileukemic quassinoids from *Brucea antidysenterica J. Org. Chem.* 38: 178-179.

Acknowledgements

Special thanks to my mentor, Christine Salomon, for her guidance and support, Chris Gelbmann, and the remaining members of the Salomon group (Center for Drug Design, University of Minnesota). This research was supported by the Undergraduate Research Opportunities Program at the University of Minnesota.