Exploring the treasures of the sea – bacterial marine natural products

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Drug development from marine natural products

<table>
<thead>
<tr>
<th>Year</th>
<th>Product</th>
<th>Source</th>
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<th>Product</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>1969</td>
<td>Cytarabine</td>
<td>Sponge NP inspired</td>
<td>2004</td>
<td>Prialt</td>
<td>Cone snail</td>
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<tr>
<td>1976</td>
<td>Vidarabine</td>
<td>Sponge NP inspired</td>
<td>2007</td>
<td>Yondelis</td>
<td>Ascidian</td>
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</tbody>
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Common traits of MNPs = complex structures and limited supply

Other applications of marine natural products

• As biomedical probes...
  okadaic acid (phosphatase inhibitor produced by dinoflagellates)

• As cosmetics...
  pseudopterosin (antiinflammatory skin care additive produced by corals)

Yondelis (ET-743) is related in structure to bacterial products

ET-743 from ascidian
Renieramycin E from sponge
Saframycin B from bacterium

• Yondelis is produced commercially by semi-synthesis from saframycin
• Related examples also exist from cyanobacteria, myxobacteria, pseudomonads
• Potential of synthetic biology to solve long-term supply
Salinosporamide A is a potent natural proteasome inhibitor produced by *Salinispora tropica*

Salinosporamide A was discovered by Fenical & Jensen in 2003 (Angew. Chem.) and is currently in phase Ib human clinical trials for multiple myeloma and other cancers with Nereus Pharmaceuticals.

Actinomycetes are antibiotic factories

- Soil-dwelling microbes notable for their production of natural products, including over 60% of all natural antibiotics.
- Examples include: macrolides, polyenes, aromatic polyketides, beta-lactams, aminoglycosides, lipopeptides, glycopeptides.

Salinosporamide A – Cl is key for proteasome inhibition

Total genome sequencing reveals a treasure trove of novel biosynthetic pathways

**Salinispora tropica CNB-440**
Bahamas
accession no. CP000667
PNAS 2007
5,183,331 bp
circular
69.8% G+C
4,593 ORFs
79% orthologs in SA
9% 2° metabolism

Correlating the biogenesis of salinosporamide A to its SAR suggests the feasibility of a bioengineering approach to unnatural analogs

**SAR**

**Biogenesis**

Salinosporamide is assembled by a hybrid polyketide synthase / nonribosomal peptide synthetase pathway.
Salinosporamide biosynthesis via a novel synthetase

Where does the “chlorobutyrate” unit come from?

The salL gene product is 35% identical to FLUORINASE

Analysis of the sal gene cluster:
No oxidative halogenases present!

Discovery of SaL suggests a novel biological chlorination pathway

Salinosporamide biosynthesis via a novel synthetase
SalL is a novel SAM chlorinase

SalL initiates an unprecedented pathway to a halogenated polyketide synthase substrate

Applications of this basic discovery...

Towards the selective overproduction of salinosporamide A

- SalR2 is a two-component response regulator belonging to the LuxR-type transcriptional regulator family.
- Its location adjacent to the divergent salLM and salNO transcripts suggests that SalR2 is a selective activator of chloroethylmalonyl-CoA biosynthesis that may directly impact salinosporamide A production.

Engineering new salinosporamide chemistry

Example: Fluorosalinosporamide

Rationale: Explore the effect on replacing the Cl leaving group with F
Mutasynthesis of fluorosalinosporamide

Decoupled $^{19}$F/$^1$H NMR of crude extract

Engineering fluorometabolite production from F⁻ ion: Fluorinase expression in S. tropica

Fsal is the most active "reversible" salinosporamide (yeast proteasome IC₅₀ 1.5 nM)
Where does the 3-CHA unit come from and can it too be selectively modified?

Gene inactivation of \( \text{sal}X \) allows for entry into the salinosporamide amino acid unit

Generating a focused library of \( \beta \)-lactone proteasome inhibitors by genetic engineering

Summary

Salinosporamide A is a densely functionalized natural proteasome inhibitor in clinical trials.

Biosynthetic studies have led to the discovery of new biochemical reactions and pathways such as in halogenation and polyketide assembly.

Analogs can be accessed through synthetic biology.

Genomics has accelerated discoveries in salinosporamide regulation and proteasome resistance.
Natural product biosynthesis moves in vitro - the total (bio)synthesis of enterocin

The longest linear series of reactions from BA to enterocin involves the formation of 10 C–C bonds, 5 C–O bonds, and 7 chiral centers and utilizes 10 recombinant proteins in ~25% overall yield in a single reaction vessel.

Diversity oriented biosynthesis of enterocin-wailupemycins

Inspiration from the sea: Marine actinomycetes

Salinosporamide team

Collaborators

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