**Comparative Docking Study of Stilbene Derivatives in the Colchicine site of β-Tubulin**

One of the leading causes of death is cancer1. With malignant growth accounting for just over 20% of all deaths in the United States each year. Depending on the type of cancer the patient has, the recommended treatment can range from removal surgery to harsh chemotherapy and radiation therapy2. While modern medicine has made advances in cancer therapies, the therapies themselves can also be incredibly toxic to the human body and studies show that certain cancers have a resistance to the most commonly used treatments.

In patients with lung cancer survival rate is still staggeringly low at approximately 16%. With the most common treatments being surgery, radiation, and chemotherapy3. With the effectiveness of surgery and radiation therapy limited by tumor radio-resistance and progression and growth of the cancer outside of the target area. Chemotherapy targeting DNA and RNA can also be of limited effectiveness due to the presence of ERCC1 a repair protein found in 46% of patients2,3. In addition patients who relapse can also see a Chemotherapy resistant tumor3.

Microtubule inhibitors are a group of compounds that target cancer in a different way. Instead of targeting DNA and RNA replication in cells, Microtubule inhibitors target tubulin proteins to disrupt microtubule formation4. These compounds show potential in clinical trials through actions at four distinct active sites on tubulin5. Of the four sites, the colchicine site is of interest due to its nature to not only inhibit the formation of microtubules but also unwind already formed microtubules due to the depolymerization of β-Tubulin6. This site was named after a naturally occurring compound that is used in the treatment of gout that is extremely toxic, the toxicity is partly due to the fact that in addition to the interactions involved in gout symptom relief it also binds to microtubules, effectively preventing mitosis. While colchicine is used as a cancer treatment the problem of offsite reactions remains. A substantial number of studies have been done on the various aspects of colchicine to try and reduce toxicity and lower free binding energy without significant progress. The work being done on colchicine has enabled the scientific community to research other ligands in the same active site. A new 2.3 angstrom structure of tubulin was published in Prota, A.E et al (2014)7 allowing for more accurate computational studies to take place. In addition to attempting to improve colchicine it is also important to explore alternative compounds for interaction in the colchicine site that may have a lower toxicity or significantly lower free binding energy.

the results of Ashutosh Tripathi et al (2009)8 showed that stilbene, a diarylethene with a central ethylene with a predicted free binding energy that shows a strong correlation with antiproliferative activity similar to colchicine. Extensive studies have not been done to determine if a derivative exists with lower free binding energy than colchicine. Additionally, a higher resolution (2.3 angstrom vs 3.1 angstrom) model of tubulin has been released since the studies on stilbene were conducted. The purpose of the experiment described within this proposal is to extensively test stilbene and its derivatives to determine its docking potential with the colchicine site using the 2.3 angstrom model.

Experiment

The goal of this experiment is to computationally determine the stilbene derivatives most likely to have a strongly favorable interaction with the colchicine site by simulating the stilbene ligands docking with the active site and comparing the reported free binding energies. If stilbene reacts favorably with the colchicine active site, then I would expect to see free binding energies in the -8 kcal/mol to -10 kcal/mol range or better. Upon closer evaluation of the tested derivatives additional ligands could be synthesized and simulated to attempt to take advantage of the most favorable interaction in their peers.

Model building

The X-ray crystal structure (2.3 angstrom) of αβ-tubulin complexed with colchicine (PDB code: 402B) will be used. C and D subunits will be removed, and hydrogen atoms added to the model with positions optimized while keeping the heavy atom position fixed. Models for stilbene derivatives will be constructed in Sybyl and optimized in the same manner as the tubulin model.

Computational docking

The initial stage of the experiment will be carried out by GOLD, a protein-ligand docking simulation that aims to minimize free binding energy, considering van der waals interactions, hydrogen bonds, shape, and electrostatic interactions. GOLD stands out as a leader in the possible choice of docking software with 90% accuracy in Pagadala NS et al (2017)9 and the ability to treat the protein as nonrigid. GOLD uses a genetic algorithm to explore the flexibility of the ligand side chains by changing the position of the hydrogen bond donor and acceptor groups and will also displace loosely bound water resulting in simulations returning the experimentally determined binding. Following the approach of Tripathi A et al8 and Nguyen et al10, the active site is determined by utilizing the position of the colchicine complexed in the model including radius of 7 angstroms around the docked colchicine. However due to the new model’s significant improvement in resolution template similarity will not be enforced allowing the ligands to dock in any orientation. I propose 1000 runs per ligand with early termination switched off to allow for novel confirmations to emerge from the genetic process of GOLD. All other parameters will be defaults. As a baseline the ligand of colchicine docked in the model will be removed and redocked using GOLD to establish the accuracy of the docking simulation similar to Fig 1.



Results of docking R-bicalutamide into 1Z95 binding site, taken from the Astex Diverse Set. The native ligand pose is shown colored by element, the top ranked GOLD pose is shown in green.

Following the baseline, the derivatives of stilbene will be docked using the same procedure as the included colchicine ligand. HINT will then be used to score each derivatives docking positions and iteratively optimized by hand.

HINT

HINT scoring is a method to score ligand protein docking through hydropathic interactions. HINT was chosen as the scoring method as it is intuitive to use and allows the exploration of per atom interactions in the active site. HINTs algorithm shown in fig 2 evaluates specific interaction between two molecules considering distance and interaction strengths. Each atom-atom interaction is then given an identifier based on interaction type. These scores can be used in the design of new derivatives as it allows you to see side chains of the derivative that react poorly within the pocket.



Fig 2. The formula used by HINT to describe interaction between two molecules where A is the hydrophobic atom constant derived from LogPo/w, S is the solvent accessible surface area, T is a function that differentiates polar-polar interactions (either acid-acid, acid-base or base-base), and R, r are functions of distance between atoms i and j. the binding score, bij describes the specific atom-atom interaction between atoms I and j whereas B is the total interaction of the molecule.

Additionally, HINT will be used to consider the potential of water molecule forming a bridge between polar atoms of the ligand and polar atoms of the active site potentially lowering the energy requirements of that confirmation.

Analysis

The ligands free binding energies will then be compared to both previous studies of stilbene and colchicine included in the model. As well as a report outlining the areas which react most favorably and least favorably in the proposed ligands.

Discussion

Following the modelling of all the compounds there will be a ligand or perhaps a few ligands that have lower free binding energies than their peers. However, it is possible that none of the compounds bind favorably at all, especially when compared to colchicine and base stilbene. One issue not addressed lies in the fact that the experiment only tests one of the many isotopes of tubulin found in the human body, the model of tubulin used is derived from cows although extremely similar to the protein in humans, while a decent facsimile Majcher U et al (2018)11 has shown that ligands can have a large range in free binding energy depending on the exact isotope they react with. Even if the simulations show a clearly favorably interaction between the target site and the ligand, the road to an effective medicine is still long.

This study does not consider toxicity, although stilbene has not been studied to the extent colchicine has the amount of off target interactions does appear to be lower12,13. Even if that holds true in further testing, more work will need to be done to mitigate toxicity as much as possible. Additionally, physical trials will have to be done to show that the ligand performs as expected in the real world. The matter of designing a carrier for the ligand also must be addressed for a viable medical trial to be considered.Carriers are important for maximizing exposure to the tumor while minimizing exposure to the healthy cells14.

While computational studies are only the first step of many in the development of new treatments, they are invaluable in narrowing down the number of possible compounds with predicted interactions at a given active site. The speed and scope with which computational studies can be performed allow for thousands of compounds to be tested at a relatively low cost and in a comparatively short amount of time compared to synthesizing and performing assays on every experimental ligand15. If the results of the stilbene study are strong enough it is possible that one of stilbenes derivatives could be a future treatment for some of the most resistant cancers in humans.

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