**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. The effectiveness of surgery and radiation therapy are limited by tumor radio-resistance, as well as progression and growth of the cancer outside of the treatment area. Chemotherapy targeting DNA can also be of limited effectiveness due to the presence of ERCC1 a repair protein found in 46% of patients2,3. ERCC1 is a repair protein with the ability to repair double stranded DNA breaks preventing DNA damage from Chemotherapy to cause enough damage to DNA to induce cell death. In addition patients who relapse can also see a Chemotherapy resistant tumor return due mechanisms similar to microbial resistance. The cancer cells that give rise to the returning tumor are the cells that were resistant to the treatments the patient first received3.

One approach being explored to treat radiation resistant and DNA targeted chemotherapy resistant cancers is to inhibit mitosis of cancer cells via microtubule inhibition. Microtubules are vital during mitosis as they pull the mitotic spindles to the poles during anaphase separating the replicated chromosomes and allowing cleavage to occur as well as providing structure to the cell. Microtubules are made up of a protein called tubulin. It is through interactions with tubulin which we can inhibit the formation of microtubules. Microtubule inhibitors are a group of compounds that target tubulin instead of targeting DNA and RNA replication in cells4. These compounds show potential in clinical trials through interaction at four distinct active sites on tubulin5. Interestingly Tubulin can be both destroyed due depolymerized or prevented from binding with other tubulin molecules by changing the shape of the active site though overstabilization. Of the four sites commonly studied active sites, one, the colchicine site, is of interest due to its nature to not only inhibit the formation of microtubules but also unwind or destroy already formed microtubules due to the depolymerization of β-Tubulin, one of the subunits of the tubulin dimer6. This site was named after a naturally occurring compound that is used in the treatment of gout. However it 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, meaning the usage of colchicine as a cancer treatment inevitably has off target effects as well as the intended ones. While colchicine is currently in use as a cancer treatment, the problem of offsite reactions is a significant one. 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. Free binding energy is a common metric used when evaluating chemical reactions as the lower the free binding energy, the more likely the reaction is to take place. For drugs the more negative the free binding energy the more favorable the interaction as a negative free binding energy means the intended reaction will occur spontaneously or without the input of energy. Additionally in an active site associated with multiple compounds the compound with the lowest free binding energy is favored over others as it will bind more easily with the active site.

The results of Ashutosh Tripathi et al (2009)8 showed that stilbene, a diarylethene with a central ethylene, has 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 significantly higher resolution (2.3 angstrom vs 3.1 angstrom) model of tubulin has been released since the studies on stilbene were conducted. This higher resolution model will allow for more accurate tests to be 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 through free binding energy.

Experiment

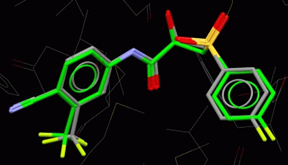
The goal of this experiment is to computationally determine the stilbene derivatives most likely to have a strongly favorable interaction, ie a low free binding energy, with the colchicine site by simulating the stilbene ligands docking or interaction with the active site. Followed by comparisons of the reported free binding energies. If stilbene or its derivatives have a compatible polarity and shape when docked 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 by examining which side chains of stilbene derivatives have poor interactions with the active site. Primarily optimizing the side chains to have polar atoms near opposite charge polar atoms or allow the gap to be large enough to be bridged by water in the active site and nonpolar molecules near nonpolar molecules in the active site. These new derivatives will be simulated in an attempt to take advantage of the most favorable interaction in their peers and create a derivative with the lowest free binding energy possible.

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 as only the colchicine active site is of importance to the simulation. Hydrogen atoms will be added to the model with positions optimized while keeping the heavy atom position fixed to allow for hydrogen bonding without significantly affecting the shape of the active site. Models for stilbene derivatives will be constructed in Sybyl and optimized in the same manner as the tubulin model. Sybyl was chosen as the molecular modeling software works in conjunction with HINT9, the scoring model discussed below, as well as allowing you to build and edit molecules atom by atom and then optimize the shape of the molecule by finding the lowest energy state with the option to account for hydrogen or to ignore them. Another important feature of sybyl is the ability to extract compounds from crystal structures. This allows you to redock ligands that are included in the model without having to create them yourself reducing human error. It also allows for the labeling and visualization of each atom in a compound which will be vital in understanding the results of the scoring process.

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 tests conducted in Pagadala NS et al (2017)10 and the ability to treat the protein as nonrigid, this allows changes in the rotation of the side chains possibly lowering the overall free binding energy. 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. These features result in simulations returning are able to find the experimentally determined binding of compounds. Following the approach of Tripathi A et al8 and Nguyen et al11, the active site is determined by utilizing the position of the colchicine complexed in the model including a 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, early termination would potentially reduce computation time as it would stop running simulations if multiple runs return very similar results, however it is possible that those results are just a local minima and additional runs would overcome the energy barrier and produce better results. 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. The results with the lowest free binding energy from GOLD for each derivative as well as base stilbene and the colchicine ligand included in the model will then be loaded into Sybyl docked in the tubulin complex. A 7 angstrom radius will then be isolated around the ligand and any atoms outside that radius will be removed from the simulation to minimize computation time. This is acceptable because interaction strength falls off nearly exponentially as distance from the ligand increases, therefore beyond 7 angstroms interaction with the molecules is negligible. HINT is then run on the atoms of the ligand by selecting them in the Sybyl environment and HINT calculates the reaction with all atoms not selected using the formula described below. The results will then be used to more closely examine each derivatives docking positions for side chains that increase free binding energy and iteratively improve the side chains 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 are representative of interaction strength with a positive score being associated with an interaction that increases free binding energy and a negative score indicating a decrease in free binding energy. The results can be used in the design of new derivatives as it allows you to see which side chains of the derivative react poorly within specific amino acids in the active site and what type of bond is the major contributor to the positive score being examined on an atom by atom basis.



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.9

Additionally, HINT will be used to consider the potential of water molecules forming a bridge between polar atoms of the ligand and polar atoms of the active site that would otherwise be too far to interact potentially lowering the energy requirements of the 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)12 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 lower13,14. 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 cells15.

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 ligand16. 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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