Progress Toward the Total Synthesis of Tubulysin D and its Analogues

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A General Look at the Cell Cycle

- Cell cycle is divided into four main parts
  - G₁ or Gap 1 phase
  - S or Synthesis phase
  - G₂ or Gap 2 period
  - M or Mitosis phase
- Cell cycle contains two major control points
  - G₁/S is the point at which cells commit to replicate genetic material, enter quiescence (G₀), or terminally differentiate and die
  - G₂/M is the point at which cells commit to division
  - Tubulin inhibitors typically cause treated cells to accumulate at the G₂/M checkpoint

http://www.biology.arizona.edu/cell_bio/tutorials/cell_cycle/cells2.html
A Brief Introduction to Tubulin

• Tubulin exists as a heterodimeric structure of the α and β tubulin proteins.
  – tubulin proteins are highly conserved structures in eukaryotes
  – the α and β-subunits are of similar secondary and tertiary structure
  – each subunit is ca. 55 kD in mass
  – the heterodimeric structure is tightly bound together and dissociates only under denaturing conditions

• Each tubulin monomer is capable of binding a molecule of GTP (guanosine triphosphate)
  – α-tubulin binds GTP and retains it in the heterodimer
  – β-tubulin binds a molecule of GTP and hydrolyses it to GDP during or shortly following the incorporation of the heterodimer into a protofilament
Structure of the αβ-Tubulin Dimer from Electron Crystallography

- Purple subunits represent bound GTP (α-tubulin), and GDP (β-tubulin) each bound within a Rossman fold.
- Subtle differences between intradimeric and interdimeric binding contacts remain somewhat ambiguous.

Introduction to Microtubules

• Microtubules exist as helical entities consisting protofilaments
  – microtubules are “3-step” helices consisting of 13 protofilaments each
  – standard microtubules are 25 nm in diameter and vary in length depending on environmental stimuli (dynamic instability)
  – protofilaments are polar entities consisting of a “+” (at β-tubulin) and “−” (at α-tubulin)
    • growth of the microtubule occurs at the “+” end
    • degradation of the microtubule occurs at “−” end
The Basics of Tubulin Polymerization

Dynamic Instability

http://www.ch.ic.ac.uk/local/projects/a_abowath/Tubulin.html
Microtubule Decomposition and Growth in Real Time

A Survey of Tubulin Inhibitors – Colchicine Domain

- Alkaloid produced by the meadow saffron (Colchicum autumnale).
- Inhibits microtubule-dependant cellular processes by strongly binding to β-tubulin, thus interfering with polymerization of tubulin protomers.
- Arrests mitotic cycle in plants and animals at metaphase.
- Commonly used in the treatment of Gout.
- The agent is not selectively toxic to cancer cell lines.

A Survey of Tubulin Inhibitors – Colchicine Domain

- Obtained as an extract from the plant, *Podophyllum peltatum*.
- Binding to tubulin is more rapid and reversible than colchicine derivatives.
- Behaves as a competitive inhibitor of colchicine binding to tubulin.
- Inhibits tubulin dependent GTP hydrolysis (*vide supra*) thus suppressing the dynamic instability properties of microtubules.

A Survey of Tubulin Inhibitors – Colchicine Domain

- Isolated from the marine cyanobacterium *Lynbya majuscula*.
- Inhibitor of mitosis, tubulin polymerization, and a competitive inhibitor to colchicine binding.
- Interaction with tubulin characterized by rapid binding, slow dissociation, and induction of GTP hydrolysis uncoupled from normal tubulin assembly.
- Although an inhibitor of normal tubulin polymerization, treatment with curacin A often results in the formation of abnormal tubulin polymers.

A Survey of Tubulin Inhibitors – Vinca Site

- Isolated From the Madagascan periwinkle *Catharanus roseus* (formerly *Vinca rosea*).
- Inhibits of microtubule polymerization through binding to the vinca binding site on α- tubulin.
- Characteristically aberrant tubulin polymerization reactions result following *in vivo* treatment with *Vinca* alkaloids.
- The alkaloids strongly inhibit tubulin-dependent GTP hydrolysis, and weakly inhibits the binding of GTP and GDP at the exchangeable nucleotide site.
- Widely used in combination chemotherapy, often in conjunction with DNA damaging agents like bleomycin.

Vinblastine $R = CH_3$

Vincristine $R = CHO$

A Survey of Tubulin Inhibitors – Vinca Site

- Isolated from fermentation cultures of the fungus *Rhizopus chinensis*.
- Reversibly binds to tubulin at 37 °C
- Agent demonstrates plant, fungal, and cellular toxicity.
- Under clinical evaluation for treatment in human cancers.
- Binding of radiolabeled rhizoxane was competitively inhibited by vinblastine ($K_i = 3 \mu M$).
- Noncompetitive inhibition of Rhizoxin was observed with phomopsin A.

A Survey of Tubulin Inhibitors – Vinca Domain

• A toxic secretion from the fungus *Phomopsis leptostomiformis*.
• Strong inhibitor of tubulin polymerization.
• The agent behaves as a noncompetitive inhibitor in the binding of $[^3]H$ vincristine to tubulin ($K_i = 2.8 \, \text{M}$), and as a competitive inhibitor to dolostatin 10 / tubulin binding.
• Inhibits tubulin-dependent GTP hydrolysis, nucleotide exchange, and formation of the cys12 – cys 201/211 cross-link.
• Phomopsin strongly stabilizes tubulin conformation.

A Survey of Tubulin Inhibitors – Vinca Domain

- An unusual peptide isolated from the shell-less mollusk *Dolabella auricularia*
- Agent causes mitotic arrest *in vivo*, with visible degradation of intracellular microtubules.
- The agent behaves as a noncompetitive inhibitor in the binding of $[^3\text{H}]$ vincristine to tubulin ($K_i = 1.4 \text{ nM}$), and as a competitive inhibitor to radio labeled phomopsin A–tubulin binding.
- Inhibits tubulin-dependent GTP hydrolysis, nucleotide exchange, and formation of the cys12 – cys 201/211 cross-link.

A Survey of Tubulin Inhibitors – Vinca Domain

- Isolated from the marine sponge *Spirastrella spinispirulifera* and *Hyrtios altum*.
- Highly toxic to a variety of human cancer lines in culture. (IC_{50} = 20 pM for L1210 murine leukemia cells).
- Noncompetitively inhibits the binding of both dolostatin 10 and vinblastine to tubulin.
- The agent is a strong inhibitor of GDP nucleotide exchange.

Spongistatin 1

Microtubule Stabilizing Agents – Taxol

- Originally isolated from the plant *Taxus brevifolia* as a cytotoxic agent.
- The agent was the first to derive its mechanism of action from the *stabilization* of microtubules rather than by the inhibition of their synthesis.
- Binding of Taxol occurs only with the tubulin polymer, and in a roughly 1:1 stoichiometry.
- Taxol binds to the polymer in a reversible fashion with a $K_D = 1 \, \text{M}$. 

(b) *Nature*, 1979, 277, 665-667.  
(c) *J. Biol. Chem.*, 1988, 263, 1342-1346.  
(f) Khalil, M.-W. M. *Dissertation 1999*, Technical University of Braunschweig.
Microtubule Stabilizing Agents – Epothilones

- Isolated from cultures of the myxobacterium *Sorangium cellulosum*.
- Epothilones were demonstrated to induce tubulin polymerization *in vitro*, in the absence of added GTP, and in a concentration dependent manner.
- The epothilones inhibited the binding of radiolabeled paclitaxel to microtubules.

Microtubule Stabilizing Agents – Discodermolide

- Isolated from the marine sponge *Discodermia dissoluta*.
- Arrests the cell cycle of treated cells in G₂/M phase.
- The agent was more potent in microtubule stabilization than Taxol under a variety of reaction conditions.
- Discodermolide stabilized microtubules were morphologically distinct (shorter) from those resulting from their Taxol stabilized counterparts.

Tubulysins: Potent Antimitotic Agents from *Archangium gephyra*, and *Angiococcus disciformis*

Tubulysin A  \( R = \text{CH}_2\text{CH(CH}_3)_2 \quad R' = \text{OH} \)
Tubulysin B  \( R = \text{CH(CH}_3)_2 \quad R' = \text{OH} \)
Tubulysin C  \( R = \text{CH}_2\text{CH}_3 \quad R' = \text{OH} \)
Tubulysin D  \( R = \text{CH}_2\text{CH(CH}_3)_2 \quad R' = \text{H} \)
Tubulysin E  \( R = \text{CH}_2\text{CH}_3 \quad R' = \text{H} \)
Tubulysin F  \( R = \text{CH}_2\text{CH}_3 \quad R' = \text{H} \)

Cytotoxicities of Various Selected Tubulin Inhibitors

Cytotoxic activity of tubulin inhibitors for sensitive and resistant cell lines (IC$_{50}$ ng/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>L929$^a$</th>
<th>K562$^b$</th>
<th>KB–3.1$^c$</th>
<th>KB–VI$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxol</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>150</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>15</td>
<td>6</td>
<td>7</td>
<td>120</td>
</tr>
<tr>
<td>Epothilone B</td>
<td>0.7</td>
<td>0.3</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Dolostatin 10</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Tubulysin A</td>
<td>0.2</td>
<td>0.07</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Tubulysin B</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Tubulysin D</td>
<td>0.03</td>
<td>0.02</td>
<td>0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Tubulysin E</td>
<td>0.1</td>
<td>0.05</td>
<td>0.03</td>
<td>0.1</td>
</tr>
</tbody>
</table>

$^a$ Fibroblast cell line from connective tissue of a mouse.
$^b$ Human myelogenous leukemia cell line.
$^c$ Human cervix carcinoma cell line.
$^d$ Multidrug-resistant KB clone.

Degradation Studies: Total Acidic Hydrolysis of Tubulysin D

6N HCl

(R)-N-methyl piperolinic acid

(S)-isoleucine

formaldehyde

H2C=O

3-methylbutyric acid

tubuphaline (1:1 mixture)

Pure Appl. Chem. 2003, 75, 167-178
Determination of the Absolute Stereochemistry of the Tubuvaline Subunit

Pure Appl. Chem. 2003, 75, 167-178
Determination of the Absolute Stereochemistry of the Tubuphenylalanine Subunit

**Chemical Reaction Diagram**

1. LiAlH₄
2. Swern oxidation
3. Ph₃P=CH(CH₃)COOMe

**Compounds**

- **BocHN**
- **H₃C**
- **O**
- **OMe**
- **BocN**
- **CH₃O**

**Complex Molecular Structure**

**Reference**


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Dolostatin 10 and Tubulysin D: An Interesting Comparison

Dolostatin 10

Tubulysin D