The T-Taxol Conformation

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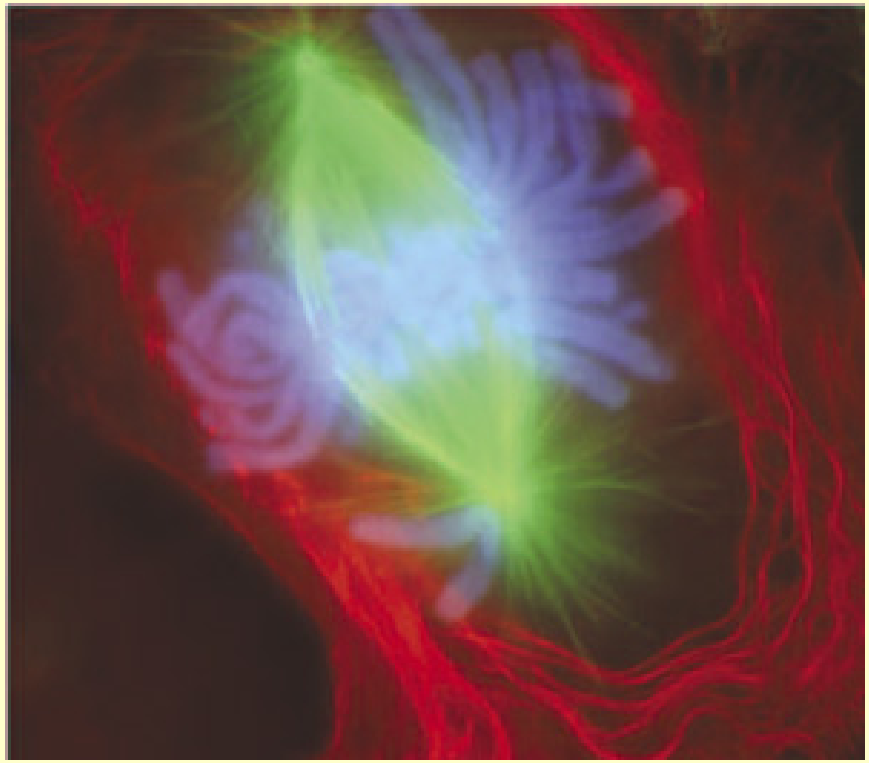
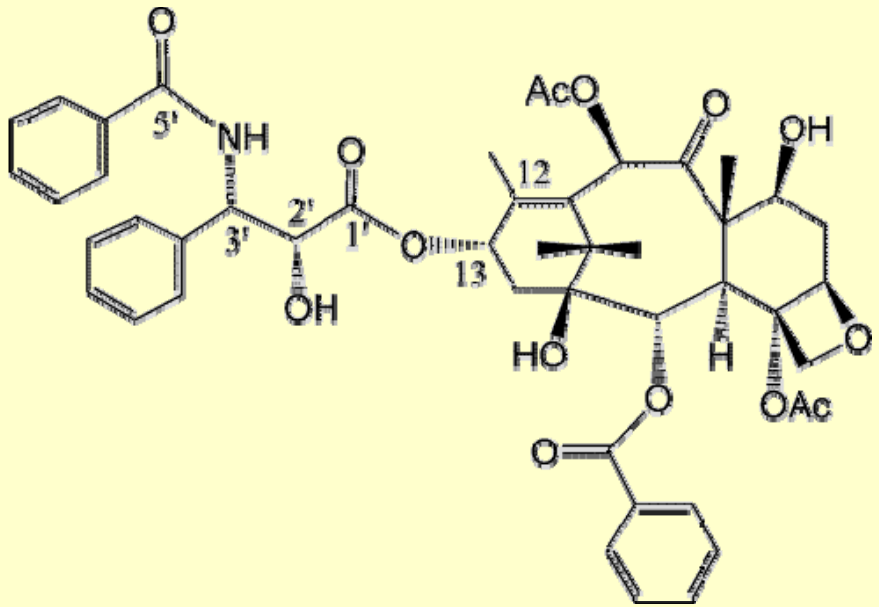
What Is Paclitaxel?

- Mitosis requires microtubules that form the mitotic spindle.
- Microtubules must be dynamic (able to grow and then disassemble).
- Microtubules consist of polymerized strands of dimers of α-tubulin and β-tubulin.



What Is Paclitaxel?

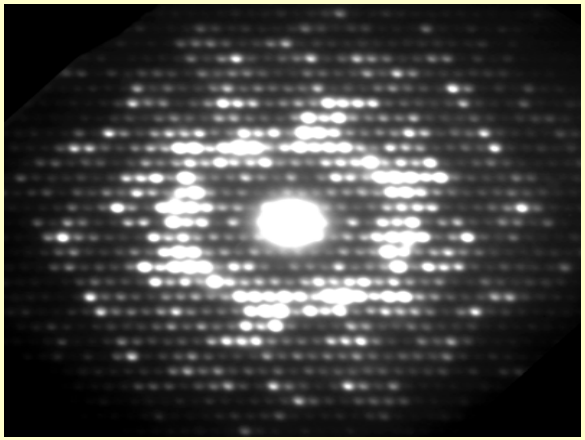
- Paclitaxel (PTX) is a complex organic compound that binds to ***β***-tubulin.
- The binding of paclitaxel stabilizes bonds between tubulin dimers, preventing disassembly of microtubules.

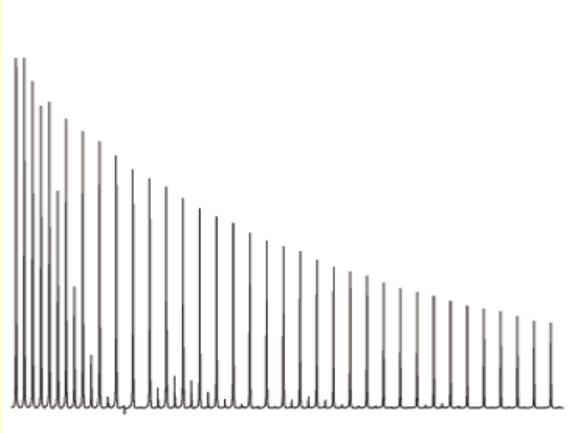


Why Is Paclitaxel Important?

- Prevents separation of duplicated chromosomes, which involves disassembly of microtubules that form the mitotic spindle
- Stops mitosis, ultimately leading to apoptosis (programmed cell death)
- Destroys tumor cells much more quickly than healthy cells, because the tumor cells divide very rapidly and their microtubules are more dynamic than most normal cells
- Approved since 1992 (under trade name Taxol®) for treatment of ovarian cancers; now also used in breast and lung cancers.

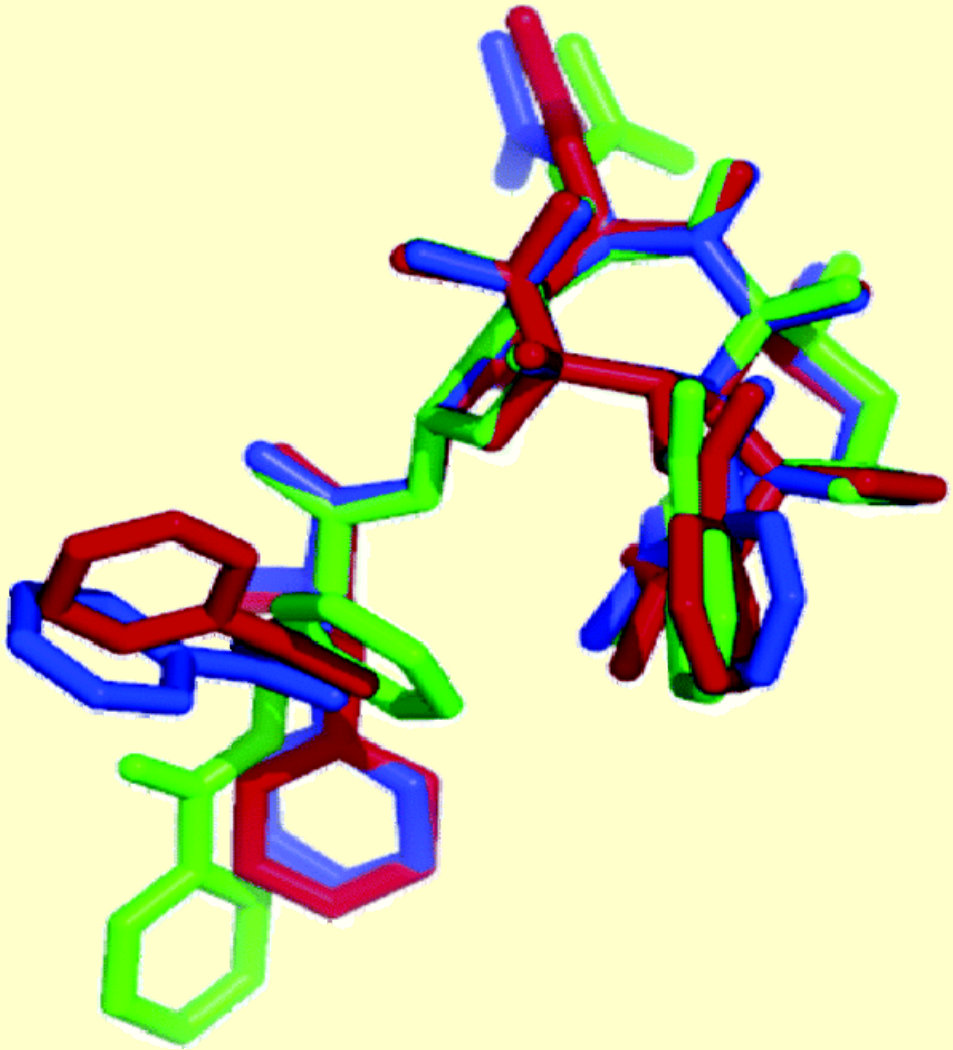
The Problem





- Lack of high-resolution model of the conformation of bound PTX despite cryoelectron crystallography and nuclear magnetic resonance studies
- Better understanding of bound conformation could help rational design/discovery of other anti-tumor agents with similar modes of action.

Background



Several Possible Conformations Proposed for PTX Bound to beta-tubulin Active Site:

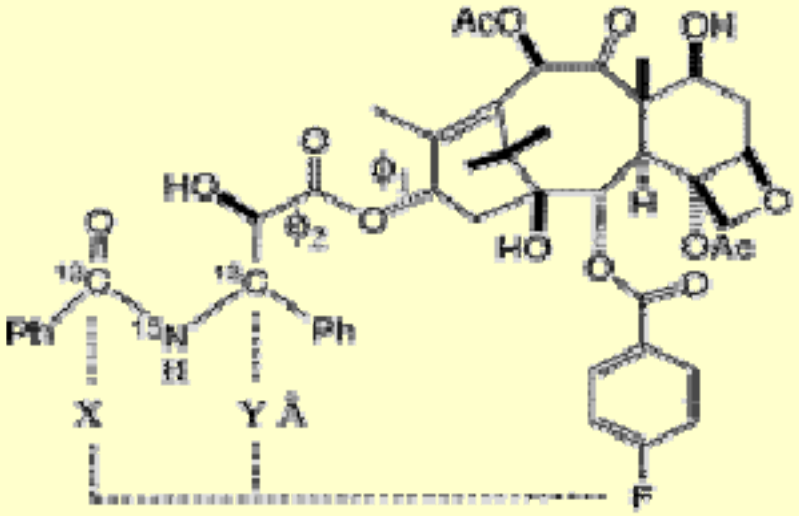
- Polar (Green)
- 1JFF (Red)
- T-Taxol (Blue)
- PTX-NY

Background

- Polar model (from first published structural analysis of tubulin-bound PTX) has little support due to the inactivity of bridged analogues that held the molecule in the conformation that it proposed.
- Currently, competing conformational models are T-Taxol, 1JFF (both based on EC analysis and quite similar to one another), and PTX-NY.
- Alcaraz et al. argue that T-Taxol is the best-supported conformational model to date, based on several lines of evidence.

Results of NMR Analysis

- Solid-state NMR experiment carried out by Schaefer, Bane, Kingston measured two intramolecular distances from ^{19}F to two labeled ^{13}C atoms (X,Y: see graphic)
- 9.8Å and 10.3Å, respectively, each with estimated uncertainty of $\pm$ 0.5Å



Conformer	r (X (F---C(O))	Y), Å (F---C-3')	deg Φ_1	Φ_2
REDOR[8]	9.8	10.3		
Polar[8]	10.4	9.6	-101	103
IJFF[11]	8.1	9.3	-94	56
T-Taxol[10]	9.1	9.9	-103	70
T-Taxol (i)	9.9	10.3	-89	70
T-Taxol (ii)	9.8	10.1	-103	82
T-Taxol (iii)	9.8	10.2	-97	76
PTX-NY[13]	9.4	10.0	-160	-100

Monte Carlo Investigation

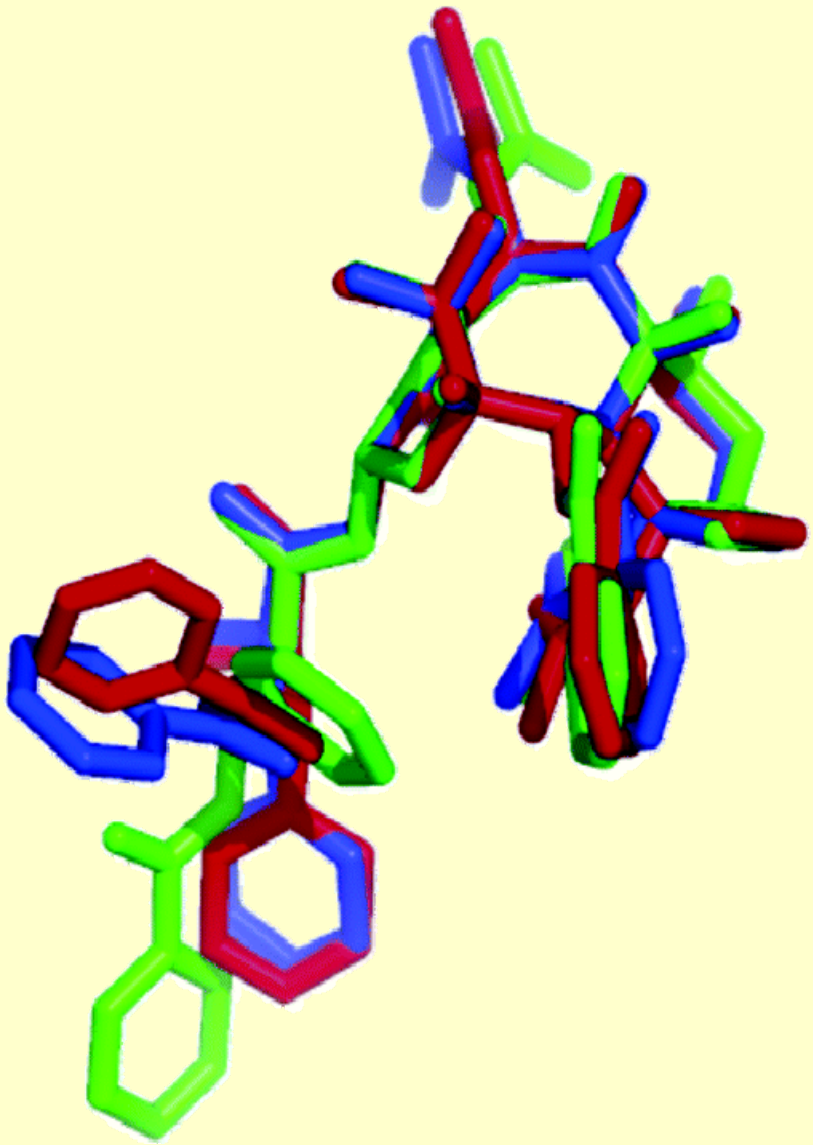
- Alcaraz et al. performed two Monte Carlo conformational searches using molecular mechanics force field methods, locking the X and Y distances at those determined by the NMR analysis
- **After unbound structures were optimized, 84 stable structures were found that matched the NMR-based intramolecular distance measurements to within ± 0.3Å**

Monte Carlo Investigation

- This analysis shows that the NMR measurements do not define PTX's conformation very specifically, because the two side-chain phenyl groups have considerable freedom of movement even within the limitations of the two intramolecular distances defined by the NMR studies.
- This freedom of movement is sufficient to prevent the NMR results from distinguishing between the T-Taxol and polar models.

Comparison to NMR Data

None of the bound conformations strictly match the NMR distances to within the ± 0.5Å error estimate, although T-Taxol and the polar conformation come within ± 0.7Å. 1JFF is less of a match, probably due to the model's relatively low resolution (3.5Å). These observations needed to be explained.



Limitations of the NMR Data

- Molecular dynamics (Tripos force field) simulations of thermal motion revealed that, by small changes in dihedral angles (6-14°), T-Taxol could be brought into agreement with the NMR data.
- Also, motion of labeled atoms (^{13}C and ^{19}F in this case) is known to sometimes weaken dipolar interactions. Since the ^{13}C-^{19}F distances were measured based on these interactions, this may have led to overestimation of these distances.

Limitations of the NMR Data

- The sample of tubulin-bound PTX used in the NMR experiment was lyophilized to remove excess water.
- It is assumed that this leaves a few fairly uniform layers of water molecules surrounding the protein, but there are problems with this assumption.

Limitations of the NMR Data

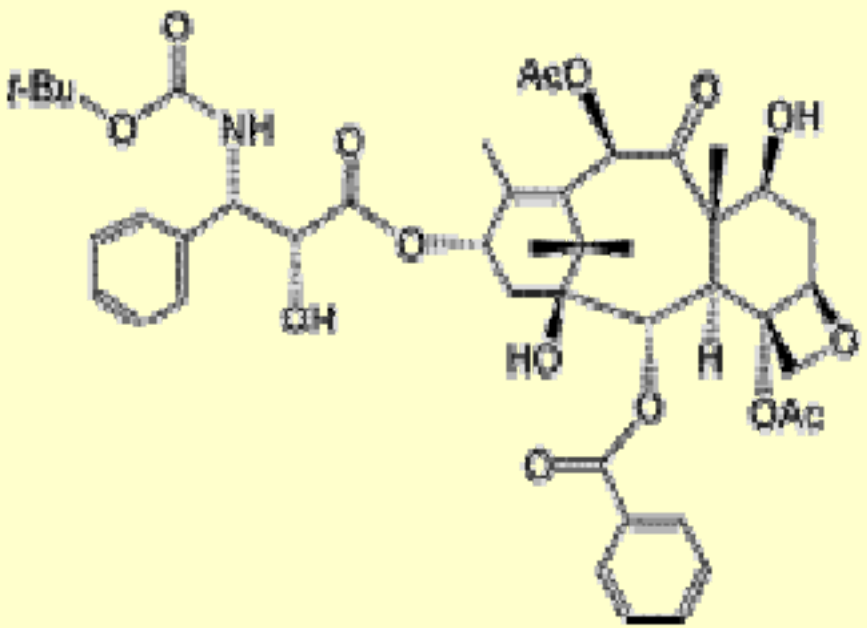
- There is no way to determine the exact amount of water remaining, and the layers may be disrupted by interaction with hydrophobic/ hydrophilic groups.
- Since PTX may share the binding pocket with a small number of water molecules, disruption of the innermost layer could change the way PTX is oriented in the pocket.

Limitations of the NMR Data

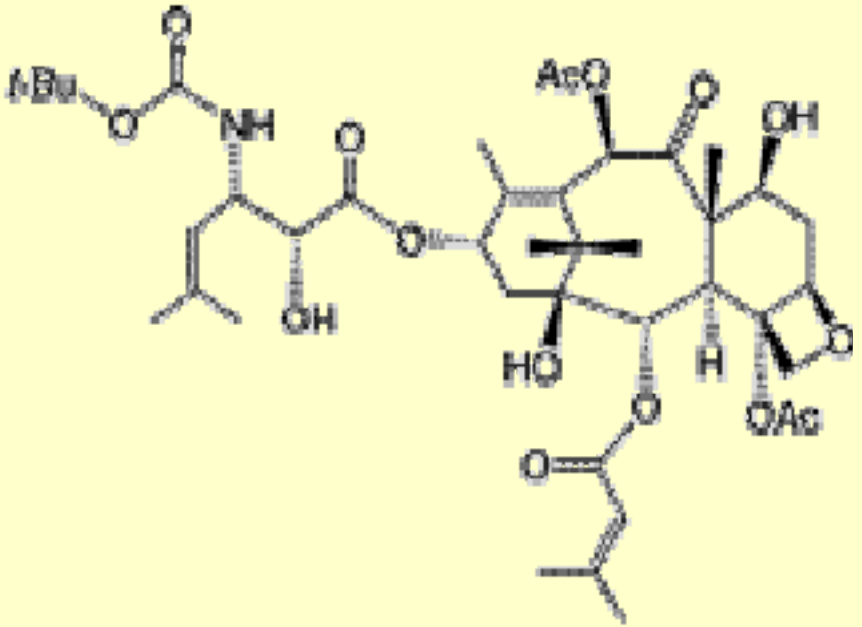
- All of the limitations mentioned thus far were amplified by the fact that only a single measurement was obtained from the experiment due to expense and time constraints (three months/measurement).
- Alcaraz et al. do not argue that these limitations invalidate the NMR results from Schaefer et al., but that the original error estimate was too conservative. Thus, the results do not invalidate the T-Taxol model as some have claimed.

PTX Analogues

- Docetaxel (DTX) and Nonataxel (NTX) are two analogues of PTX in which certain side chains of PTX are modified as shown below.

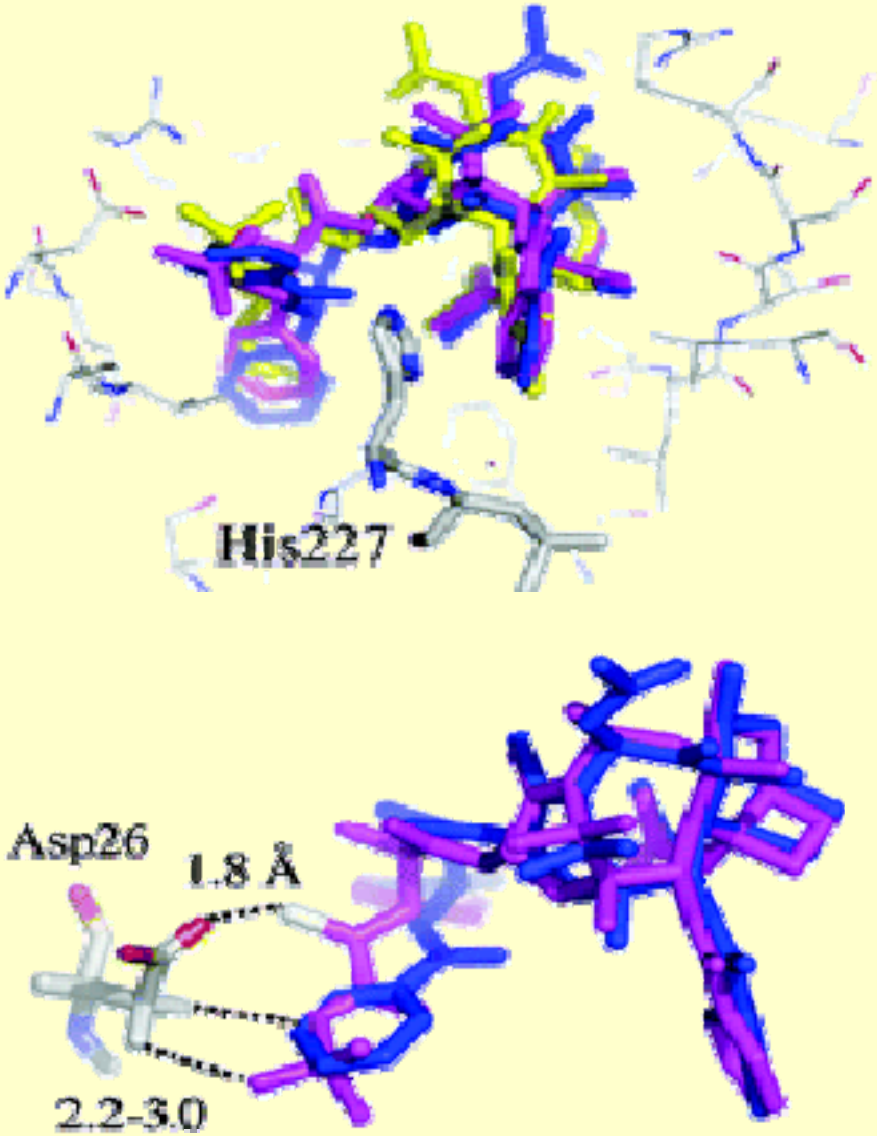


Docetaxel



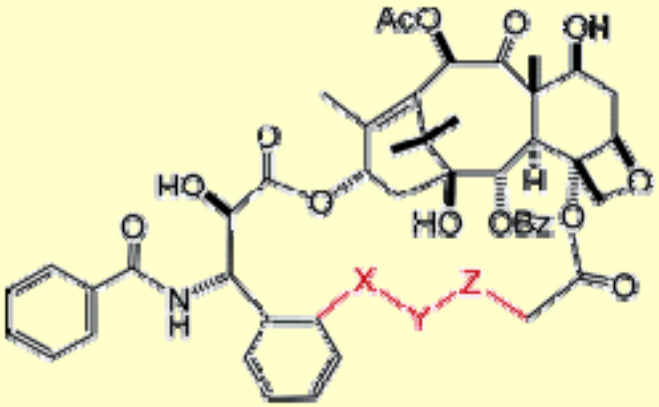
Nonataxel

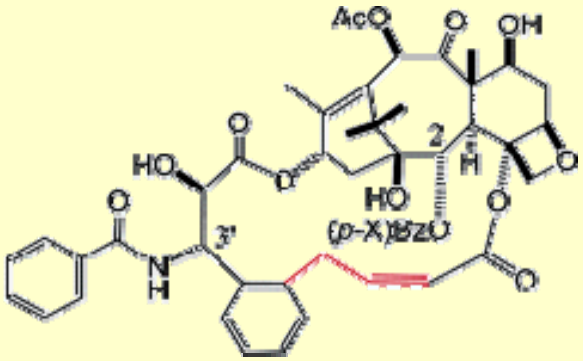
PTX Analogues

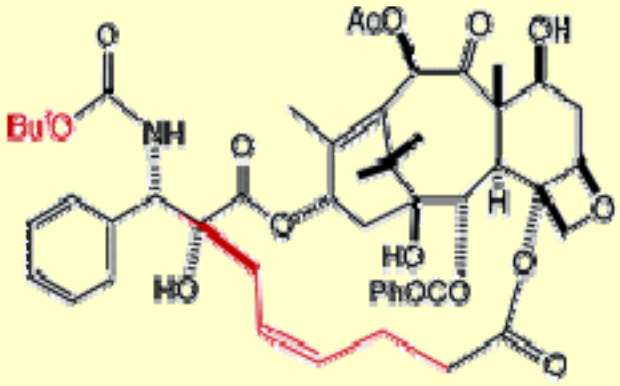


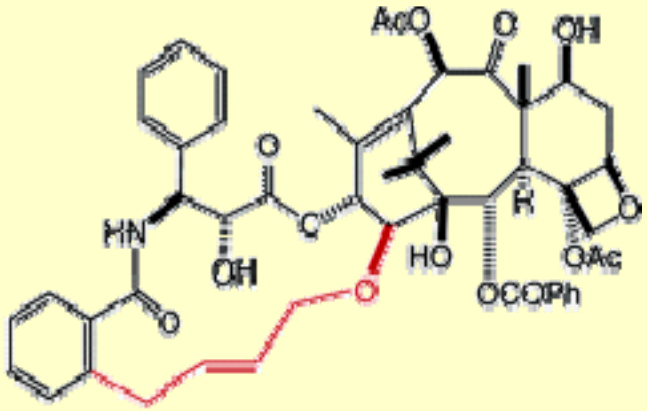
- DTX: 2x as cytotoxic as PTX
- NTX: 2-7x as cytotoxic as PTX
- Molecular modeling of DTX and NTX in T-type conformation shows H-bonding that may explain greater effectiveness.

Bridged PTX Analogues



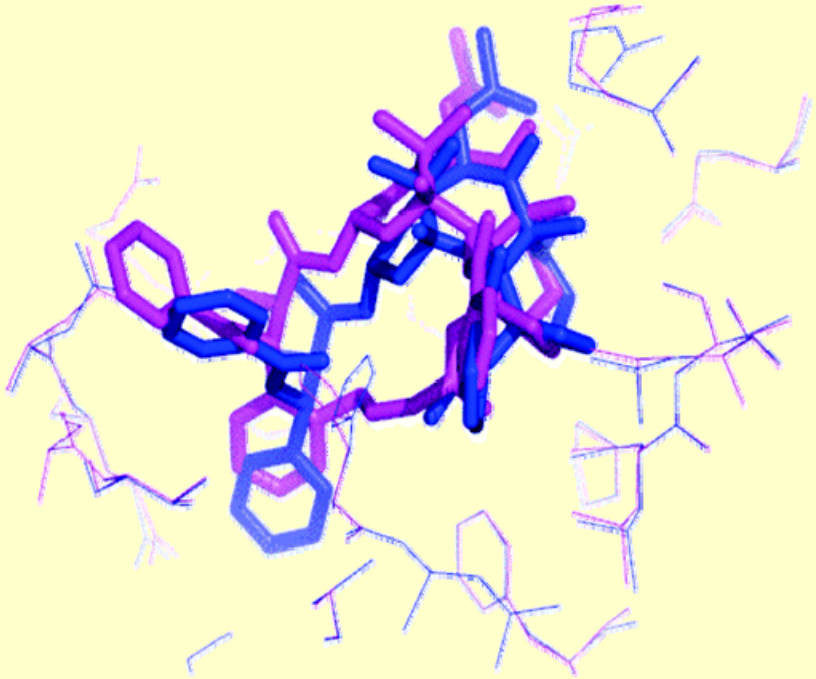


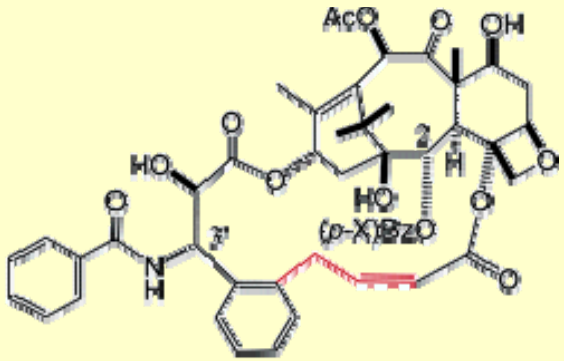




Bridged PTX Analogues

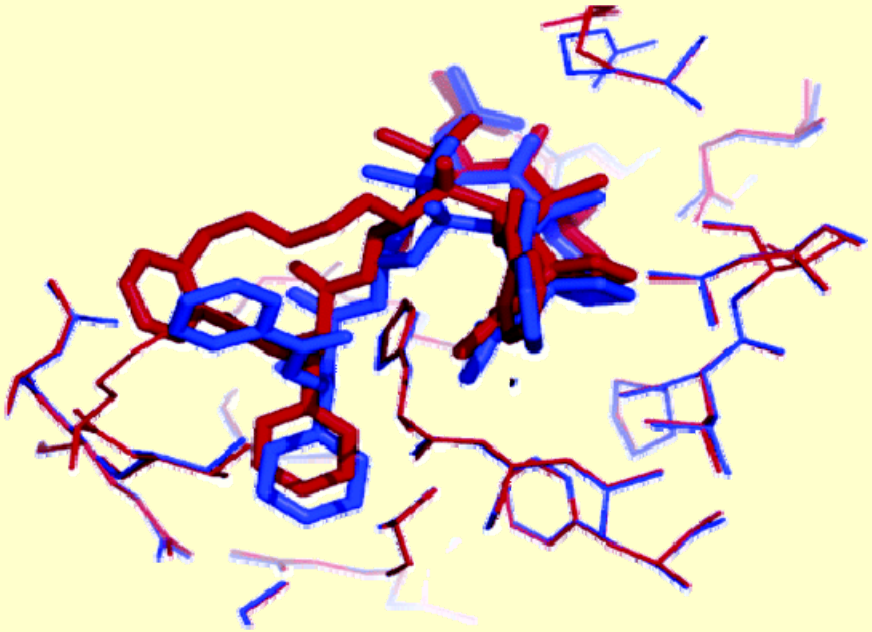
This bridged PTX analogue is up to 20x as effective as PTX and closely resembles the T-Taxol conformation.

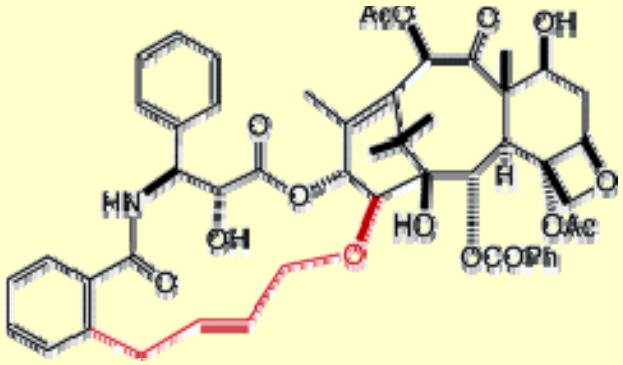




Bridged PTX Analogues

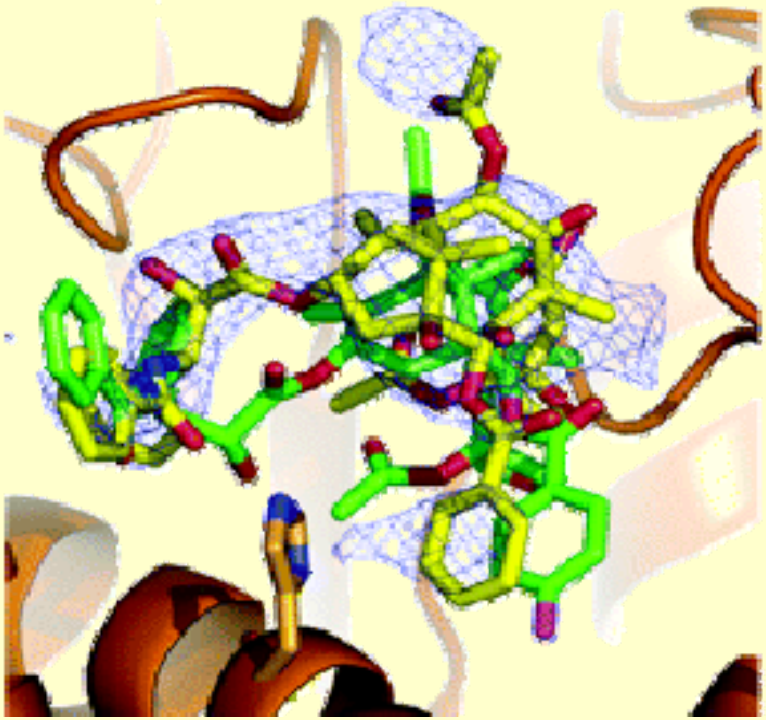
Several PTX analogues based on other models (such as PTX-NY) have been up to 20x less effective than PTX itself in certain cell lines.

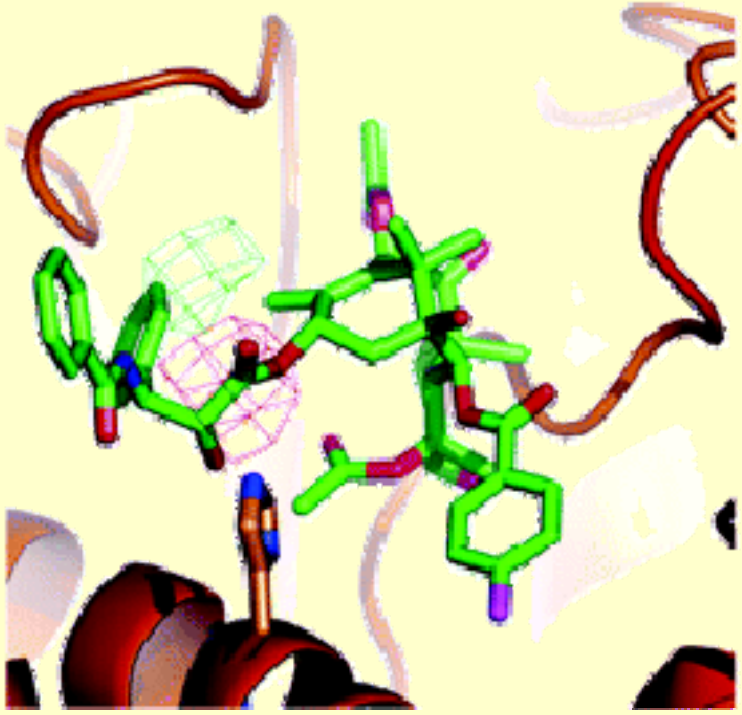




T-Taxol vs. PTX-NY

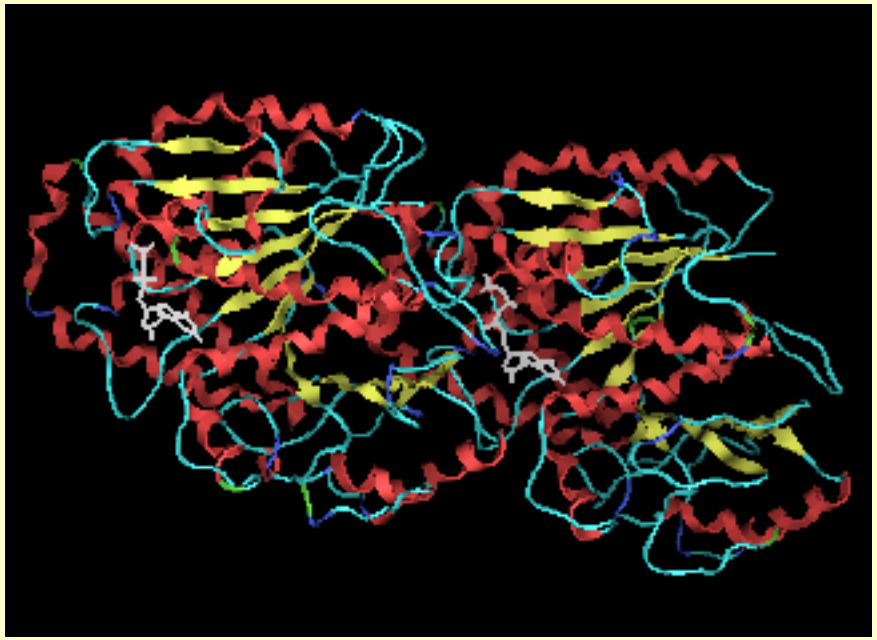
Although the EC density that T-Taxol is based upon has weaknesses in certain areas, it supports the T-Taxol over the PTX-NY conformation in the region in which they differ most.





Conclusions

When considered as a distribution of forms that represent the dynamic structure of paclitaxel, the T-Taxol conformation is a well-supported model with considerable predictive power.



References:

Technical Material:

1. Alcaraz et al., *The T-Taxol conformation. J. Med. Chem.*, 49: 2478-2488 (2006).
2. Hu, Rong; Day, Billy. ***Docking of the Dictyostatin, 16-Normethyldictyostatin, and the Discodermolide, 14-Normethyldiscodermolide, to β-Tubulin. BBSI@Pitt 2005.***
3. ***Nogales, Eva; Whittaker, Michael; Milligan, Ronald A.; Downing, Kenneth H. High-Resolution Model of the Microtubule. Cell, January 1999; 96: 79-88.***

Images:

http://www.nigms.nih.gov/moleculestomeds/images/newtcells.jpg

http://www.varianinc.com/image/vimage/docs/products/nmr/applications/app_solids/shared/900redor-data.gif

http://www.gfe.rwth-aachen.de/sig-ec/weirich/Ti11_c.gif