Total Synthesis and Biological Evaluation of C16 Analogs of (−)-Dictyostatin


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What is Dictyostatin?

- It is a potent anticancer agent that was discovered from a marine sponge of the genus *Spongia* over a decade ago.

- It is one of the most potent microtubule stabilizing agents discovered to date.
Dictyostatin (cont.)

- It rapidly became admired with the discovery that its structure shared great similarity to that of the anticancer agent discodermolide, which was a clinical candidate for cancer chemotherapy due to its high potency in microtubule stabilization and its strong activity against multiple drug resistant cancers.

  - Unfortunately, discodermolide only made it to Phase II clinical trials when tested in humans, where it failed due to unexpected toxicity.
Dictyostatin – Structurally quite similar to discodermolide and also has very high affinity for the taxoid binding site on tubulin.

A detailed NMR study showed dictyostatin to have the given structure, which shares identical configurations at all common stereocenters with discodermolide.

Discodermolide is a very promising potent agent with high affinity for the taxoid binding site, but failed clinical trials.

\(\text{YSS631} \quad (-)\text{-dictyostatin}\)

\(\text{(+)-discodermolide}\)
So... Why dictyostatin?

- It has proven to be somewhat more active than the already very active discodermolide.
- It has been identified as potent microtubule-stabilizing agent (MSA), which binds to the taxoid binding site on beta-tubulin.
- It might be less toxic than discodermolide.
- It is a promising antimitotic natural product drug lead for cancer chemotherapy development.

The isolated stereocenter at C16 of dictyostatin (red) is of special interest because discodermolide does not have the corresponding stereocenter (blue).

Instead, discodermolide has a C13-C14 Z-alkene. Note that the carbon backbone of dictyostatin is 2 atoms longer than that of discodermolide, so C13 and C14 of discodermolide correspond to C15 and C16 of dictyostatin.

Also, the bottom chain of dictyostatin, the C1-C9 region (purple), is important because there are many active analogs of discodermolide with modifications in that part of the molecule.
Studies have demonstrated that dictyostatin arrests cells in the G2/M phase of the cell cycle.

Image from: http://www.physiomics-plc.com
Microtubules

- polymers of α and β tubulin heterodimers
  - (+) end (β subunit exposed)
  - (−) end (α subunit exposed)

Functions:
- Movement
- Intracellular transport
- Cytoskeleton support

Image from: www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/microtub.htm#animat
Drugs with Activity Against Microtubules

Microtubules are essential in the function and structure of cells and in cell division.

Discodermolide and dictyostatin hyper-stabilize microtubules by binding to the β-tubulin of the microtubule and prevent the disassembly from the (−) end.

Therefore, they prevent the disassembly of microtubules that is crucial for cell division.
Biological Evaluations

- Minimum detectable effective concentration (MDEC) of the test agent in HeLa cells after 21 h of continuous exposure.
- This is the concentration of the test agent necessary to cause a detectable change in tubulin polymer mass.
- This is the lowest (best looking) # of importance, because it is an indication that the analog is a microtubule stabilizer.

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>MDEC for Tubulin Polymer Increase, nM ± SD (N)</th>
<th>GI₅₀,₈ nM (fold-resistance) (N = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.4 ± 1.9 (4)</td>
<td>0.69 ± 0.80</td>
</tr>
<tr>
<td>2</td>
<td>65 ± 0 (2)</td>
<td>1.7 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>29 ± 21 (2)</td>
<td>3.7 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>25 ± 9 (3)</td>
<td>0.41 ± 0.52</td>
</tr>
<tr>
<td>5</td>
<td>1278 ± 181 (3)</td>
<td>61 ± 6</td>
</tr>
<tr>
<td>Secco-5^a</td>
<td>nd</td>
<td>9140 ± 3290</td>
</tr>
<tr>
<td>6</td>
<td>11 ± 2 (3)</td>
<td>8.3 ± 0.8</td>
</tr>
<tr>
<td>27</td>
<td>&gt; 5000 (3)</td>
<td>7800 ± 1410</td>
</tr>
<tr>
<td>53</td>
<td>647 ± 106 (4)</td>
<td>210 ± 110</td>
</tr>
<tr>
<td>54</td>
<td>&gt; 5000 (1)</td>
<td>4260 ± 400</td>
</tr>
<tr>
<td>55</td>
<td>&gt; 5000 (1)</td>
<td>&gt; 50000</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>5.2 ± 0.4 (4)</td>
<td>0.71 ± 0.11</td>
</tr>
</tbody>
</table>

- Fifty percent growth inhibitory concentration (GI₅₀) in 1A9 cells after 72 h of continuous exposure.
- This is the concentration of the test agent that decreases the growth of the cell culture by 50% as compared to an untreated culture.
- Note: Lower concentration = a more potent analog.
Both 16-normethyl-15, 16-dehydrodictyostatin (6), and 16-normethyldictyostatin (4) were effective in the low nanomolar range, comparable to dictyostatin 1, 14-normethyldiscodermolide 3, and paclitaxel.
1A9/Ptx10 cells have a Phe270->Ala mutation in the taxoid binding site of β-tubulin.

Note the very large cross resistance of the 1A9/Ptx10 cell lines towards compounds 4, 6 and 53. Also be aware of the surprising fact that 14-normethyldiscodermolide (a direct analog of structure 4) experienced no cross-resistance in this cell line...

...this revealed some potentially telling clues about the orientation of the dictyostatin macrocyclic core w/in the binding site.

Specifically, it suggests the possibility that the dictyostatins and discodermolides may not adopt the same orientations w/in the taxoid binding site.
Conclusions

- Total synthesis of multimilligram quantities of the C16 analogs of (−)-dictyostatin was achieved by a versatile synthetic strategy.
- 16-Normethyldictyostatin and the C16-normethy-C15-Z analog had biological activities near those of the parent compound.
- 14-Normethyldiscodermolide (a direct analog of 16-normethyldictyostatin) did not experience the same cross-resistance in a cell line where an amino residue is mutated in the taxoid binding site, suggesting that the dictyostatins and discodermolides may not adopt exactly the same orientations when bound to the protein.
Thank You