Computational and Molecular Modeling
Evaluation of the Structural Basis for Tubulin Polymerization Inhibition by Colchicine Site Agents

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Changes in Microtubules during Mitosis

http://petrus.ncl.ac.uk/urchins/tubulina.html
http://www.wadsworth.org/BMS/SCBlinks/mcewen/dynamics.htm
Surface that faces the inside of the MT

GTP bound at the intradimer interface in $\alpha$
never hydrolysed

GTP bound at the plus end of the MT in $\beta$
($=$ exchangeable site, E site)
hydrolysed during assembly

Catalytic residue on $\alpha$ activates
GTP hydrolysis on $\beta$

Current Opinion in Structural Biology
8: 785 (1998)
CASE and MultiCASE

**CASE**
- Subdivides the molecule into 2-10 continuous non-H atoms
- These substructures were used to calculate TPI activity

**MultiCASE**
- Identifies substructural fragments of a group of chemicals
- Invokes modulators
- MultiCASE programs can also predict potency
The Learning Set

- Consists of 536 chemicals tested for TPI activity
- Their potency and chemical structures were obtained from literature or generated in this study
- The chemicals analyzed generated 202 active, 307 inactive, and 27 marginally active fragments.
Table 1. Major CASE biophores associated with the inhibition of tubulin polymerization

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CH&quot;—CH =C. —C. =</td>
<td>58</td>
<td>9</td>
<td>1</td>
<td>48</td>
<td>&lt;0.001 + + +</td>
</tr>
<tr>
<td>2. CH₂—CH₂—CH —C. =</td>
<td>44</td>
<td>3</td>
<td>1</td>
<td>40</td>
<td>&lt;0.001 + + +</td>
</tr>
<tr>
<td>3. CO —O —CH₂—CH —CH₂—</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0.031 + + +</td>
</tr>
<tr>
<td>4. CH₂—CH₂—CH —C. =CH —</td>
<td>&lt;3-NH&gt;</td>
<td>41</td>
<td>2</td>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>5. N —C =CH —CH =C —CH =</td>
<td>30</td>
<td>4</td>
<td>2</td>
<td>24</td>
<td>&lt;0.001 + + +</td>
</tr>
<tr>
<td>6. OH —C =C —CH =CH —C&quot; —CH₂—</td>
<td>&lt;3-O&gt;</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>7. CH₂—O —C. =CH —C =C —CH =</td>
<td>&lt;5-O&gt;</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>8. O —C. =CH —C =C —CH =C —O —</td>
<td>&lt;4-O&gt;</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>9. NH —CH —C. =CH —CO —C =CH —CH =</td>
<td>&lt;6-S&gt;</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>10. CH₃—O —C =C —CH =C —CH =CH —C =</td>
<td>24</td>
<td>5</td>
<td>1</td>
<td>18</td>
<td>0.003 + + +</td>
</tr>
<tr>
<td>11. CH —CH —CH =CH —C =C —NH —C. =CH —</td>
<td>&lt;5-CH═&gt;</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>12. CH =CH —CH =CH —CH =CH —C =C&quot; —NH —C. =CH —</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>0.033 + + +</td>
</tr>
</tbody>
</table>

*C. Indicates a carbon atom common to two rings. C" Indicates a substituted carbon. SUB indicates a substituent on the biophore, e.g. <3-NH> is an NH on position 3 from the left. The table lists the number of times the fragment was encountered in the learning set and its distribution among active, inactive and marginally active molecules. This distribution was used to predict the likelihood that the presence of that fragment contributes to the inhibition of tubulin polymerization. Also listed in the table are the probability values associated with the significance of these distributions: + + + , $p < 0.01$ (F-test). Localization of the fragments in selected molecules are shown in Figure 1.
<table>
<thead>
<tr>
<th>Fragment</th>
<th>No. of Fragm.</th>
<th>Inact.</th>
<th>Marg.</th>
<th>Active</th>
<th>Average IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CH₂=CH₂=CH=CH=C=CH</td>
<td>41</td>
<td>2</td>
<td>1</td>
<td>38</td>
<td>3.0 ++ +</td>
</tr>
<tr>
<td>2. CH₃-N=C=CH=CH=CH=C=CH=C=CH=C</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>5.6 ++ +</td>
</tr>
<tr>
<td>3. CH₂-O-C=CH=CH=CH=C=CH=C=CH=C=CH=C</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>5.1 ++ +</td>
</tr>
<tr>
<td>4. CH₃-O-C=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>22</td>
<td>4</td>
<td>1</td>
<td>17</td>
<td>5.9 ++ +</td>
</tr>
<tr>
<td>5. OH-C=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>5.4 ++ +</td>
</tr>
<tr>
<td>6. CO-O-CH₂=CH=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2.1 ++ +</td>
</tr>
<tr>
<td>7. CH=CH=CH=CH=C=CH=C=CH=C-JH=C=CH=C</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>6.6 ++ +</td>
</tr>
<tr>
<td>8. CH₃-CH₂=O-C=CH=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>5.1 ++</td>
</tr>
<tr>
<td>9. OH-C=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5.9 +</td>
</tr>
<tr>
<td>10. O-CH₂-O-C=CH=CH=C=CH=C=CH=C=CH=C</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>5.0 ++ +</td>
</tr>
<tr>
<td>11. O-C=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>12. O-CH₂-O-C=CH=CH=C=CH=C=CH=C</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>4.2 ++</td>
</tr>
<tr>
<td>13. S-C=CH=CH=CH=C=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>29</td>
<td>6</td>
<td>0</td>
<td>23</td>
<td>4.5 ++ +</td>
</tr>
<tr>
<td>14. NH-C=CH=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>9.3</td>
</tr>
<tr>
<td>15. CH=C=CH=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>5.3</td>
</tr>
<tr>
<td>16. O-C=CH=CH=CH=C=CH=C=CH=C=CH=C=CH=C</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5.0 ++ +</td>
</tr>
<tr>
<td>17. C=CH=CH₃-C=CH=C=CH=C=CH=C</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6.5 ++ +</td>
</tr>
</tbody>
</table>

* Indicates a carbon atom common to two rings. C\textsuperscript{\textprime} Indicates a substituted carbon. SUB indicates a substituent on the biophore, e.g. \textless 3-NH\textgreater is an NH on position 3 from the left. The table lists the number of times the fragment was encountered in the learning set and its distribution among active, inactive and marginally active molecules. This distribution was used to predict the likelihood that the presence of that fragment contributes to TPI activity: +, \( p < 0.125 \); ++, \( p < 0.05 \); +++, \( p < 0.01 \) (F-test). Also listed in the table are the average IC\textsubscript{50} values of the molecules containing the given fragment. Localization of fragments in selected molecules are shown in Figure 2.
Figure 1. Examples of the 12 biophores (bold) identified by CASE: 1, 2, 4: colchicine; 3: deoxypodophyllotoxin; 5: E-1-(2,5-dimethoxy-phenyl)-3-[4'-(dimethylamino)-phenyl]-2-methyl-2-propen-1-one; 6: dihydrocombretastatin A-4; 7, 8: NSC 350102; 9: thiocolchicine; 10: combretastatin A-4; 11, 12: 6-methoxy-2-phenyl-4-quinolone. Note: known stereochemistry of the chemicals is intentionally not shown in Figures 1–5 and 7 (see second paragraph of Results).
Figure 2. Examples of the 17 biophores (bold) identified by MultiCASE: 
1: colchicine; 2: E-1-(2,5-dimethoxyphenyl)-3-[4′-(dimethylamino)-phenyl]-2-methyl-2-propen-1-one; 
3: NSC 321567; 4: combretastatin A-4; 5: dihydrocombretastatin A-4; 6: deoxypodophyllotoxin; 
7: 6-methoxy-2-phenyl-4-quinolone; 8: E-1-(23,4-trimethoxyphenyl)-3-[4′-(diethylamino)phenyl]-2-methyl-2-propen-1-one; 
9: podophyllotoxin; 10: deoxypodophyllotoxin; 11: 6-methoxy-2-strylyquinazolin-4(3H)-one; 
12: combretastatin A-2; 13: thiolepho; 14: N-(3′,4′,5′-trimethoxybenzyl)-4-methoxylaniline; 15: 
6,7-dimethoxy-2-phenyl-4-quinolone; 16: E-1-(3,4,5-trimethoxyphenyl)-3-[4′-(dimethylamino)-phenyl]-2-methyl-2-propen-1-one; 
17: E-1-(2,3,4-trimethoxyphenyl)-3-[4′-(dimethylamino)-phenyl]-2-propen-1-one.
QSARs of CASE and MultiCASE Biophores

- QSAR of Colchicinoids
- QSAR of Podophyllotoxin
- QSAR of Combretastatin
Figure 3. The MultiCASE and CASE biophores present in colchicinoids, podophyllotoxins, and combretastatins. The lower right of the figure depicts the four modulators of the MultiCASE biophore present in dihydrocombretastatins. Modulator 1 increased the predicted potency (i.e. decreased predicted IC₅₀ by 1.05 μM) when present. The other three modulators decreased the predicted potency.
Figure 4. The computationally predicted potencies of the colchicinoids and podophyllotoxins in the learning set versus their experimental potencies obtained from literature sources. For the 59 colchicinoids (A), the correlation ($R^2$) was 0.85. For the 21 podophyllotoxins (B), $R^2$ was 0.87.
Discussion

- Podophyllotoxin, steiganacin, combretastatin A-2, and A-4 all competitively inhibit binding of colchicine.

- The trimethyloxyphenyl moiety
Major goal of this Work

- The screening of 5000 chemicals

- Does the computational selection of paclitaxel and discodermolide indicate unsuspected overlap or coincidence of the binding sites for these polymers stabilizing agents with the colchicine binding site?
**Figure 7.** Localization of the common MultiCASE biophore (bold) invoked to predict potent antitubulin activity for discodermolide and paclitaxel. The same biophore was found to be statistically significant in one colchicinoid, stegancin, and was located in its acetate side chain. Those marked with an asterisk (*) were accompanied by warnings due to being in an environment different from the biophore identified from the learning set.
536 Known Tubulin Polymerization Inhibitors with Diverse Structures “Learning Set”

MultiCASE/CASE QSAR Equations

(All other known microtubule stabilizing agents correctly predicted...)

5000 Chemical Database of the “Chemical Universe”

- Inhibits growth of paclitaxel-resistant (altered β-tubulin expression) and multidrug resistance P-glycoprotein overexpressing cells
- Induces apoptosis
- >100-fold more H₂O-soluble than paclitaxel
- Synthetically accessible (but not easy) QSARs provide clues for simpler, more potent analogs

Discodermid dissoluta
500 ft, Caribbean Sea

- Inhibits mitosis
- Induces spectacular microtubule bundling
- Causes microtubule assembly under conditions in which paclitaxel is inactive
- Most potent known microtubule stabilizing agent
- Competitively inhibits binding of paclitaxel to microtubules
- Enhances tubulin nucleation reactions more potently than paclitaxel

(+)-Discodermolide

Balachandran et al., *Breast J.* 1998, 4, 409-419.
Balachandran et al., *Anti-Cancer Drugs* 1998, 9, 67.
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