Docking Studies of the Binding Mode of Dictyostatin and Its Analogues to the Taxoid Binding Site on Beta Tubulin

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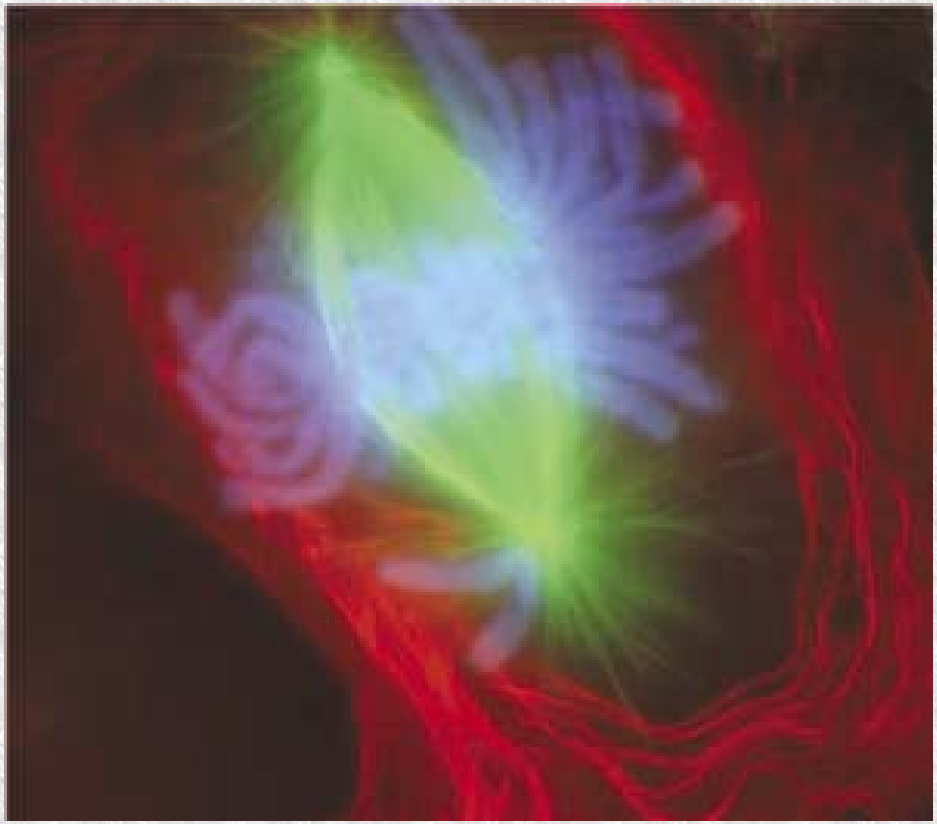
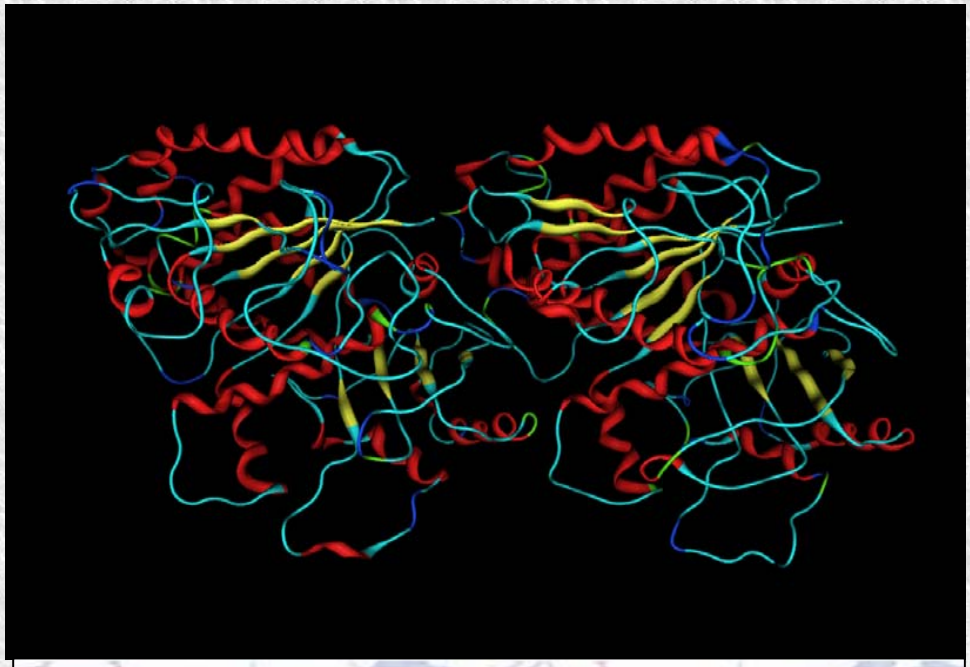
What Are Microtubules?

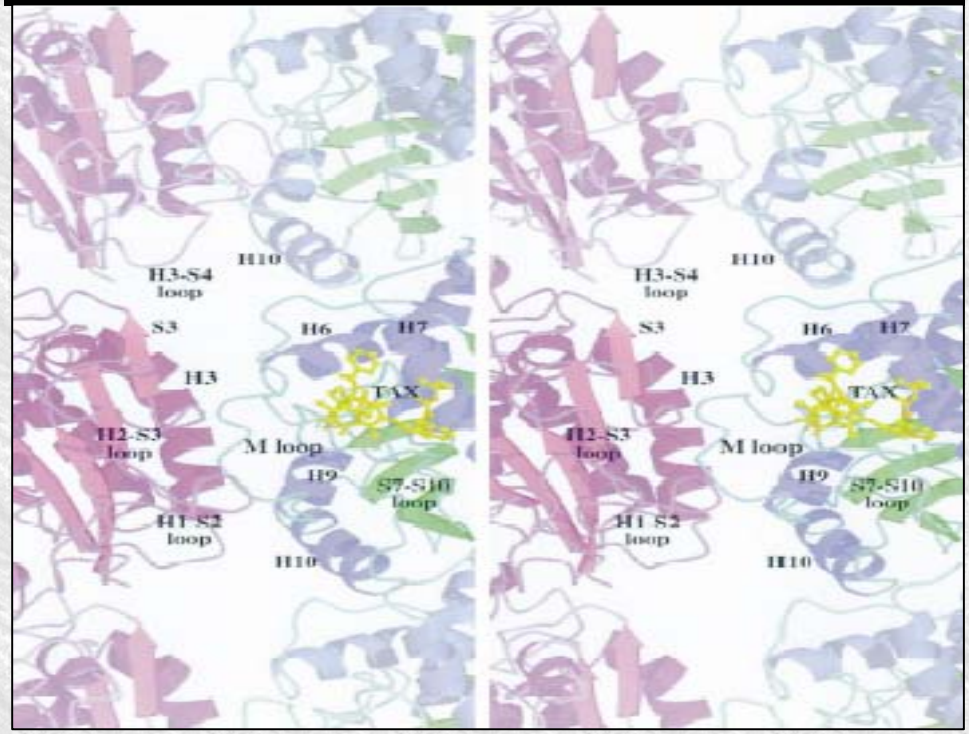
Several Functions:

- Intracellular transport
- Motility
- Structural elements
- **Mitotic spindle**



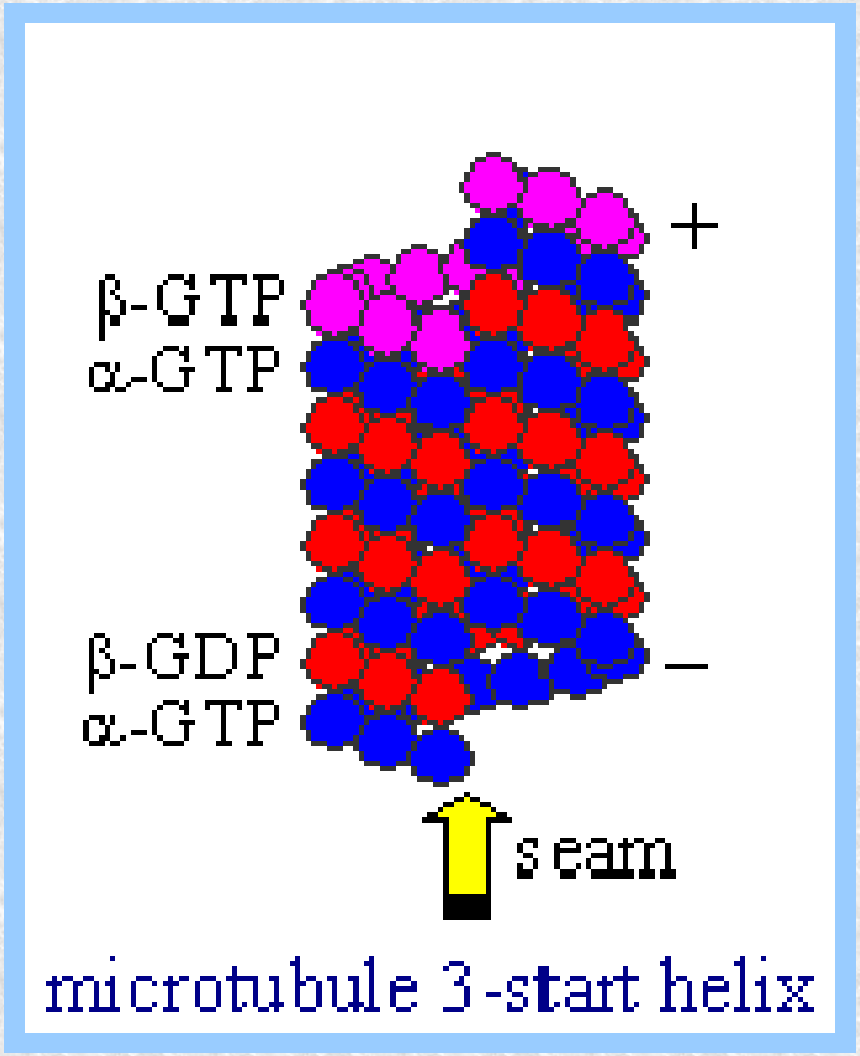
What are Microtubules?





- Protofilaments composed of α,β heterodimers
  - β-tubulin bears nucleotide GDP at interface between heterodimers, α-tubulin bears GTP
- Homogeneous lateral contacts (α,α and β,β) form microtubule walls

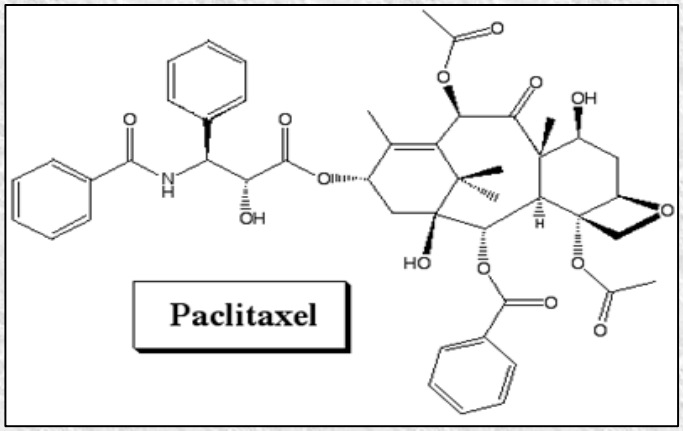
What are Microtubules?

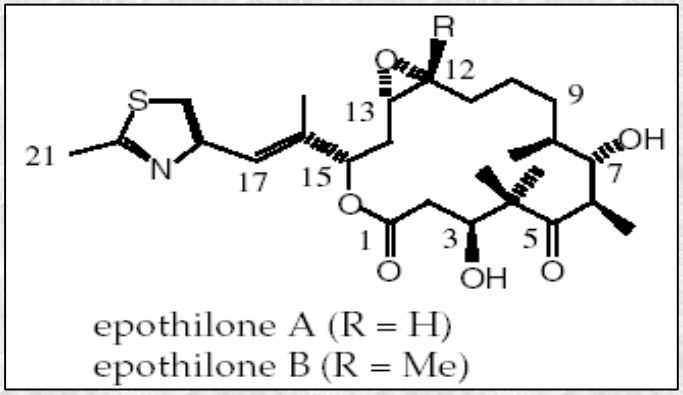


- Left-handed helix with “plus” and “minus” ends
- Minus end is capped with α-tubulin bearing GTP, and is stable
- Plus end is capped with β-tubulin bearing either GTP (stable) or GDP (unstable)

A Brief History of Antimitotic Drugs

Paclitaxel – widely used antimitotic agent approved for treatment of ovarian cancer in 1992

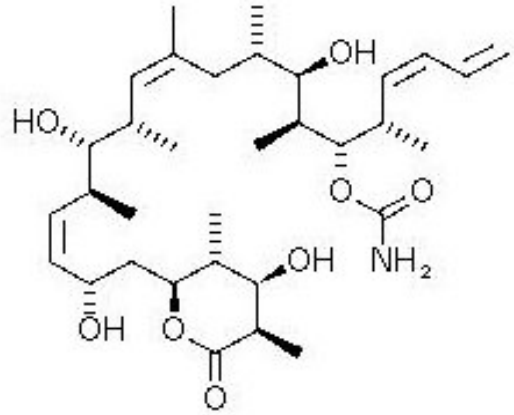




Epothilones – Alternative antimitotic agents with effects similar to paclitaxel but different binding mode

Both agents bind to same pocket in β-tubulin, hyperstabilizing lateral contacts at the plus end by preventing a destabilizing conformational change. This destroys the microtubule's normally dynamic properties, preventing completion of mitosis.

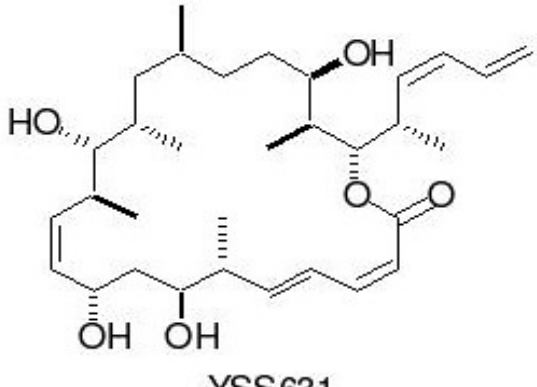
New Antimitotic Agents – Discodermolide and Dictyostatin



(+)-discodermolide

Discodermolide – Very promising, potent agent with high affinity for taxoid binding site, but was deemed dangerous in clinical trials

Dictyostatin – Structurally quite similar to discodermolide and also has very high affinity for taxoid binding site

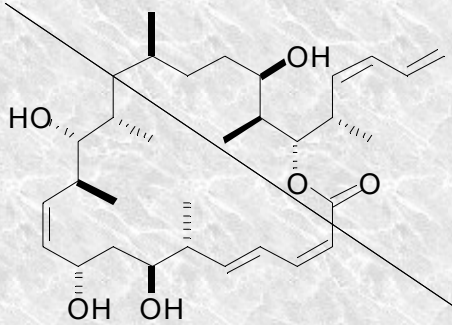


YSS631
(-)-dictyostatin

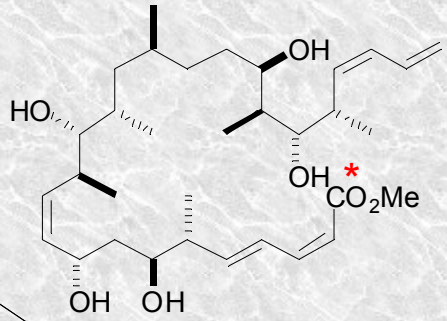
Motivation for Research

- Laboratory data indicate the mutant ovarian carcinoma cell line 1A9/Ptx10 with Phe270->Val mutation in β-tubulin shows cross-resistance to a variety of antimitotic drugs, including dictyostatin and certain dictyostatin analogues.
- Phe270 was therefore suspected to in close contact with parts of these drugs when bound to β-tubulin.
- Data has also been gathered on the potency of dictyostatin and a variety of synthetic analogues.
- Can these data be rationalized and related to structure through computer-simulated docking studies?

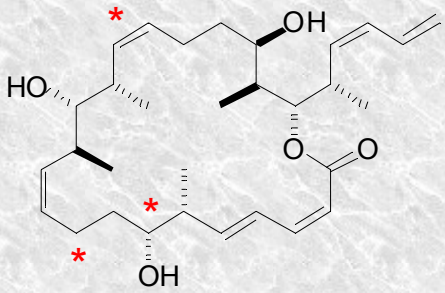
Compounds Under Study



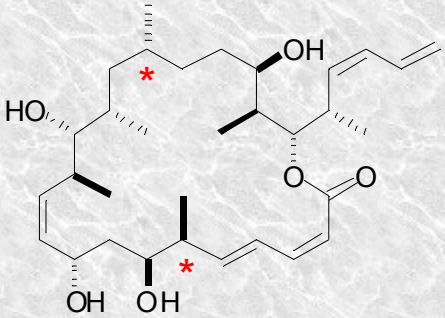
YSS631
(−)-dictyostatin



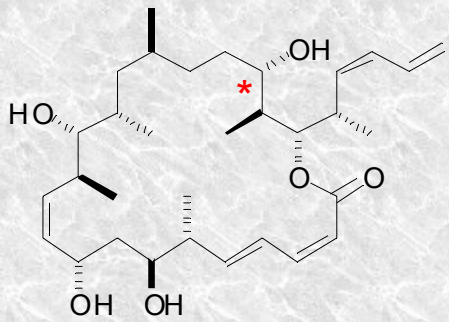
YSS629
open chain methyl ester



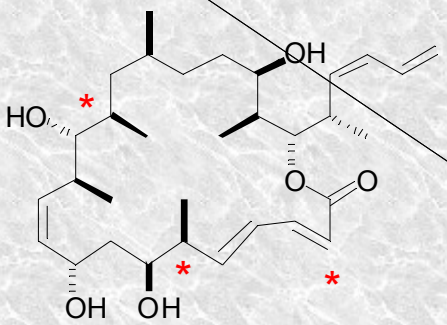
YSS133
C7-*epi*,C9,C16-*bis*-norMe,C15/C16-(*Z*)



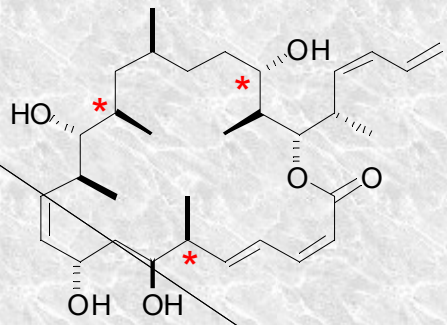
YSS479
C6-*epi*,C16-*epi*



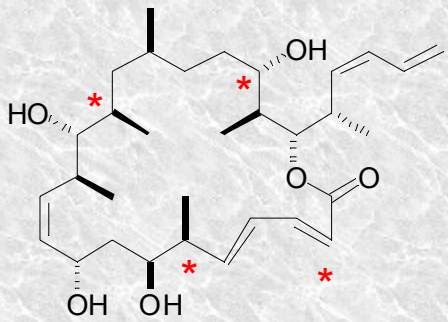
YSS652
C19-*epi*



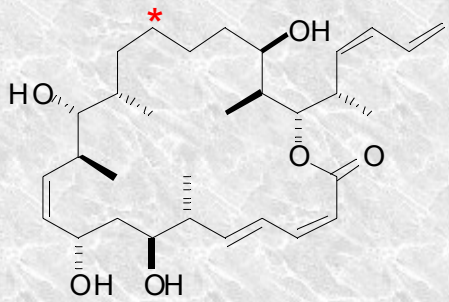
YSS665-2
C2/C3-(*E*),C6-*epi*,C14-*epi*



YSS675-1
C6-*epi*,C14-*epi*,C19-*epi*



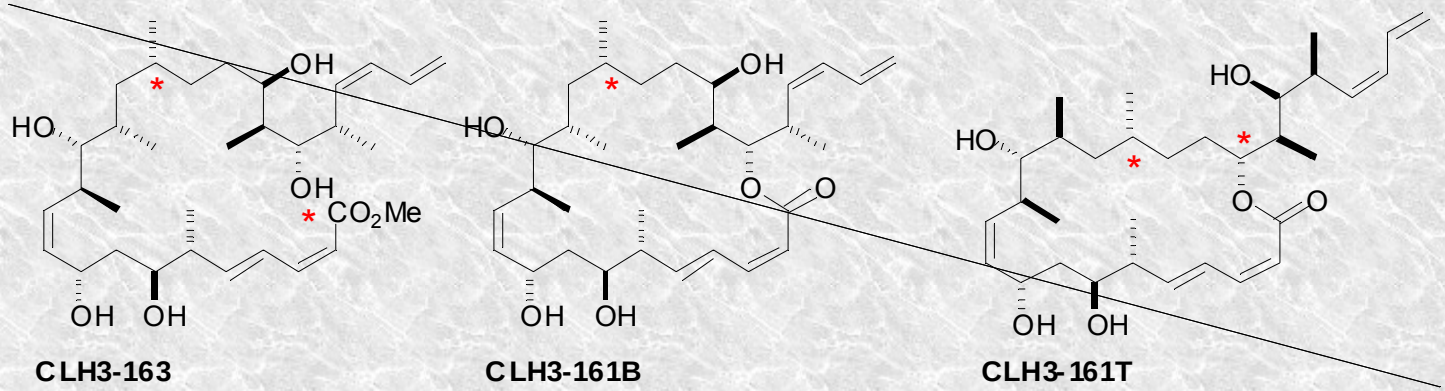
YSS675-2
C2/C3-(*E*),C6-*epi*,C14-*epi*,C19-*epi*



YSS698-2
C16-norMe

Shin, Y. *et al.* (2002) *Org. Lett.*, *4*: 4443-6.
Shin, Y. *et al.* (2004) *Agnew. Chem. Int. Ed. Engl. 43*: 4634-7.
Shin, Y. *et al.* (2005) *Org. Lett.*, *7*: 2873-6.
Shin, Y. *et al.* unpublished.

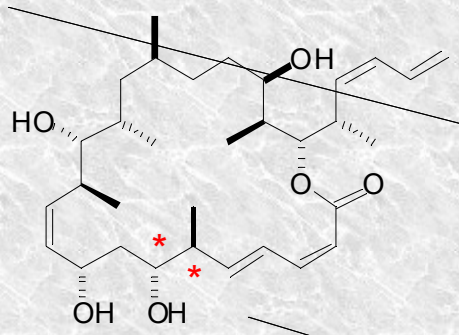
Compounds Under Study



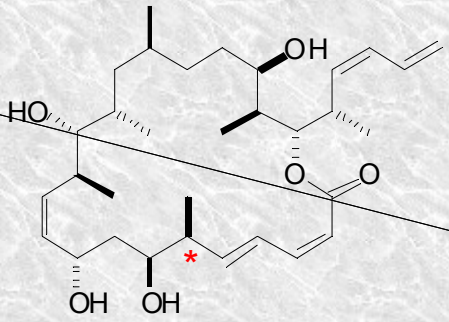
CLH3-163

CLH3-161B

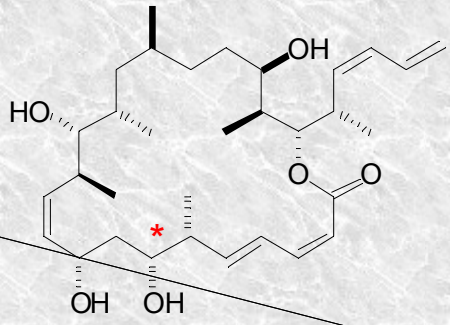
CLH3-161T



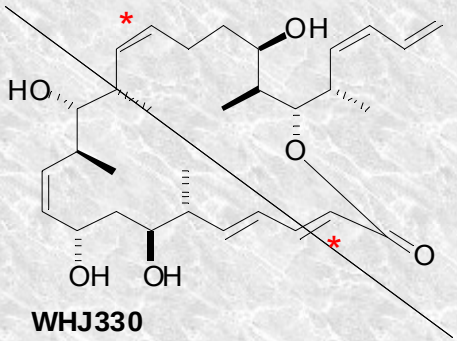
YF2-50



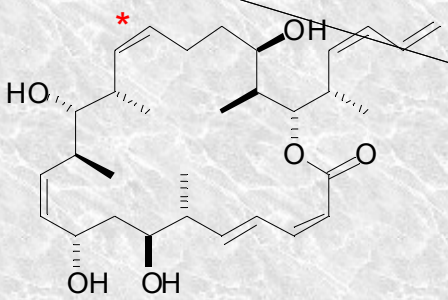
YF2-51



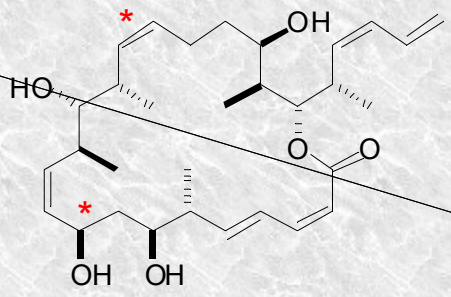
YF2-52



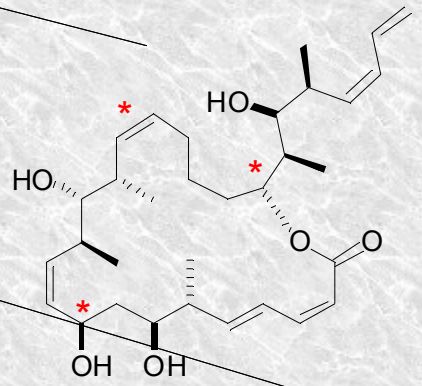
WHJ330



WHJ350



WHJ360

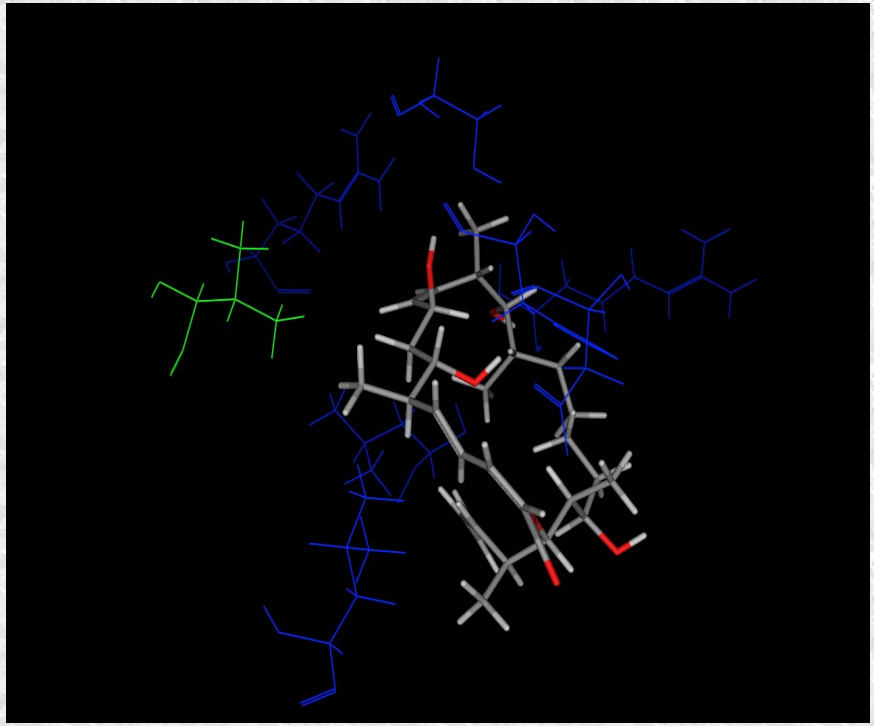


WHJ362

Fukui, Y. *et al.* (2006) *Org. Lett.* 8: 301-4.
Jung et al., in preparation

Methods

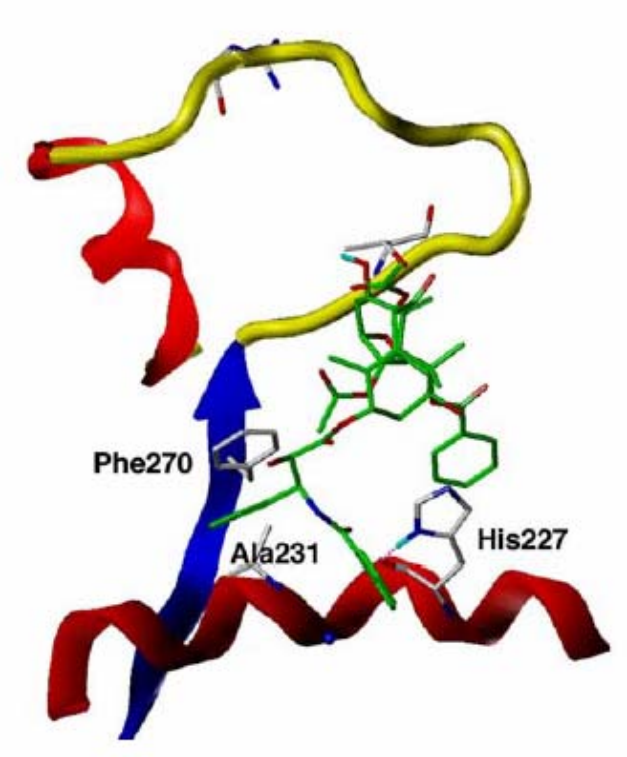
- MOE automatic docking algorithm used to find energetically favorable “poses”
- Randomly generates ligand orientations in binding pocket and scores them based on hydrogen bonds, hydrophobic interactions, hydrophobic/polar repulsion, and other intermolecular forces

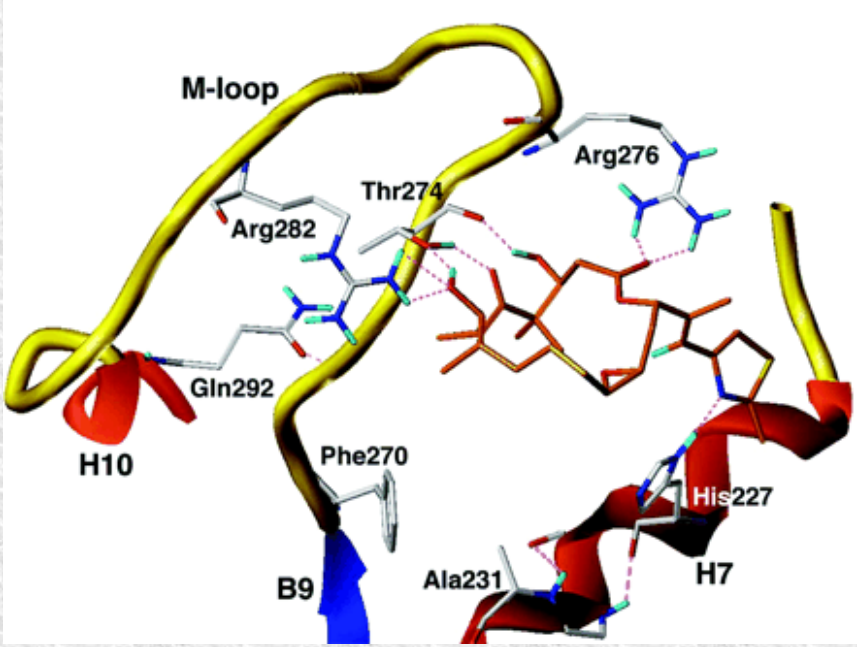


Methods

Structures for paclitaxel-bound and epothilone-bound tubulin experimentally determined and deposited in the PDB

- Paclitaxel-bound: 1JFF
- Epothilone-bound: 1TVK

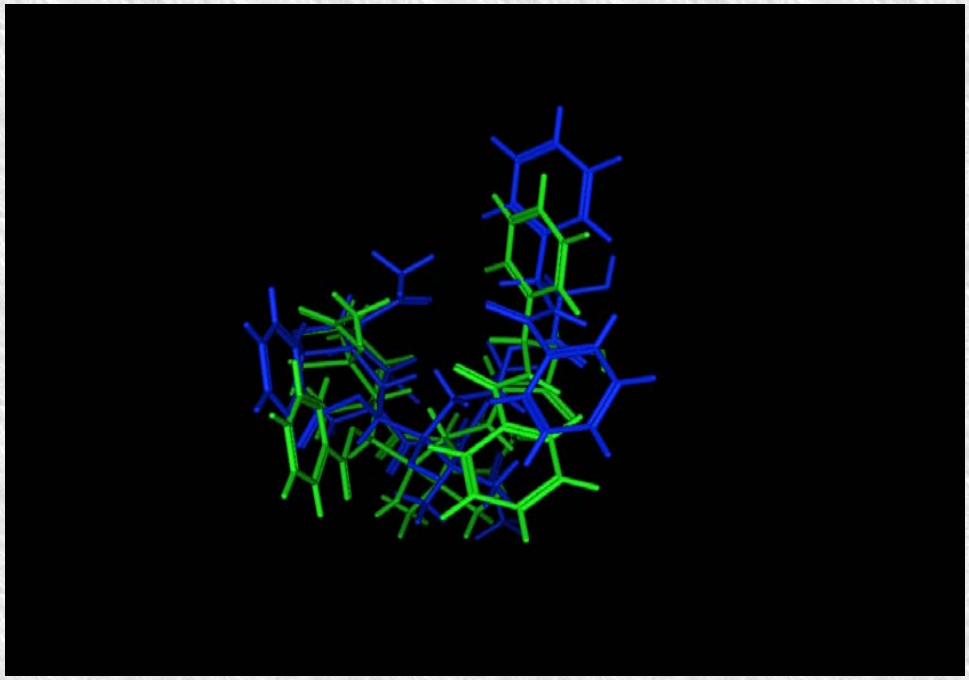


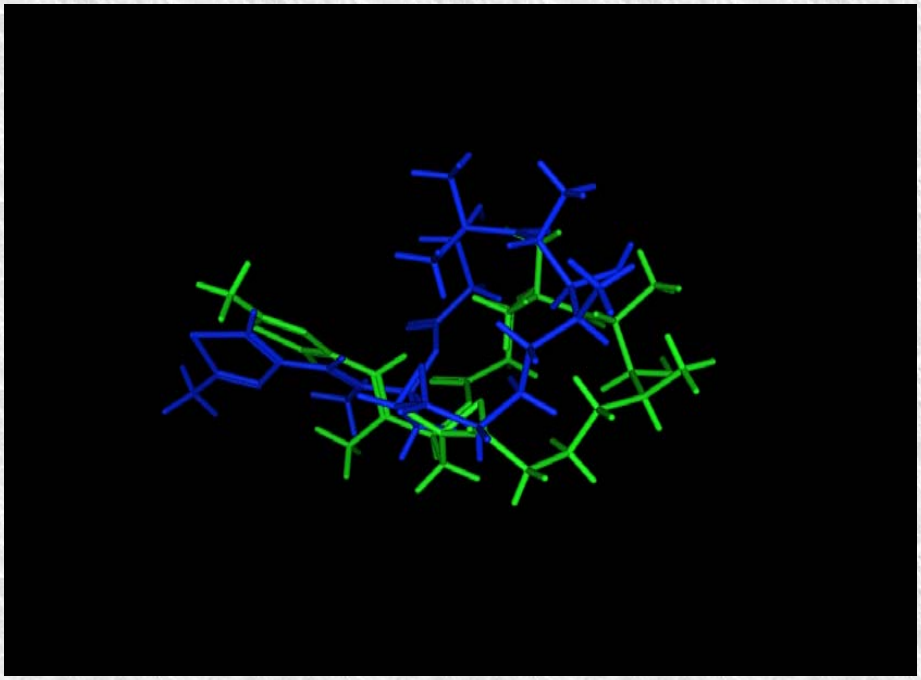


Nettles, J.H., *et al*. (2004) *Science 306*: 866-69.

Methods

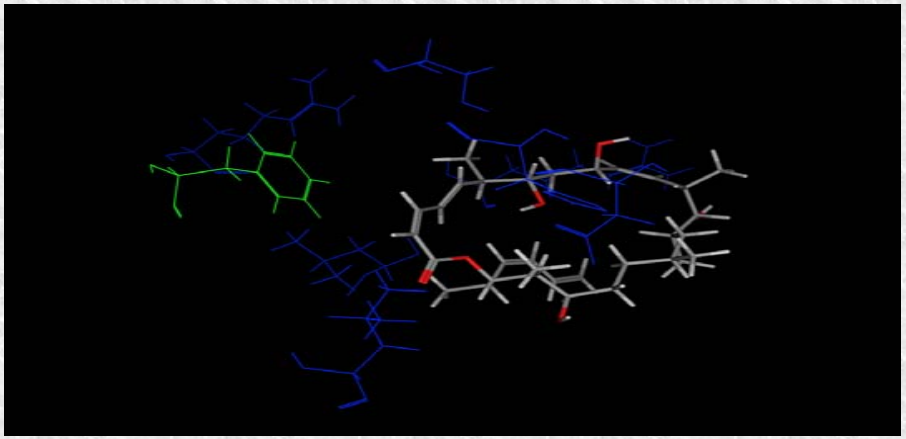
- Paclitaxel and epothilone were docked and results were compared to known binding modes to test settings of parameters.
- Best results were obtained with a rigid ligand (no conformational search).



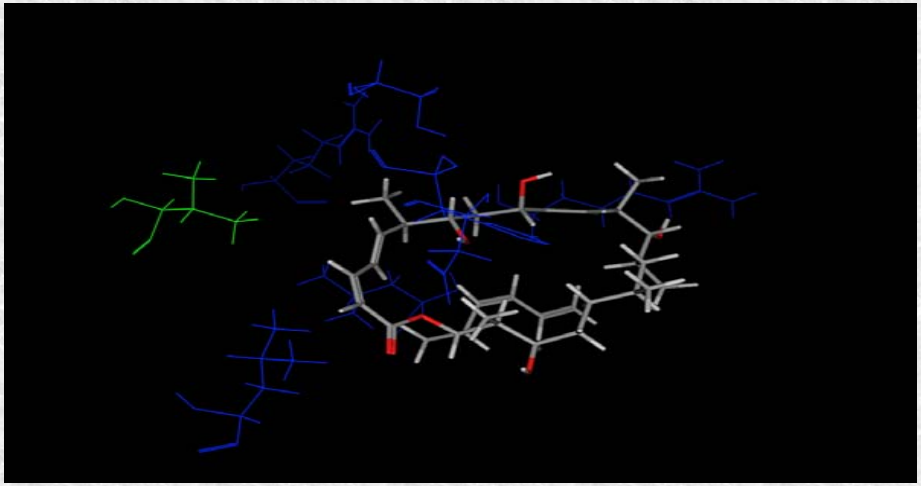


Methods

- Dictyostatin and 18 analogues were docked in wild-type β-tubulin. The "pose" that docked inside the binding pocket and received the best score was saved for further study.
- The Phe270->Val mutation expressed in 1A9/Ptx10 was introduced.
- The docking process was repeated for all ligands with the mutant β-tubulin.



Dictyostatin docked in wild-type β-tubulin

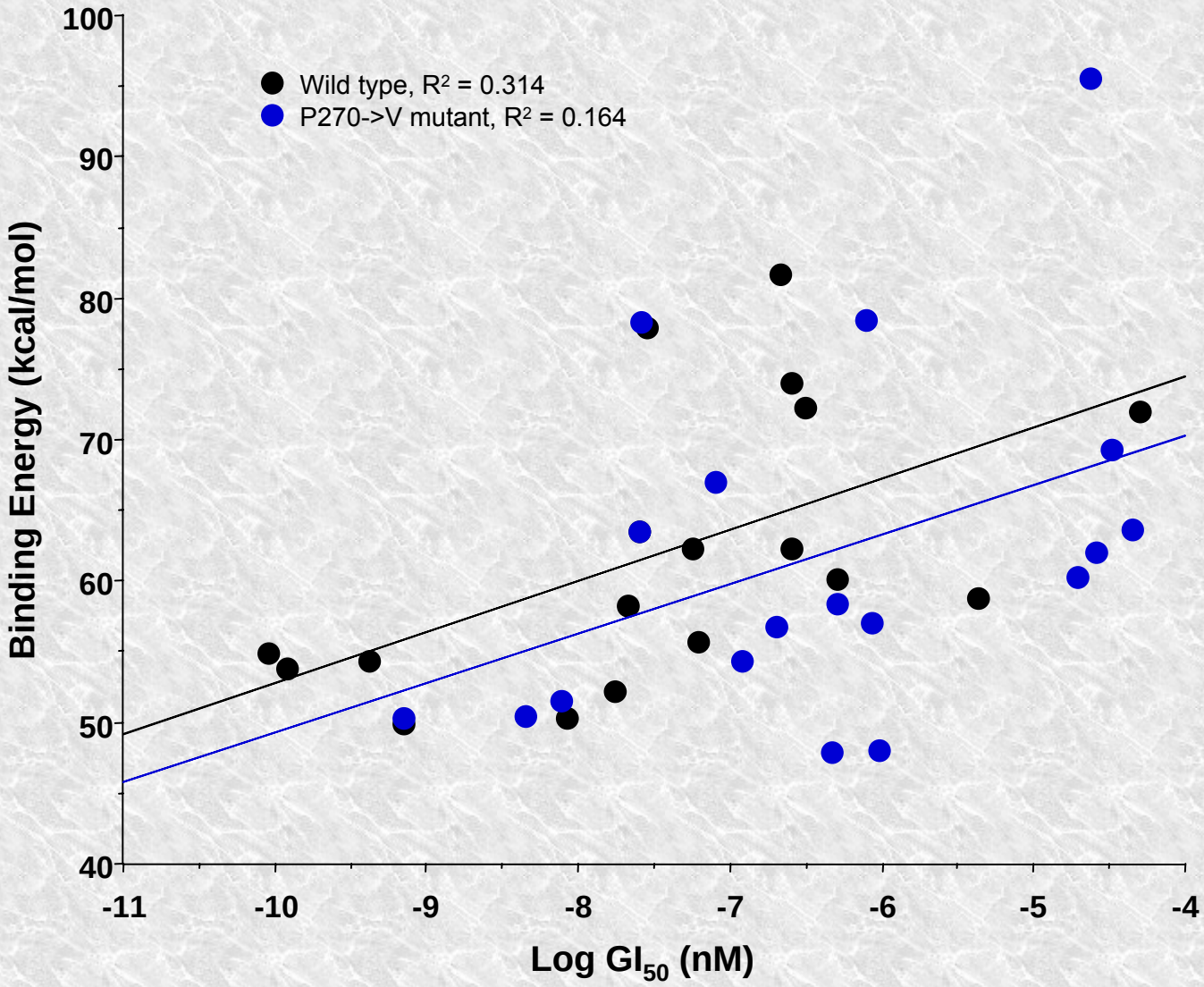


Dictyostatin docked in mutant β-tubulin

Methods

- Once all ligands had been docked in both wild-type and mutant β-tubulin, an energy minimization was performed using the MMFF94 forcefield to relax steric clashes, etc.
- The binding potential energy of each energy-minimized ligand was calculated.
- These calculated potential energies, along with the positions of each ligand's atoms after docking and energy minimization, serve as the primary data used to draw our conclusions.

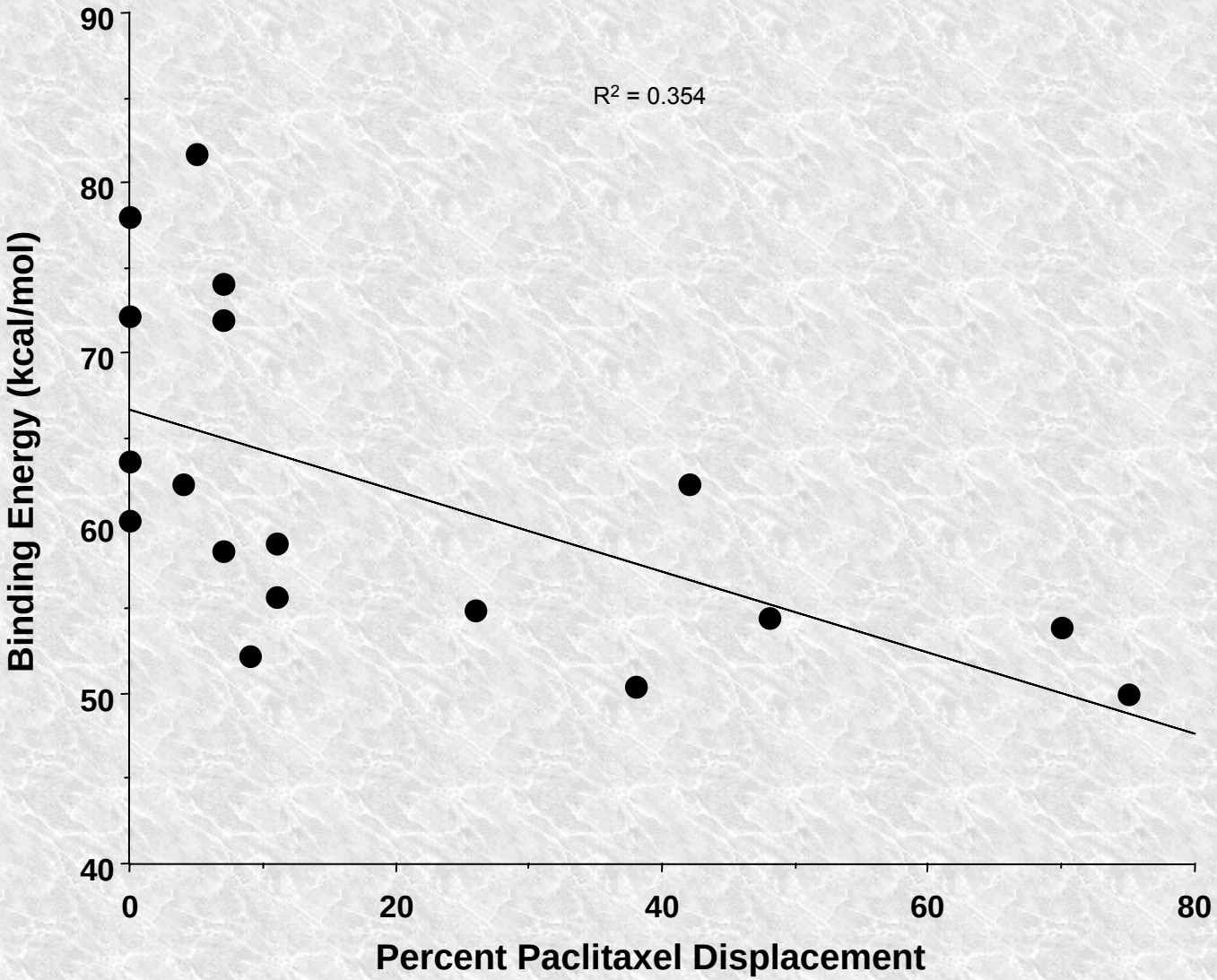
Results


Wild type, R² = 0.314
P270->V mutant, R² = 0.164
Binding Energy (kcal/mol)
100
90
80
70
60
50
40
-11
-10
-9
-8
-7
-6
-5
-4
Log GI50 (nM)

Results

- As is intuitively expected, the calculated binding potential energies were fairly well-correlated with experimentally measured GI_{50} values. In other words, as known potency increased, calculated binding energy generally decreased.
- Correlation coefficients were:
  - Wild-type data set = 0.58
  - 1A9/Ptx10 data set = 0.42

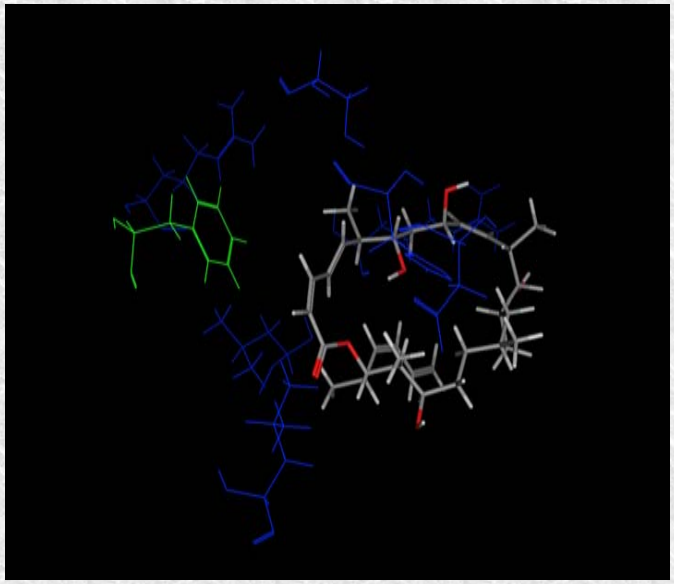
Results


R^2 = 0.354
Binding Energy (kcal/mol)
90
80
70
60
50
40
0
20
40
60
80
Percent Paclitaxel Displacement

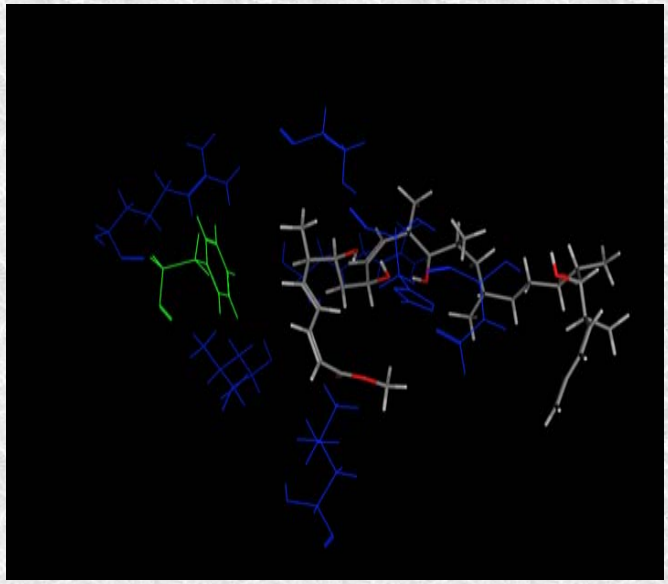
Results

- Again, the calculated binding potential energy values correlate reasonably well with experimental data. In this case, as observed percent displacement of paclitaxel (an indirect measure of a compound's affinity for the taxoid binding pocket) increases, calculated binding energy generally decreases.
- Correlation Coefficient = 0.49

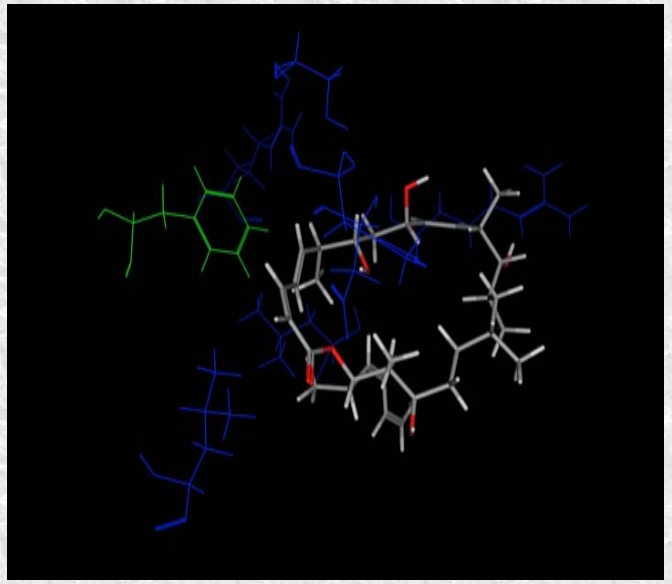
Wild-Type Tubulin



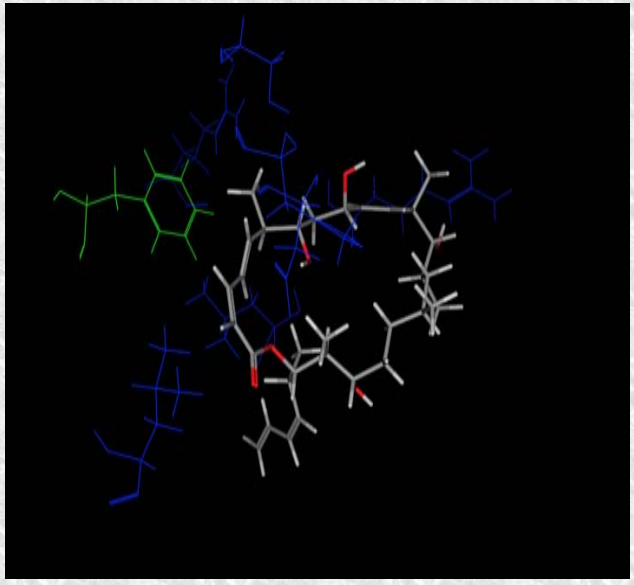
YSS631 (1A9)



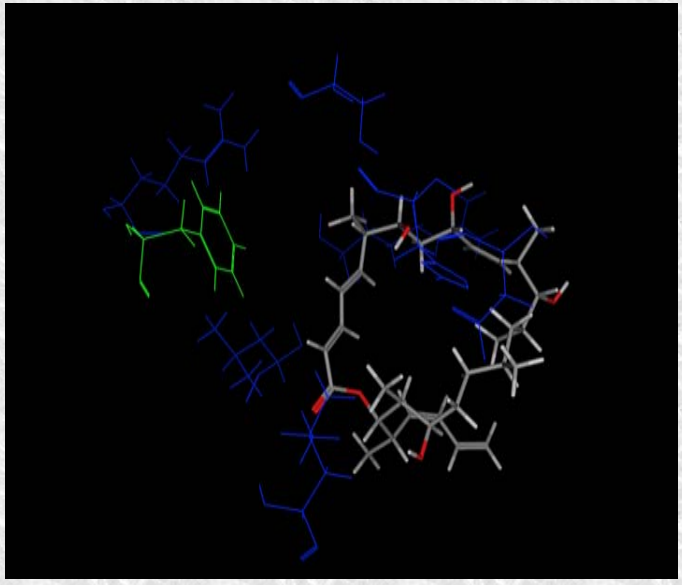
YSS629 (1A9)



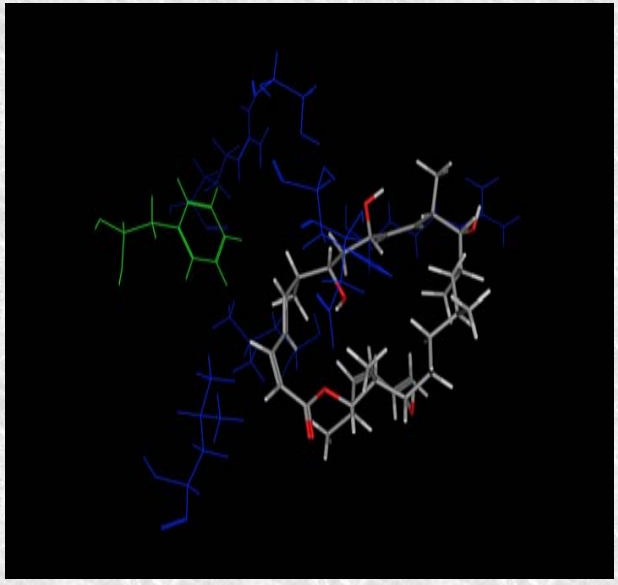
YSS479 (1A9)



YSS652 (1A9)



YSS65-2 (1A9)

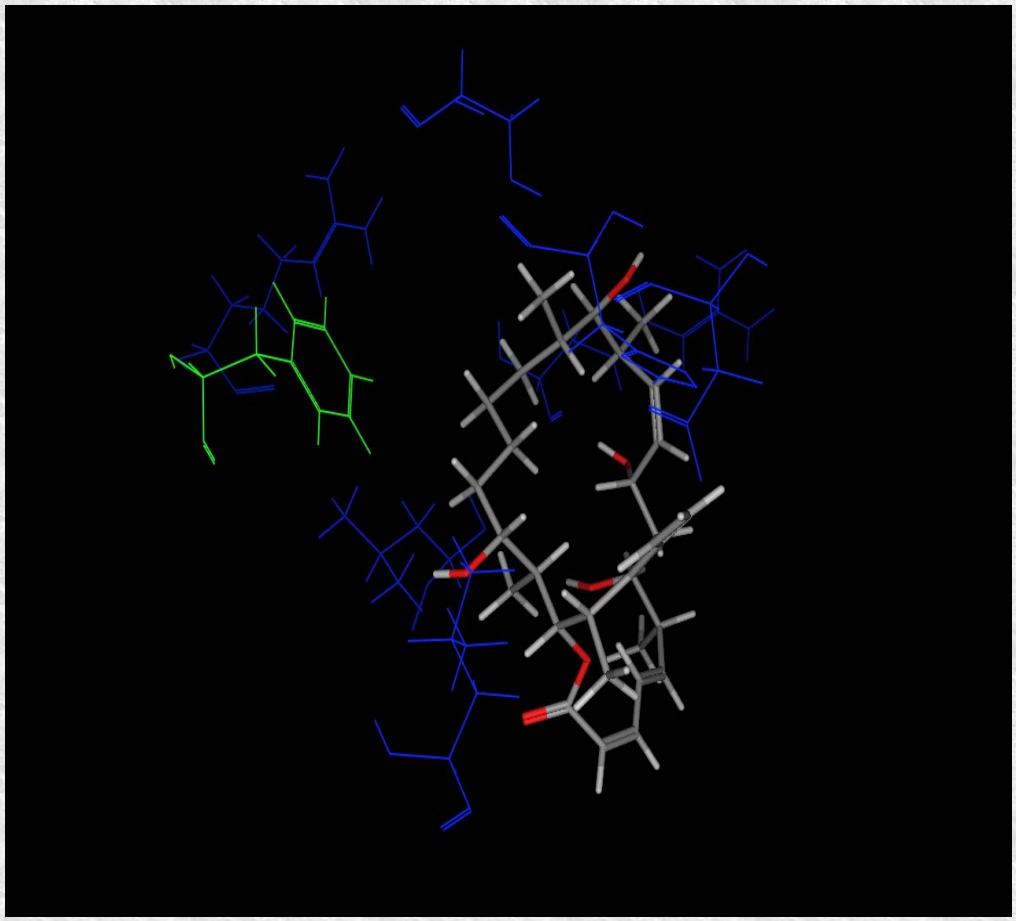


YSS675-1 (1A9)

Wild-Type Tubulin

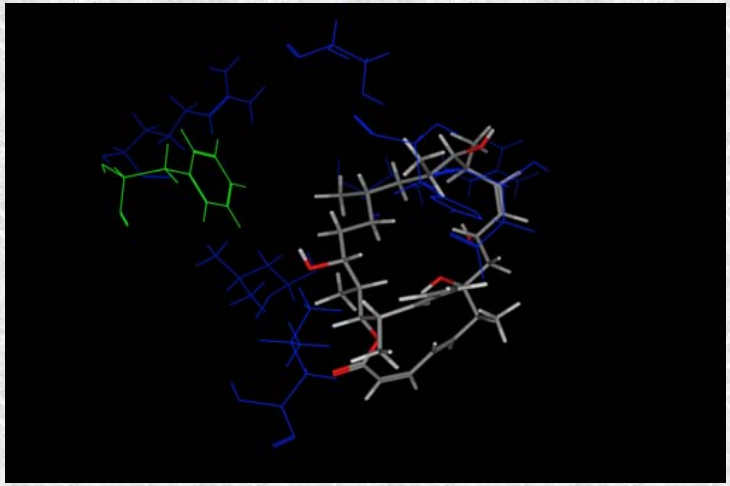
- Notice that all of the preceding ligands docked in a similar orientation, with C2-C6 in fairly close proximity to Phe270.
- This is interesting, in that it was expected based on the ineffectiveness of 16-normethyl dictyostatin in 1A9/Ptx10 cells that C16 would be in close contact with Phe270.
- Thus, it is also interesting that 16-normethyl dictyostatin docked essentially as was expected...

16-Normethyldictyostatin



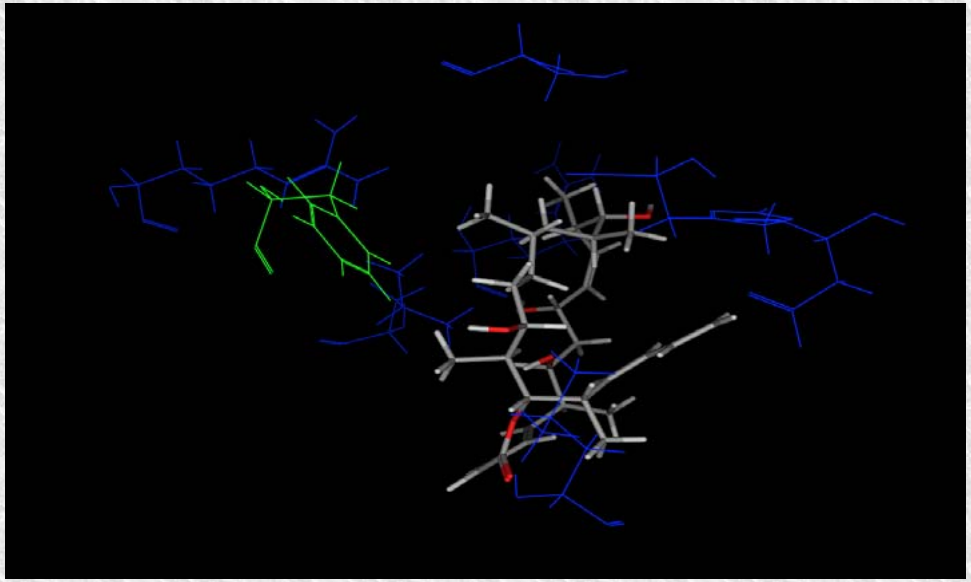
Note that the nonpolar region of the ligand from C16-C18 is in close proximity to Phe270 in the case of 16-normethyl dictyostatin, as would be expected based on the SAR, which requires the C16 methyl group for dictyostatin to remain active in 1A9/Ptx10.

YF2-50, YF2-51, and YF2-52

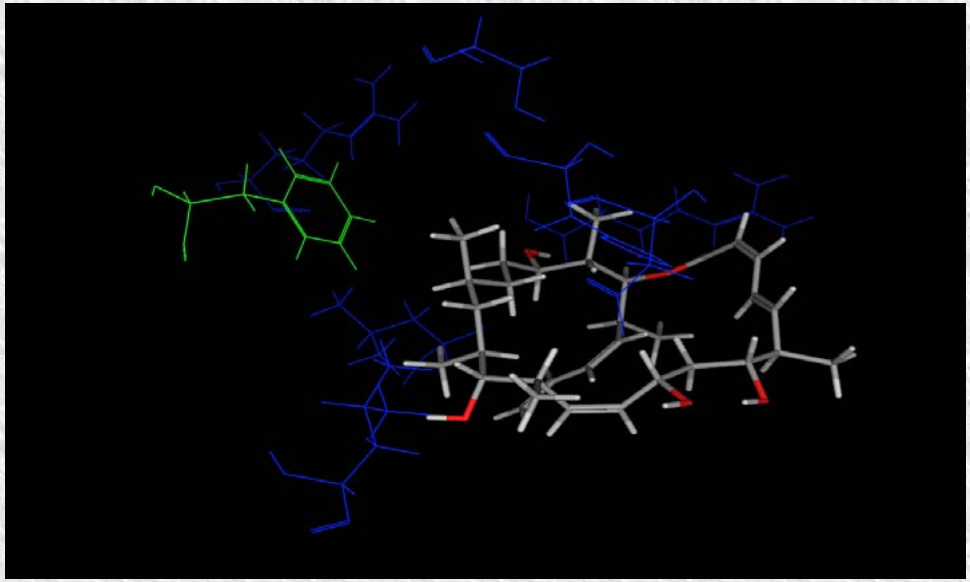


YF2-50

Similar orientations are favored with these analogues, which bear a methyl group at C16 but have stereochemistry at C6 and C7 that differs from that of dictyostatin.

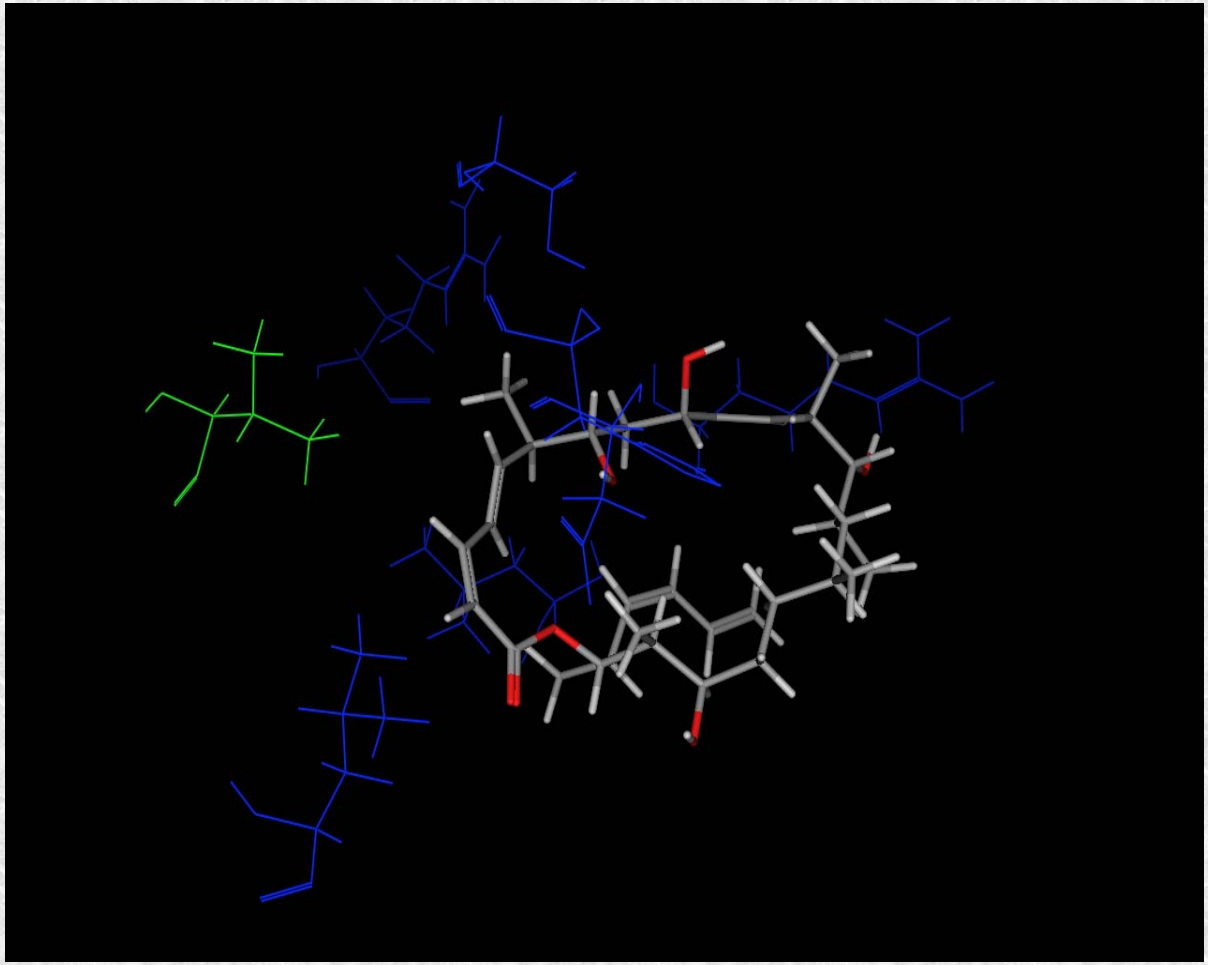


YF2-51



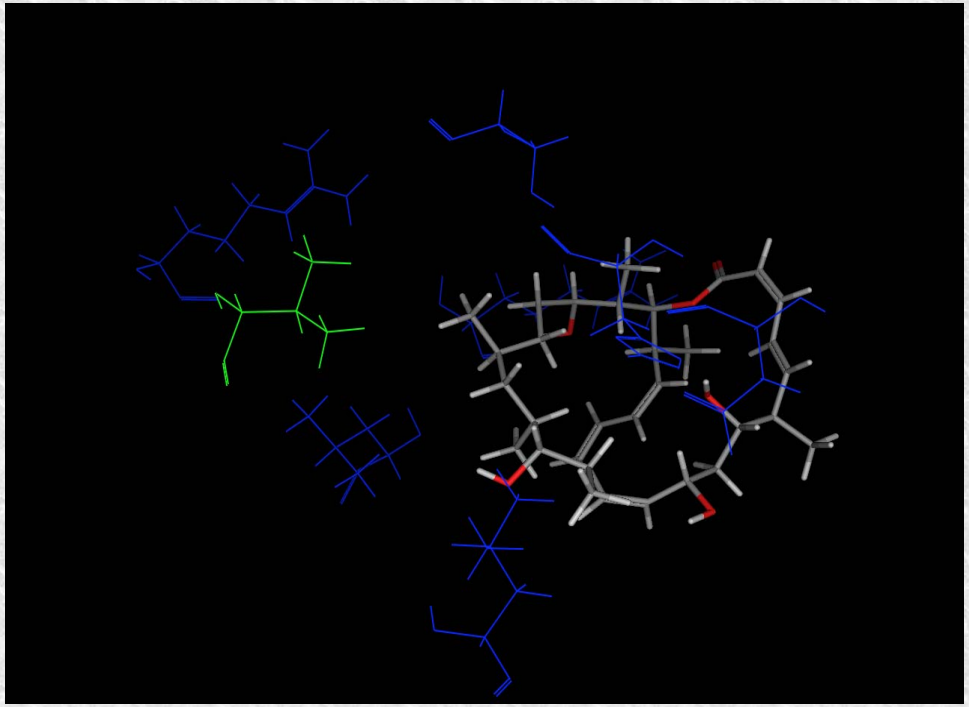
YF2-52

What about 1A9/Ptx10?

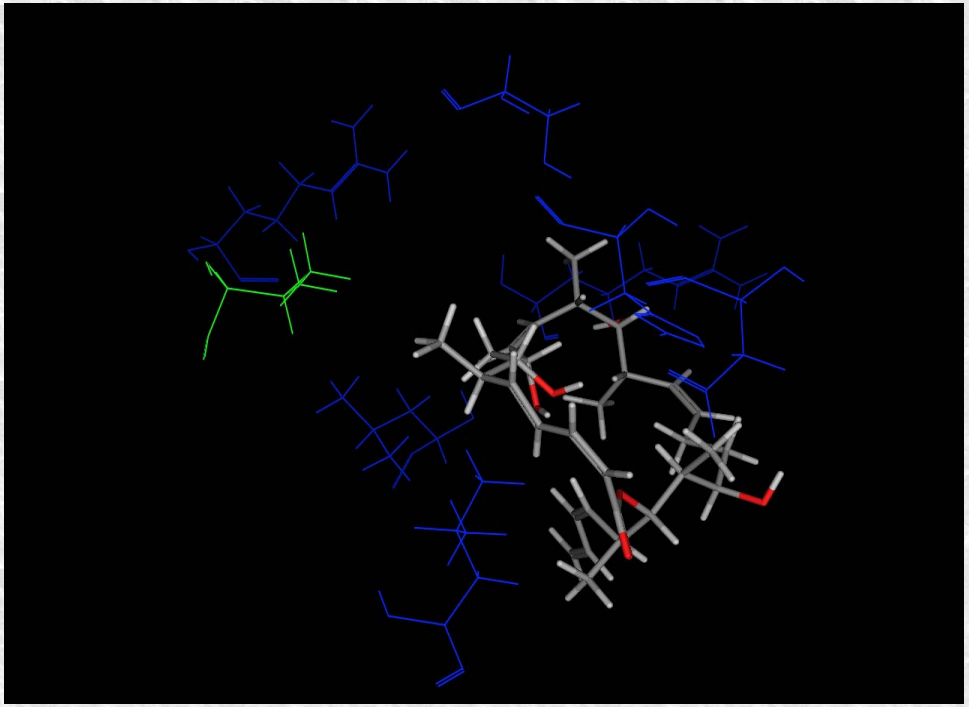


Some of the compounds studied, including dictyostatin (pictured), docked in essentially the same orientation in the mutant tubulin as they did in wild-type tubulin. However, note that valine is a less bulky amino acid than phenylalanine, meaning that it does not extend as far into the pocket.

What about 1A9/Ptx10?



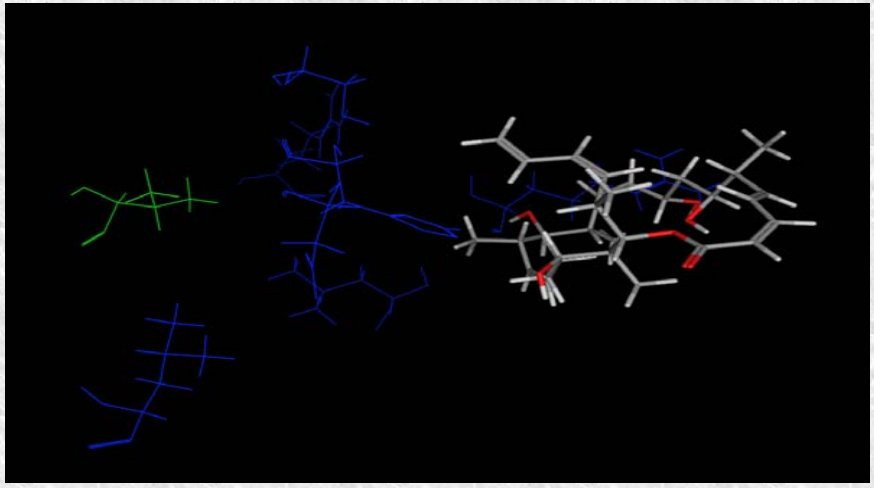
YSS652 (1A9/Ptx10)



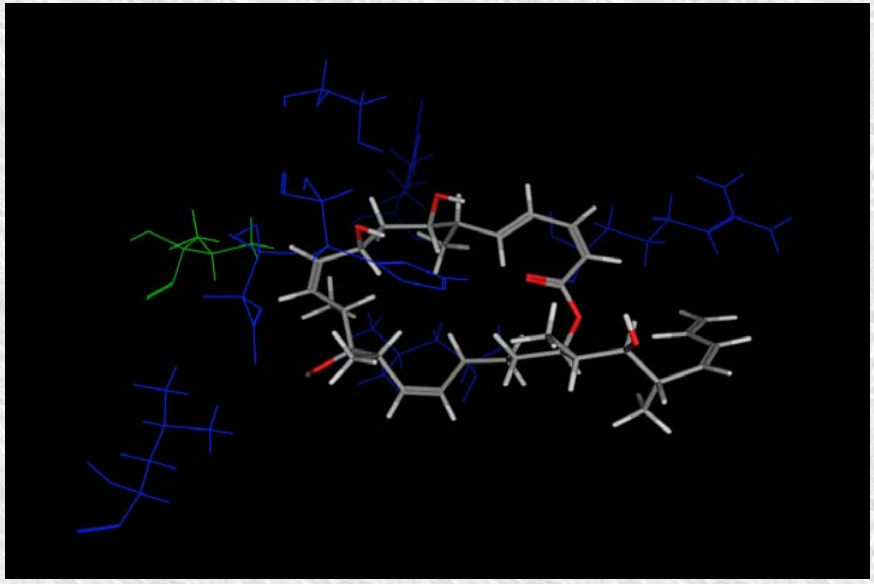
WHJ360 (1A9/Ptx10)

In some cases, a methyl group (sometimes the methyl group at C16, as with YSS652, shown above) seems to be directed deep into the pocket, presumably to maintain nonpolar contact with the less bulky Val270. This may help explain the fact that 16-normethyldictyostatin is dramatically less effective in 1A9/Ptx10 cells than in 1A9 cells which express wild-type tubulin.

What about 1A9/Ptx10?



WHJ350 (1A9/Ptx10)



WHJ362 (1A9/Ptx10)

Finally, some of the ligands either docked in unique positions unlike any others, or did not dock in the binding pocket at all. Most likely, the absence of Phe270 greatly hindered or destroyed their affinity for the taxoid binding site.

Difficulties and Limitations

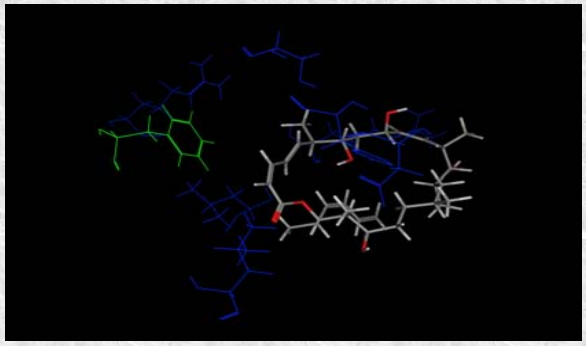
- We have no experimentally determined structure for the mutant tubulin expressed by the 1A9/Ptx10 cell line.
- Side chains within the binding pocket remain rigid during docking in MOE.
- Although MOE uses implicit solvation, explicit treatment of water molecules may give better results.
- New computational techniques (such as those being developed by Dr. Camacho) will help research such as this greatly.

Conclusions

- Computational results and wet lab data correlated more or less as expected.
- Interactions between nonpolar portions of the ligand and Phe270 almost certainly play an important role in the binding of dictyostatin and its analogues to the taxoid binding site on β-tubulin.

Conclusions

- However, our results also seem to suggest that portions of these ligands other than the C16 methyl group may play an important role in these interactions.
- It appears that the interactions involved in the binding of dictyostatin to β-tubulin may be more complex than were previously thought and are certainly worthy of further study.



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