Challenges in Marine Natural Product Drug Discovery

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Wyeth Research

Why Natural Products?

- **Of the 520 new drugs approved between 1983 and 1994, 39% were NP/NPD - 60-80% of antibacterials and anticancer agents were derived from NP.**

- **In 1999, 20 of the best-selling non-protein drugs were NP/NPD with combined annual sales of >US$16 billion.**

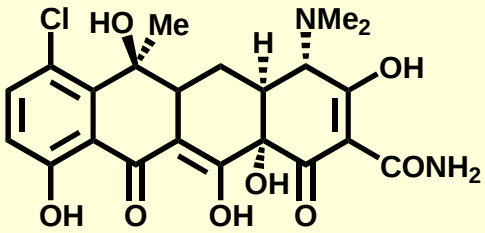
- **Chemical novelty of NP is higher than any other source: 40% of chemical scaffolds are absent from synthetics.**

- **NP that are active in biologically active assays are generally small molecules with drug-like properties.**

Alan Harvey, Drug Discovery Today Vol.5, No 7 July 2000

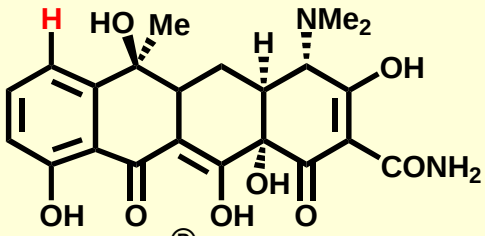
Five Generations of Tetracycline Antibiotics

1945



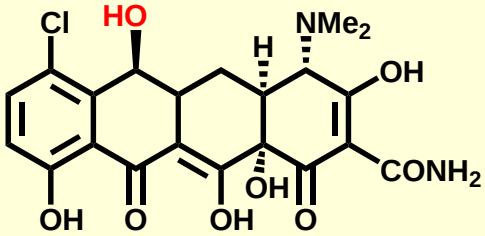
Aureomycin®

1953



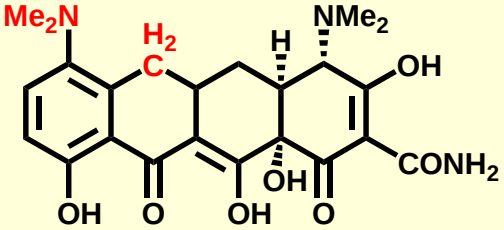
Achromycin® (tetracycline)

1959

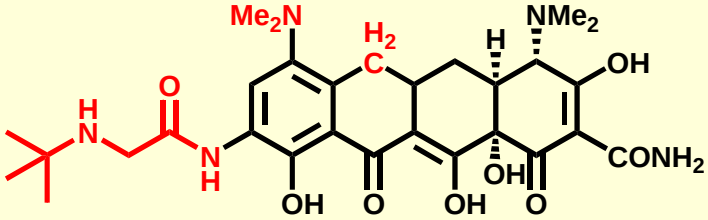


Declomicin®

1971



Minocin®



GAR-936

(active against multiple resistant gram positive and gram negative strains, presently in Phase III clinical studies)

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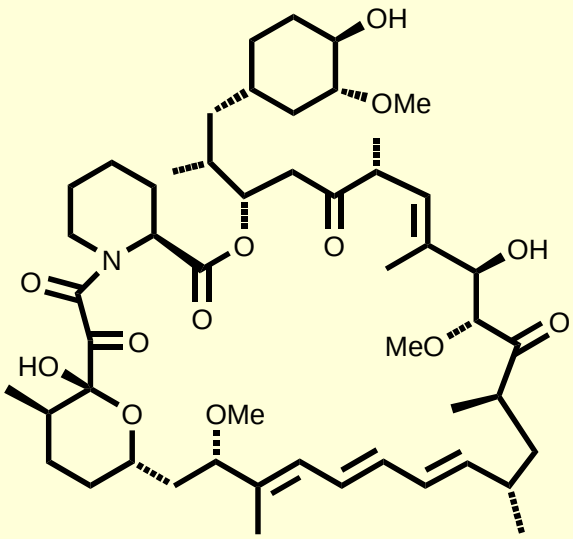
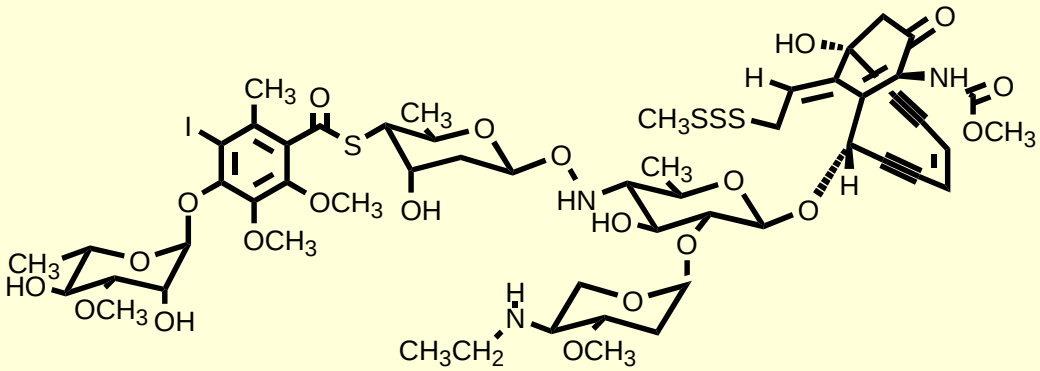
Wyeth's Other Natural Therapeutic Agents

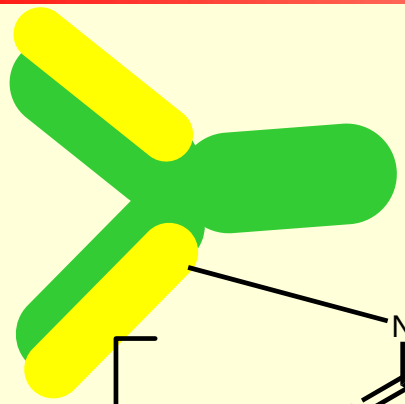


Rapamune™
Rapamycin
(sirolimus)



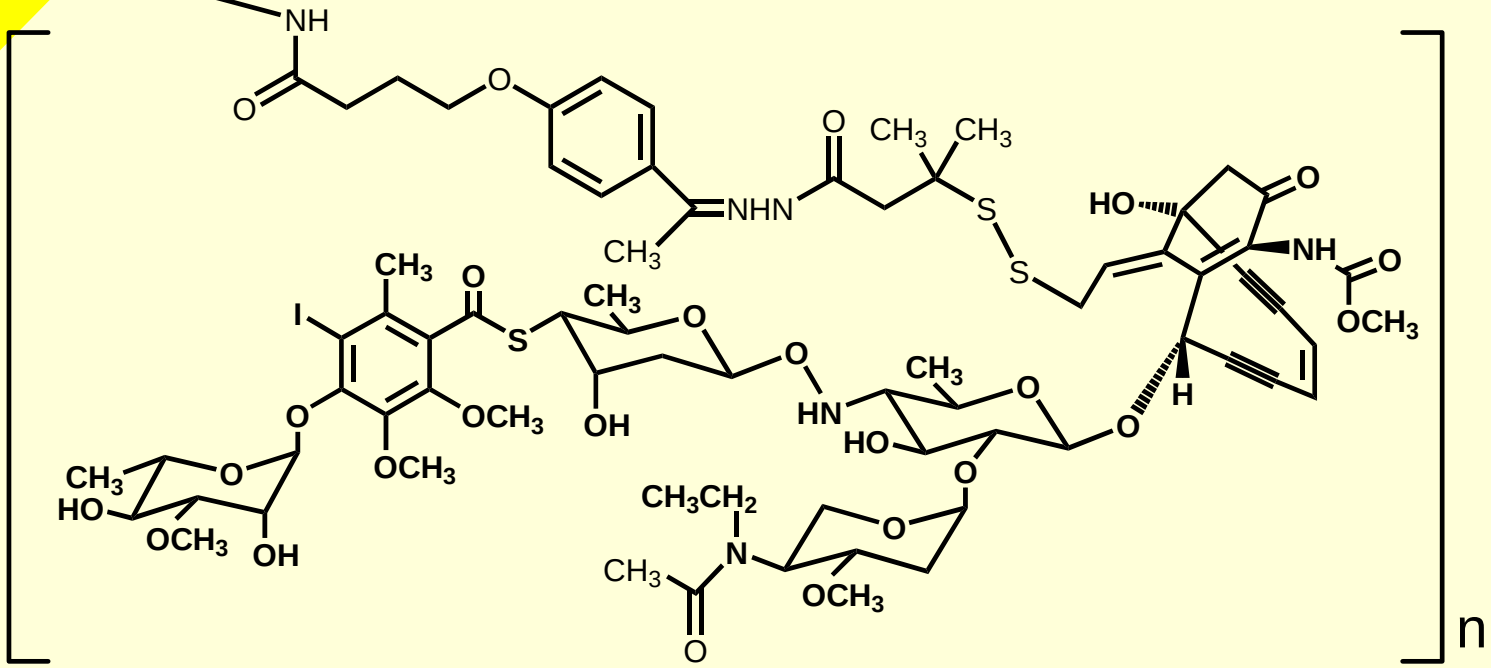
Calicheamicin γ_1^I
Mylotarg™ (as conjugate)

Mylotarg™
Gemtuzumab Ozogamicin



Anti-CD33

Acute myeloid leukemia



average n = 2 to 3

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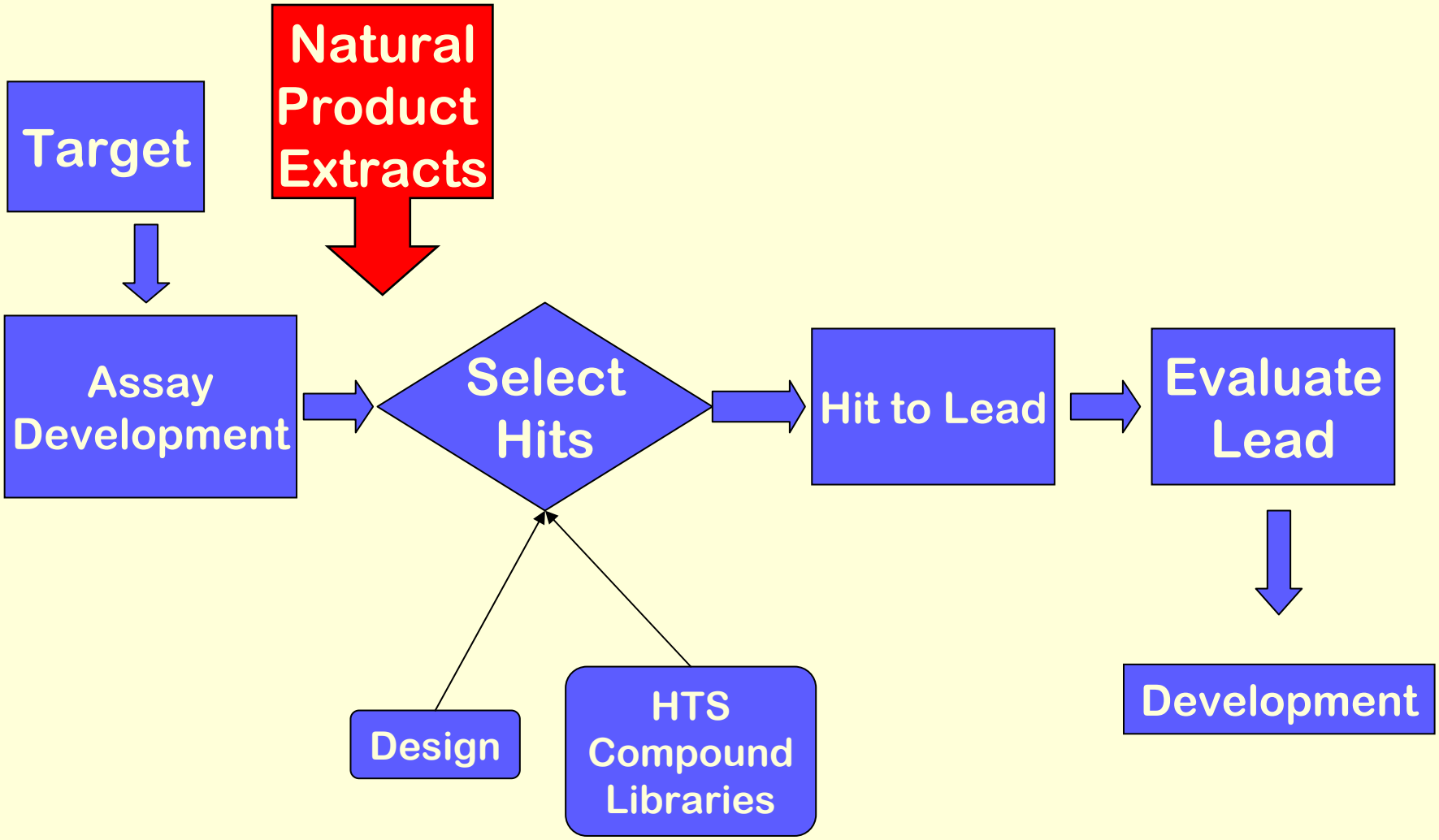
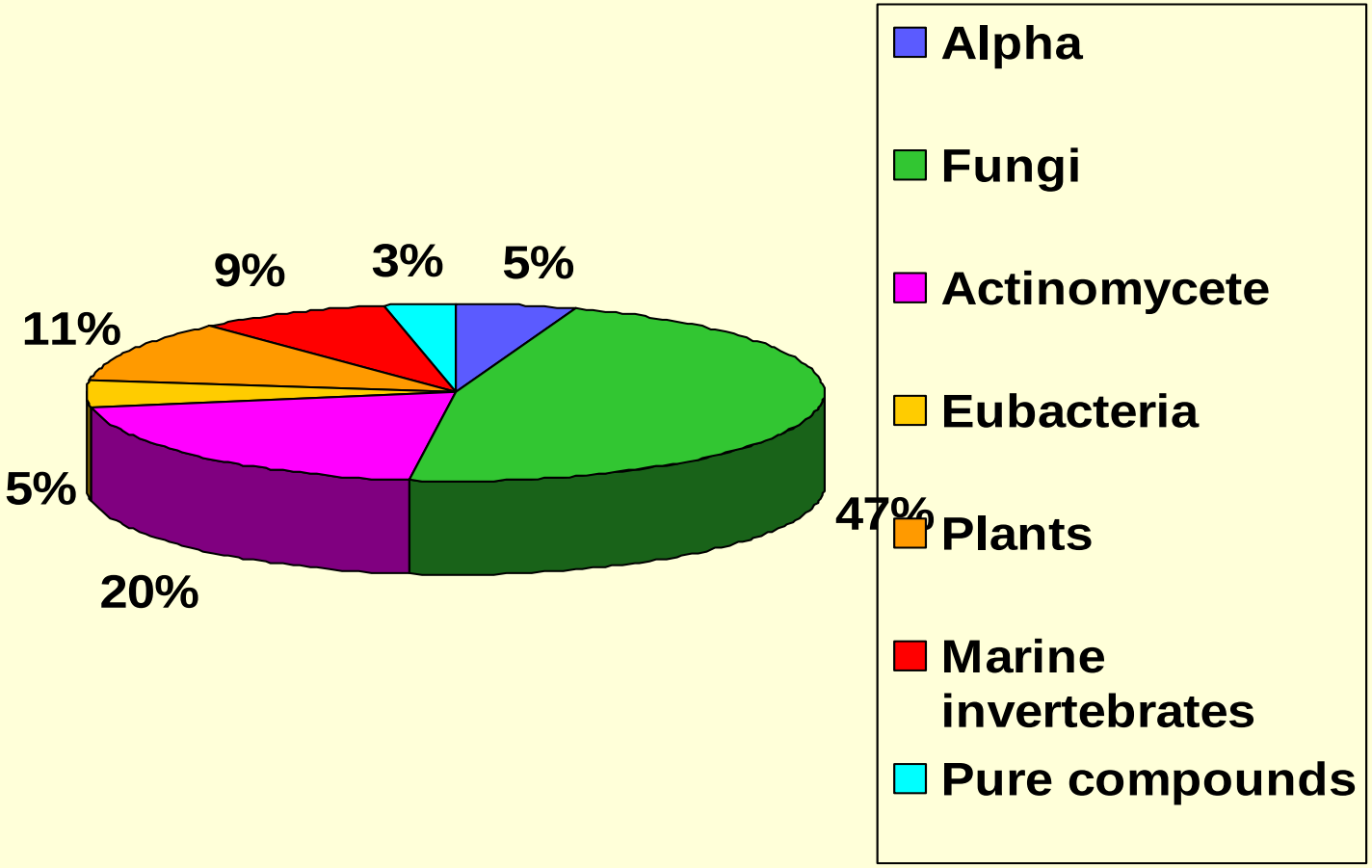
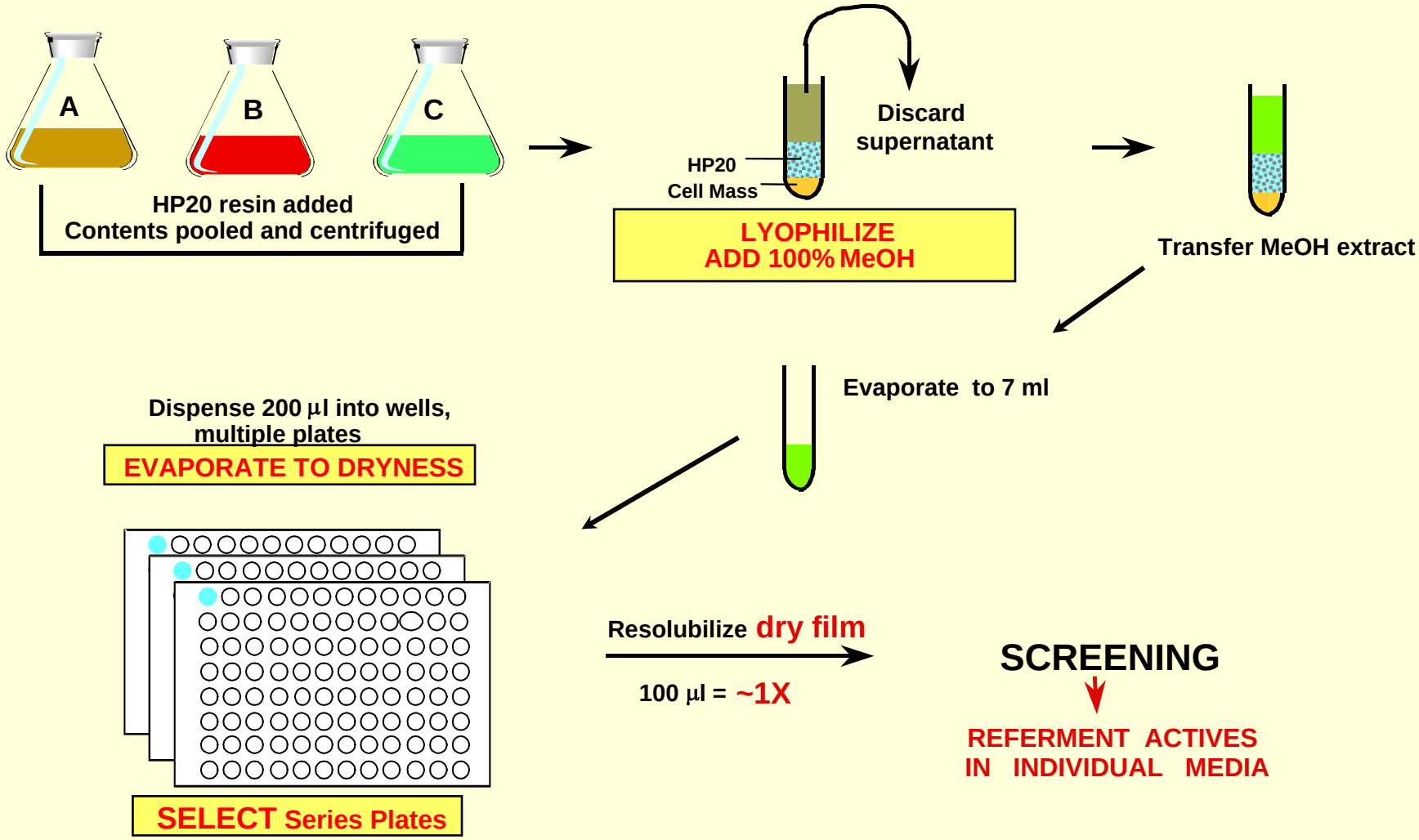
Drug Discovery Process



Natural Products Library Diversity



Microbial Fermentation Extraction Scheme



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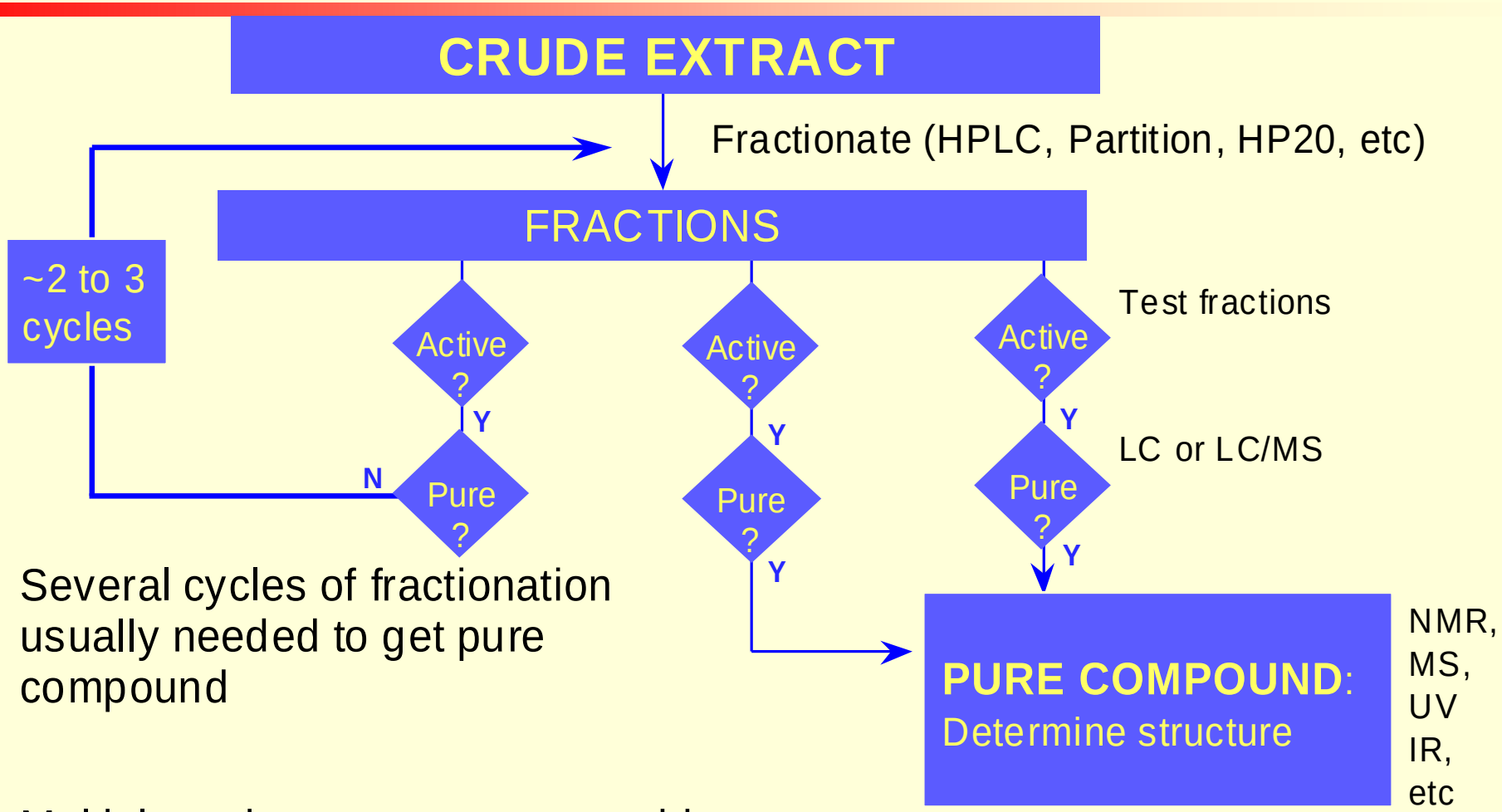
Bioassay Guided Fractionation: Generic Scheme



- Several cycles of fractionation usually needed to get pure compound
- Multiple active components provide 'NATURAL PRODUCT SAR'

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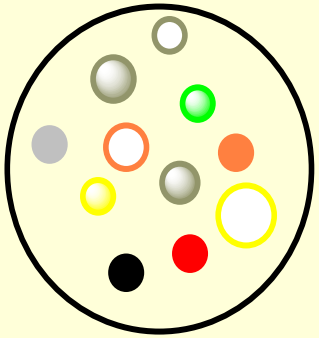
Discovery of calicheamicin

caliche soil sample

Isolation of actinomycetes

Petri plate - individual colonies



Screening

Flask fermentations of individual organisms

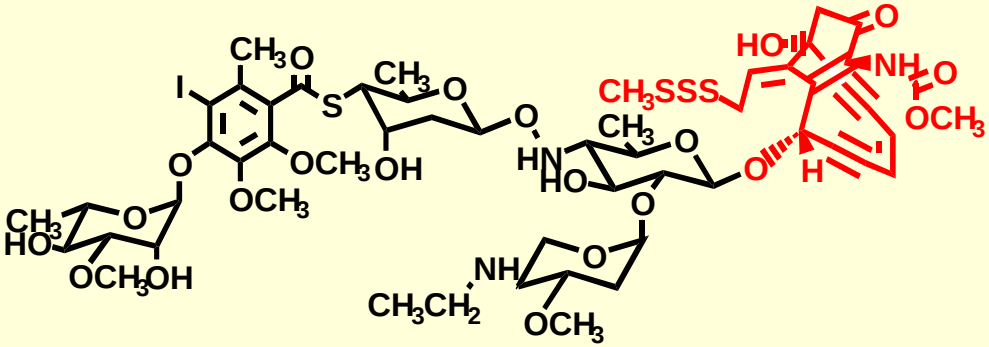
calicheamicin @ 0.1 ng/mL

Tank Fermentation
(50,000 liters)
Refining
Purification

calicheamicin
(10 mg)

Structure elucidation

NMR
Mass Spec.
X-ray



Calicheamicin $\gamma 1^{I}$ (1987)

Highly cytotoxic (1000 X > adriamycin)

M. D. Lee, et. al., JACS, 1987

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Natural Products Drug Discovery

- **New sources of biodiversity**
  - Required to fuel the screening engine
  - Premise: novel biodiversity => novel chemodiversity
  - Abundant diverse species
    - Terrestrial plants and animals - most have been studied
    - Terrestrial microorganisms
      - **Investigate rare genera & unique habitats**

Marine organisms: plants & animals

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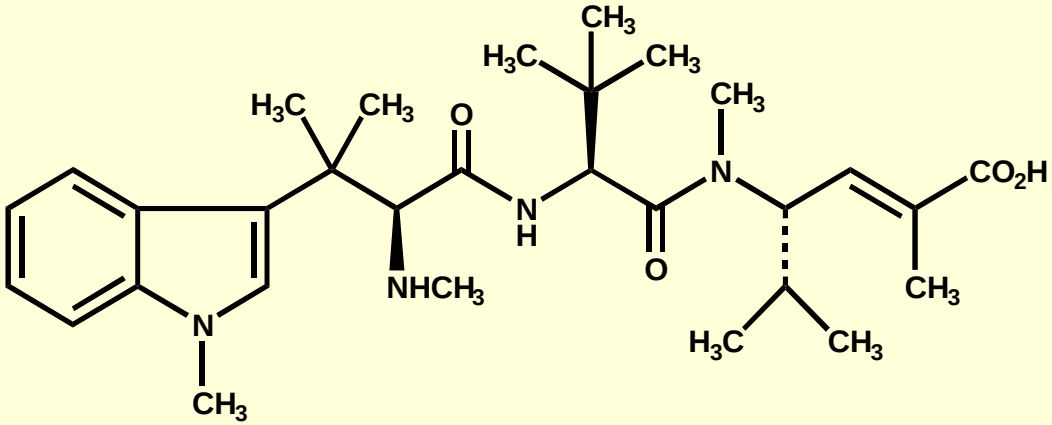
Research

Hemiasterlin antitumor agents



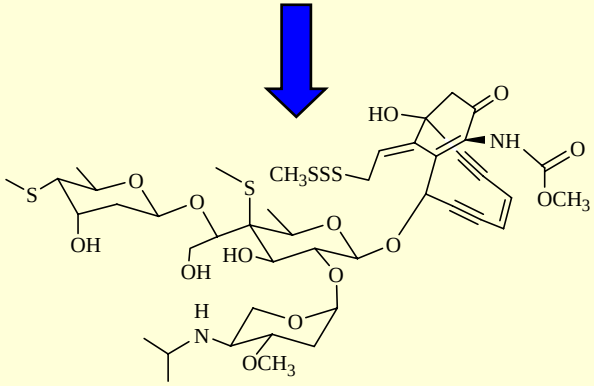
- Isolated independently by Andersen and Kashman
- Synthesis and preliminary SAR by Andersen
- Mechanism of action by Andersen, *et. al.* and NCI
- Congener currently in Phase I Clinical studies by Wyeth

Supply issue was solved by total synthesis of the agent

Namenamicin from Ascidian *Polysyncraton lithostrotum*

- **Didemnid ascidian *Polysyncraton lithostrotum* from Namenalala Island, Fiji**
  - Extract had SOS induction (DNA damage) in Biochemical Induction Assay (BIA)
  - Extract showed exceptionally potent cytotoxicity in tumor cell lines
  - Namenamicin isolated
    - **First "marine" enediyne isolated**
    - **Produced in low yields (10^{-4}%)**
    - **Suggests microbial source - symbiont?**

If a microbial source could be isolated, then the supply issue would be resolved



Namenamicin

McDonald et al., J. Am. Chem. Soc. **1996**, *118*, 10898.

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Research

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Research

Isolation Plate - Marine Microbes

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Types of Organisms Isolated from *Polysyncraton lithostrotum*

Organism Type	Total Number of Isolates	Number of unique isolates
Fungi	2	2 genera
Mycobacterium	2	1 species
Oceanospirillium	5	2 species
Bacillus	4	3 species
Pseudomonas	3	2 species
Agrobacterium	5	1 species
Rhodococcus	1	1 species
Micromonospora	16	3 species
Unidentified	24	6 genera; 10 species

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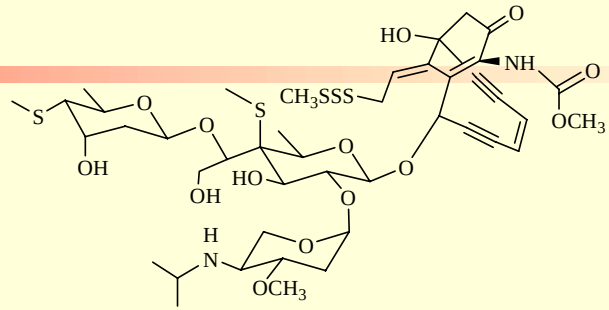
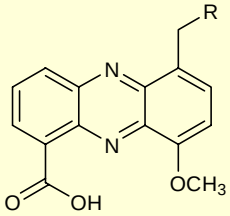
Natural Products From Microbial Inhabitants of a Marine Tunicate



→ **Isolation of Microorganisms** → **Fermentation and Assay**

Actives

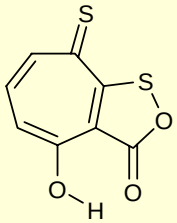
Anticancer BIA+



Phenazines from *Pelagiobacter variabilis*

Singh et al., *J. Antibiot.* **1997**, *50*, 785.

Antibacterial Gm +



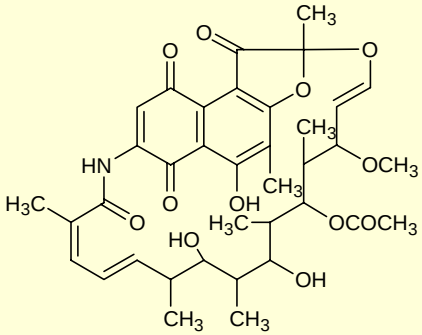
Thiotropocin from *Agrobacterium* sp.

Kawano et al., *J. Mar. Biotechnol.* **1998**, *6*, 49.

Anticancer BIA+

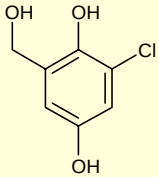
Lomaivitacins
Micromonospora lomaivitiensis

Antibacterial Gm +



Rifamycin S from *Micromonospora* sp.

Antibacterial Anticancer



Chlorogentisyl alcohol and epoxydons from a fungus

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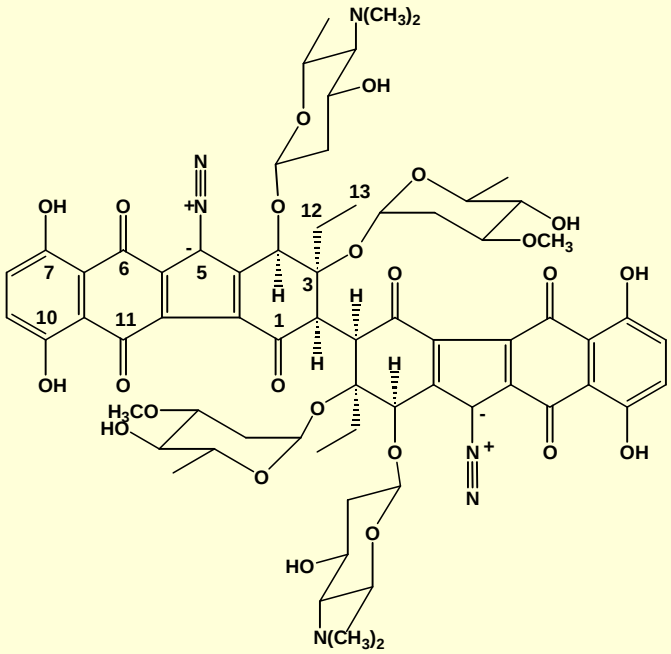
Lomaiviticin A



Novel compound with potent cytotoxicity

Mechanism of action
Structure-Activity Relationships
Synthesis of analogs
Novel delivery systems

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Marine microorganisms derived from *P. lithostrotum*

- Rich, renewable sources of bioactive metabolites
  - Both fungi and bacteria have yielded new chemistry
  - Known "terrestrial" products also isolated *e.g.* rifamycin, tropolones
  - Lomaiviticins are potent cytotoxic agents
    - IC50's for **A** 10 pg to 100 ng *versus* tumor cell lines
    - DNA-damaging mode of action
- Have not yet isolated the namenamicin producer
  - May be a true symbiont and difficult to culture

Challenge : Inability to culture majority of marine microorganisms

Challenges

- **Finding new biodiversity for screening**
  - Freedom of exploration
  - Isolation of intractable microbes
- **Culturing/cultivating organisms for production**
  - Macro- and microorganisms
  - Supply Issue
- **Expression of silent biosynthetic pathways**
  - Evidence for pathways, e.g PKS & NRPS, but no expression

Recommendations

- **How to facilitate the Discovery of new therapeutic agents derived from marine organisms**
  - Fund/conduct research focused on
    - Understanding marine microbial ecology and symbiosis
    - Marine microbial physiology
    - Fundamental activities of marine toxins and other secondary metabolites
    - Expression of silent metabolic pathways

Opportunities : New Sources of Bio/Chemo-Diversity

- **New microbes**
  - Need a renewable source to overcome supply issue
  - Isolate in the course of collections of macroorganisms, ecological studies, from core samples, etc.
- **Cultivate the "unculturable"**
  - More fundamental microbiological studies
  - Capture the biosynthetic capacity of
    - Symbiotic microbes
    - "Under-expressers"

Opportunities: Defining Biological Functions

- Novel natural products
  - Determine mechanism of action
    - Understand on the molecular level
      - **Target-based screening**
      - **Use compound to define target "orphan ligand' concept**
  - Show activity can be modulated by structural variation
    - Usually have natural SAR from congeners
    - Modify chemically or biosynthetically
    - Define the critical pharmacophore

Challenge : Lack of understanding of intrinsic biological functions

Acknowledgements

- **Natural Products Discovery**
  - Valerie Bernan
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