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

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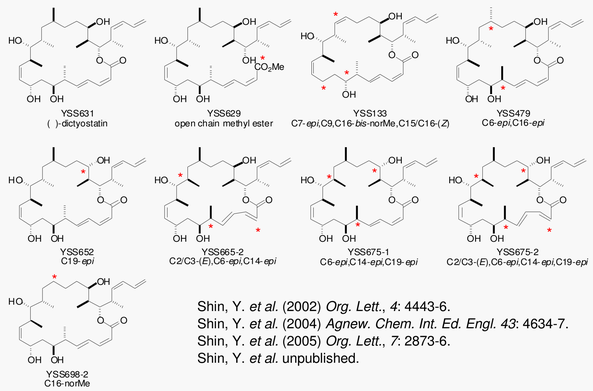
Introduction

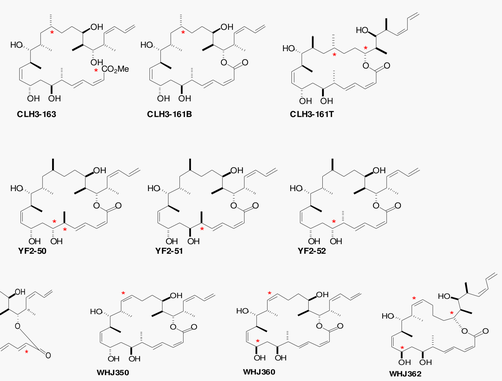
The division of a cell's contents during mitosis is highly dependent on the dynamic growth and breakdown of microtubules. Because quickly dividing cancerous cells are especially susceptible to disruption of this process, many treatments for cancer have targeted this behavior of microtubules, hyperstabilizing the tubulin polymers they are composed of to arrest the cell cycle and trigger apoptosis. These agents have been successful in some respects, but they do face certain problems, particularly the emergence of resistant tumors.

Computer docking simulations were used to examine binding modes of the promising new antimitotic agent dictyostatin and several of its analogues with wild-type tubulin and a mutated form that is resistant to dictyostatin and some similar drugs. Our goal was to discover protein-ligand interactions that could help explain the resistance of cells expressing this mutant form of tubulin and thereby facilitate the formulation of hypotheses regarding new agents to synthesize.

Compounds Studied



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Shin, Y. *et al.* unpublished.



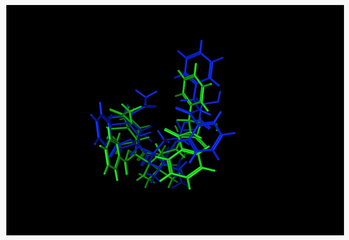
Fukui, Y. *et al.* (2006) *Org. Lett.* 8: 301-4.
Jung et al., in preparation

Methods

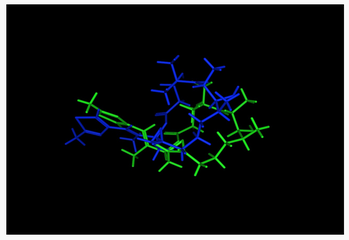
The program used to dock the ligands being studied was the Chemical Computing Group's Molecular Operating Environment (MOE). This application includes an automatic docking algorithm which generates a large number of random "poses" for the ligand in the vicinity of the specified binding site. Each of these poses is then assigned a score based on several types of interactions with the binding site, including hydrogen bonding, hydrophobic interactions, and hydrophobic/polar repulsion. After 15,000 iterations of this algorithm, the 100 poses with the best scores are retained and can be viewed as they docked in the binding site.

Paclitaxel and epothilone are two antimitotic drugs that occupy the same binding site as dictyostatin. Initially these two molecules, for which the binding modes have been determined through cryoelectron crystallography, were docked in β-tubulin to determine which program parameters would give the most accurate orientations. We discovered that a rigid ligand model generally worked best, as shown by the results to the right. This is reasonable, since nearly all of the compounds under study consisted of fairly rigid ring structures.

Dictyostatin and each analogue under study were docked to both wild-type and Phe270 -> Val mutant tubulin. The energies of the docked molecules were minimized using the MMFF94 forcefield, and the potential energy of each bound molecule was calculated. These binding energies, along with images of the docked ligands and surrounding residues generated by MOE served as the data used to draw our conclusions.



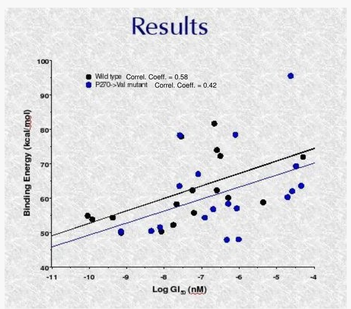
Paclitaxel: Docked Orientation (Green) and from Crystallographic Data (Blue)

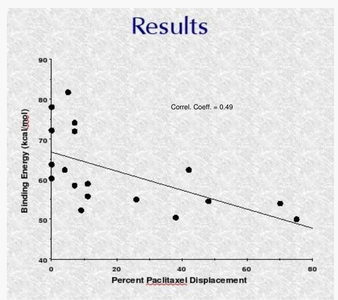


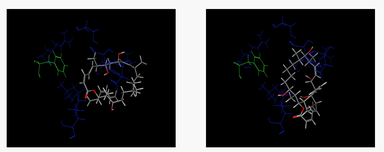
Epothilone: Docked Orientation (Green) and from Crystallographic Data (Blue)

Results

- 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.
- 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.

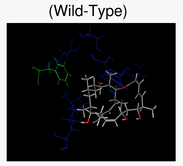




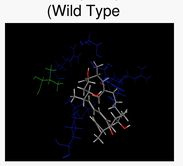


Dictyostatin (Wild-Type)

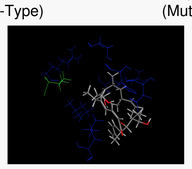
16-Normethyldictyostatin (Wild Type



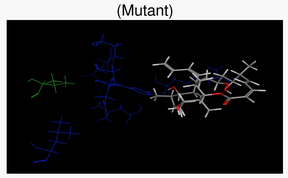
YF2-52 (Wild-Type)



16-Normethyldictyostatin (Mutant)



WHJ360 (Mutant)



WHJ350 (Mutant)

Difficulties & Future Work

- 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, but explicit treatment of water molecules may give better results.
- Currently developing computational techniques are expected to help research such as this greatly.
- QSAR currently in progress shows some signs of consistency with these results. In any case, when complete this study should shed more light on the specific roles that various structural elements of dictyostatin plays in determining its activity.

Acknowledgments

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