Trans-Sialidase's Role in Chronic Chagas Disease, and it's Potential for Infection Inhibition by Employing Natural Products

H. Wolff, P. Bourne, J. Lin, C. Krishna, N. Huang, P. Chu

Abstract

Chronic Chagas Disease is a malady affecting a highly vulnerable population, with currently no promising cure. This study looked at natural products from the Taiwan Pharmaceutical Databank, and utilized an *in-silico* approach to discover potential new drug compounds. Two structures from the PDB were identified for docking, namely 1S0J and 1MS8. The crystal structures were of Trans-sialidase, a surface protein on the protozoan, essential for establishing short and long term infection. The results of the docking were ranked, and the top three compounds *Dihalenaquinolide A, (+)-Ovigeridimerin,* and *Bisisodiospyrin* were further discussed. The free binding energy of *Dihalenaquinolide A* was -13.6 kcal/mol and -15.23 kcal/mol when docked to 1S0J, and -12.7 to -16.86 kcal/mol when docked to 1MS8. *(+)-Ovigeridimerin* had free binding energies of -12.6 kcal/mol to -13.50 kcal/mol to 1S0J, and -14.4 to -15.92 kcal/mol to 1MS8. Finally, *Bisisodiospyrin* had free binding energies of -12.3 kcal/mol to around -15.73 kcal/mol when docked to 1S0J, and -13.8 to -16.76 kcal/mol when docked to 1MS8. The compounds need to be further studied to verify their actual efficiency as potential novel drug candidates.

Introduction

Currently there are eight to ten million people worldwide infected with a parasitic infection known as Chagas Disease, and an estimated 300,000 live in the US (Bern, 2011). However, the malady mainly affects people in poor, rural areas in Latin America, where there is limited health care access. The vectors of the disease are hematophagous triatomine bugs (Bern, 2011) that carry the flagellated parasite, known as Trypanosoma Cruzi (T. cruzi). The parasite lives in the hind-gut of the bug, and once the bug has its blood meal, when its host is asleep, it defecates, and the parasite enters through the bite site or via contact with mucous membranes. At this stage the parasite is a metacyclic trypomastigote and it invades many different kinds of cells, where it evades host cell defenses, such as lytic processes. The metacyclic trypomastigote transforms into amastigotes within the host cell, and starts replicating until the cytoplasm is full. The intracellular amastigotes transform back into trypomastigotes, where they burst out of the cell and continue to infect other host cells.

The initial phase of infection is known as acute Chagas Disease, where the host can display symptoms similar to common cold, however, at times infected individuals have been symptom free, and the parasite proliferates without the knowledge of the host. The phase lasts about one to two months, until the replication is regulated by host immune responses (Bern, 2011). But the immune system fails to eliminate the parasite fully, and it continues to live within the host for decades. This is known as chronic Chagas Disease. During this phase, the parasite can live within the host without causing any further damage to its host, but in 20 to 50 percent of infected individuals the disease progresses into irreversible cardiac muscle damage, and gastrointestinal failure, resulting in death.

Currently, there has been some success in eliminating the parasite during acute stage, with a parasitological cure of about 60 to 80 percent (Bern, 2011). However, once the disease enters chronic phase, a parasitological cure has been estimated to be around 10 to 20 percent (Jose Rodrigues Coura, 2002). The drugs used to treat Chagas Disease are Benznidazole, and Nifurtimox, which were both developed around late 1960s and early 1970s and prove to have adverse side effects when treating Chagas. In fact, the use of Nifuritimox was discontinued first in the 1980s in Brazil with other countries following suit (Jose Rodrigues Coura, 2002). In the US, both drugs are not approved by the FDA, and can only be obtained for usage under investigational protocols from the CDC (Bern, 2011).

The issues with current drugs can be attributed to the fact that the drug is not uniquely targeting the parasite but also its host. Therefore, identifying a protein limited only to the protozoan is an attractive alternative. In this study Trans-sialidase (TcTS) was identified as a novel target. It's found solely on the surface of the protozoan, and it scavenges sialic acid molecules from the host, due to its inability of

synthesizing the molecule itself. The protein has been identified to be involved with host cell invasion, evade action of lytic antibodies, and even may have a role in immunomodulation, which allows the parasite to establish long term infection (Mendonca-Previato, 2010).

Therefore, TcTS was selected as a novel target to find potential drugs to inhibit its catalytic activity. These potential drugs were obtained from Taiwan Pharmaceutical Databank. It houses over 2,000 natural compounds that have been collected and synthesized in Taiwan. Natural products are lucrative in their drug design and have shown to have an immense importance in the pharmaceutical industry where they, or their derivatives have been readily approved by the FDA (David J Newman, 2012). Therefore in this study natural products were identified as putative drug candidates against to combat chronic Chagas Disease.

Methods/Materials

Ten structures from the PDB were originally picked to be analyzed, namely: 1S0J, 2AH2, 1S0I, 1MR5, 1MS8, 1MS5, 1MS9, 1MS3, 1MS4, 1MS0. In order to determine their usefulness in virtual screening all these structures were actual crystal structures of T. cruzi's TcTS, none of the structures were homology models derived from other species. Two of the structures were finally picked: 1S0J, and 1MS8, based on how accurate AutoDock Vina, and AutoDock 4.0 were able to reproduce the experimental results. The location of the ligand had to be in the same binding pocket, and the orientation of the ligand had to mimic the experimental results to some extent.

Each selected PDB structure, had its ligand striped away from the pdb file by editing the identity tags, HETATM from the file. Water molecules were removed, polar hydrogens were added, and Kollman Charges were added using AutoDock Tools 1.5.6. The complete structure was saved as a pdbqt file, and was used in both AutoDock Vina and AutoDock 4.0 virtual screening processes. A gridbox was set up around the binding pocket to specify where AutoDock Vina should attempt to dock the ligands. The ligands were obtained from the Taiwan Pharmaceutical Databank (http://tpd.mc.ntu.edu.tw/index.php), which were already in pdbqt format.

AutoDock Vina was first used in the screening process, the rational to use Vina first was due to the fact that its algorithm and processing time was a lot faster in obtaining results in comparison to AutoDock 4.0, so it was a good screening tool to obtain results fast. Once the docking was completed, the top results with lowest free binding energy were docked once again using AutoDock 4.0, in order to confirm and obtain the binding energies predicted by AutoDock 4.0. Unlike AutoDock Vina, AutoDock 4.0 accounts for the electrostatic interactions, which the majority of binding activity between ligand and Trans-sialidase are through hydrogen bond interactions. Therefore, electrostatic forces are thought to be significant in this reaction, in addition to several key residues in the binding pocket are charged.

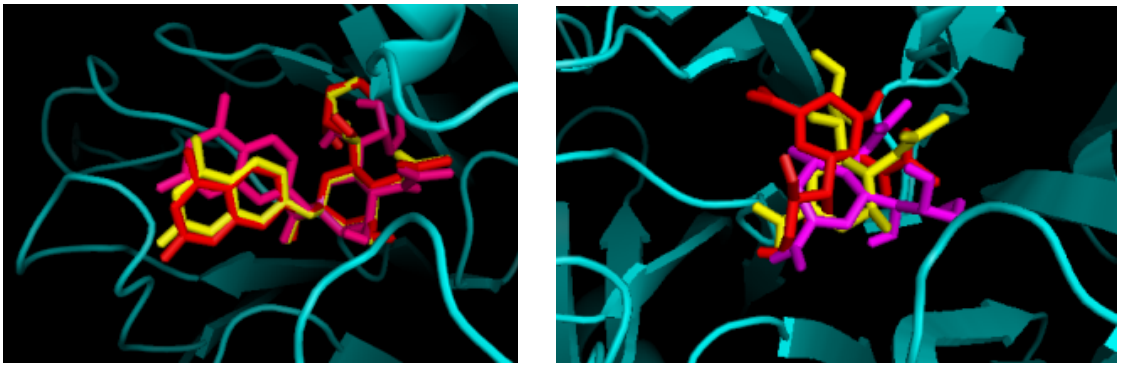


Figure 1: Key: Vina in yellow, AD4 in red, and experimental result in pink. Both Vina and AD4 were able to recreate the binding fairly accurate.

Results

After running AutoDock Vina to screen potential compounds in the Taiwan Pharmaceutical Databank, the compounds were ranked in base of their free energy results, from most negative (highest

binding affinity) to the least negative (low binding affinity). A text file was generated with all the compounds ranked accordingly. The top ten compounds are demonstrated in the Table 1 and Table 2. To the most left of the table is the compound's rank. Thereafter, the numbers following TPD is the compound's SN number, the number that can be used to find the compounds in the Databank by using a URL link (http://tpd.mc.ntu.edu.tw/compound.php?SN=) where the ten to twelve digit number follows the equal sign. The binding affinity can be found to the right of the compound's SN number.

Five compounds show up in both tables, their SN numbers are: 71184833541, 91185766573, 281010096476, 71184834376, and 91186211040. However, in this paper the top three compounds are only highlighted namely, 71184833541, 91185766573, and 281010096476. These compounds were consistently among the top three regardless of what protein structure they were docked to.

Top 10 Binding Affinities for 1S0J

Rank	File Name	Free Binding Energy
1	**TPD.71184833541_out.pdbqt**	**-13.6**
2	**TPD.91185766573_out.pdbqt**	**-12.6**
3	**TPD.281010096476_out.pdbqt**	**-12.3**
4	TPD.281011317462_out.pdbqt	-12.3
5	**TPD.71184834376_out.pdbqt**	**-12.2**
6	TPD.281010461515_out.pdbqt	-12.1
7	**TPD.91186211040_out.pdbqt**	**-12.1**
8	TPD.101185345882_out.pdbqt	-12.0
9	TPD.281011318172_out.pdbqt	-12.0
10	TPD.281011317061_out.pdbqt	-11.8

Table 1

Top 10 Binding Affinities for 1MS8

Rank	File Name	Free Binding Energy
1	**TPD.91185766573_out.pdbqt**	**-14.4**
2	**TPD.281010096476_out.pdbqt**	**-13.8**
3	**TPD.71184833541_out.pdbqt**	**-12.7**
4	TPD.281011409415_out.pdbqt	-12.4
5	TPD.281011409716_out.pdbqt	-12.4
6	**TPD.71184834376_out.pdbqt**	**-12.3**
7	**TPD.91186211040_out.pdbqt**	**-11.7**
8	TPD.281011297903_out.pdbqt	-11.6
9	TPD.281011398209_out.pdbqt	-11.6
10	TPD.281011399613_out.pdbqt	-11.6

Table 2

Ranked number one to 1S0J was *Dihalenaquinolide A* (SN: 71184833541) was derived from a Taiwanese marine sponge, *petrosia elasticia.* It's a fairly non-polar compound with ten aromatic rings. Two regions of the compound have a higher electronegativity than other regions, however, that can probably be attributed to the two ketone groups and an ester group on the aromatic rings. The mass of the compound was around 694 amu.

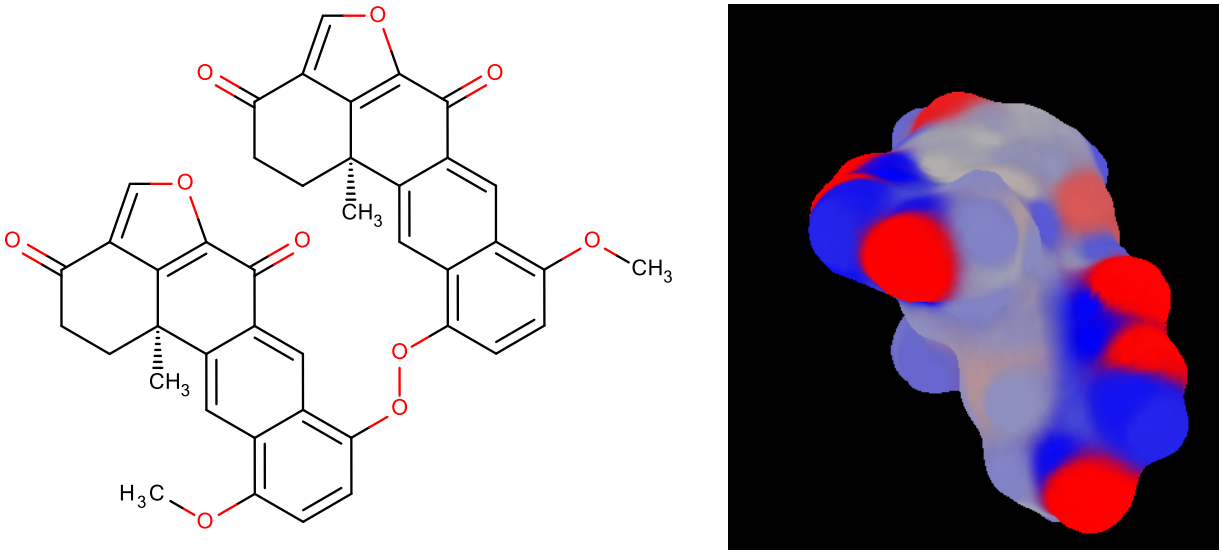


Figure 2: (left) chemical structure of *Dihalenaquinolide A*. (Right) charges distribution of the chemical.

The compound was docked successfully to both 1S0J and 1MS8. When docked to 1S0J Vina predicted a free binding energy of -13.6 kcal/mol, whereas AD4 predicted -15.23 kcal/mol. Vina's result placed *Dihalenaquinolide A* as the compound with the lowest free binding energy out of the whole compound databank, whereas when docked using AD4 the compound had the second lowest free binding energy behind *Bisisodiospyrin*. When the same compound was docked to 1M08, Vina ranked the compound as the third lowest free binding energy compound with -12.7 kcal/mol, and AD4 ranked it as number one with -16.86 kcal/mol.

Dihalenaquinolide A				
Docking Method	Rank	1S0J	Rank	1MS8
Vina (kcal/mol)	1	-13.6	3	-12.7
AD4 (kcal/mol)	2	-15.23	1	-16.86

When running a perl script it was verified that the compound interacts with key residues, Asp59, Glu230, and Tyr342 for both compounds. It should also be noted that when the compounds were docked to 1S0J there was a high agreement on chemical orientation of the compounds, whereas, when the compounds were docked to 1MS8, the binding pocket was similar but the chemical orientation was a little different.

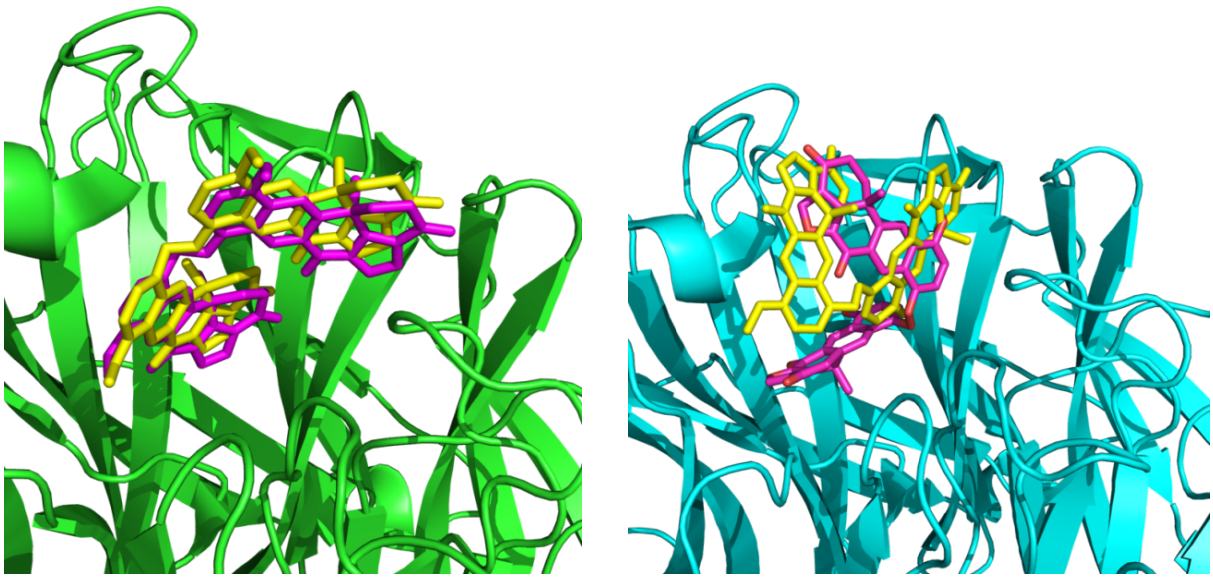


Figure 3: To the left is the compound *Dihalenaquinolide* A, docked to 1S0J, and to the right it's docked to 1MS8. Vina's prediction is the yellow structure, and the pink structure is AD4's prediction.

The next highest ranking compound to 1S0J was *(+)-Ovigeridimerin* (SN: 91185766573 and it's derived from the trunk bark of *hernandia nymphaeifolia*, collected from the green island, Taiwan. The free binding energy of the extracted compound was predicted by vina to be -12.6 kcal/mol, and ranked second lowest free binding energy, whereas AD4 predicted it to be at -13.50 kcal/mol, ranking it as the third lowest free binding energy. When docked to 1MS8, vina ranked the compound as lowest free binding energy compound with -14.4 kcal/mol, and AD4 predicted the free binding energy to be -15.92 kcal/mol. This ranked the compound

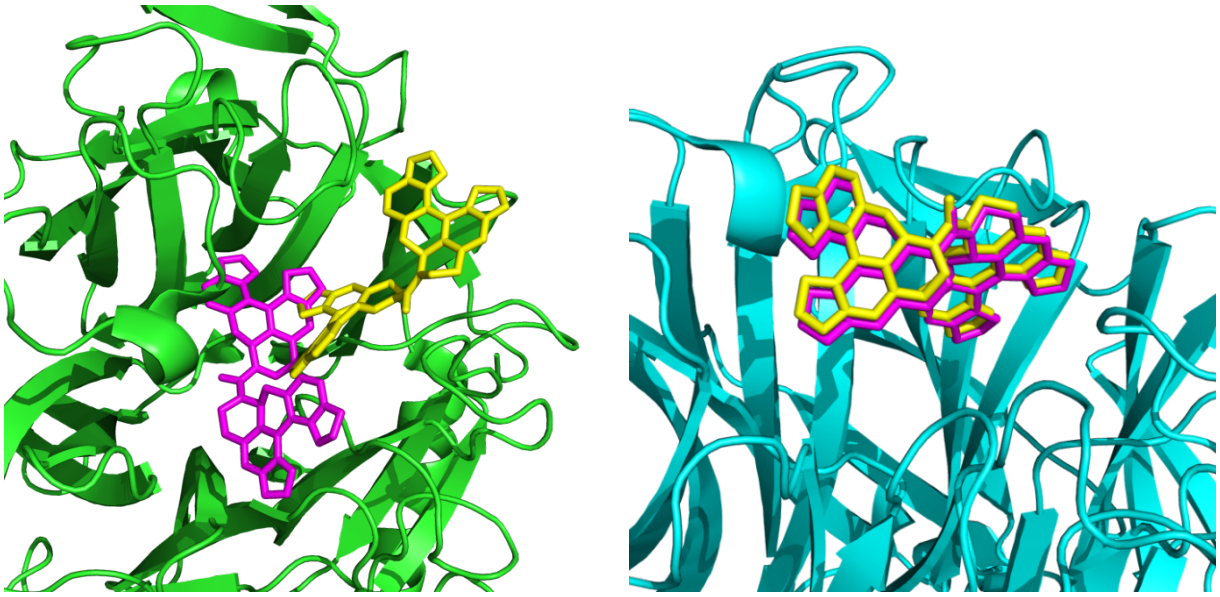
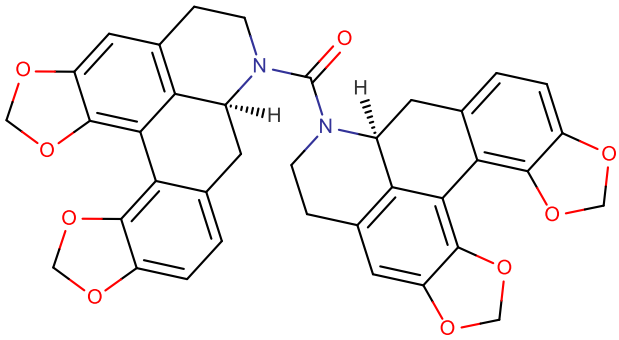
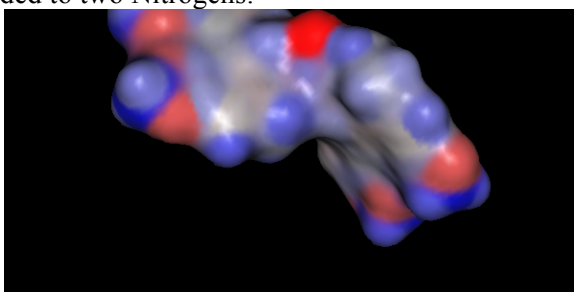


Figure 4: *(left) (+)-Ovigeridimerin* docked to 1S0J. *(Right)* same compound docked to 1MS8. The yellow compound is vina's prediction, the pink is AD4 prediction.

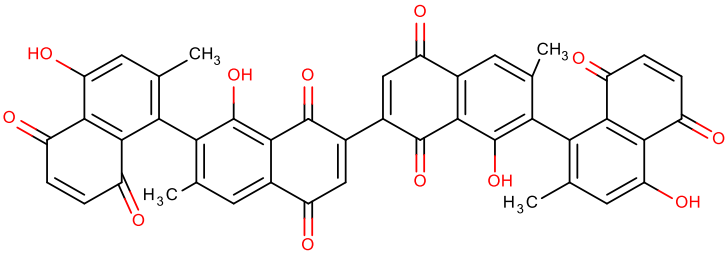
(+)-Ovigeridinerin				
Docking Method	Rank	1S0J	Rank	1MS8
Vina (kcal/mol)	2	-12.6	1	-14.4
AD4 (kcal/mol)	3	-13.50	3	-15.92

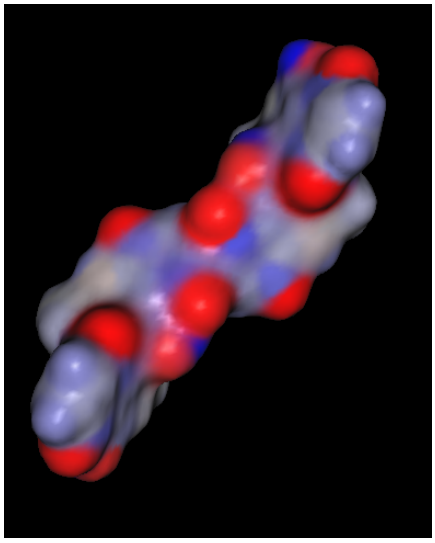
as the third lowest free binding energy compound. Moreover, a perl script determined that the same key residues had interactions between the compounds and the protein structure. The chemical composition of *(+)-Ovigeridimerin* is C37H28N2O9, and it has a mass of 644 amu. The compound is fairly non-polar with the except for one region on the compound, the ketone bonded to two Nitrogens.





Next compound has the chemical name *Bisisodiospyrin* (SN: 281010096476,) and it's from the stem of *diospyros maritime*. The compound was collected in Linkou, Taiwan. The compound is fairly non-polar, however, due to many ketone functional groups, and alcohol groups, there are significant more areas on this compound that causes some polarity to be significantly different from the other two compounds. The mass of the compound is estimated to be around 746 amu.





The compound ranked third with a free binding energy of -12.7 kcal/mol to 1S0J, however, when using the same model, AD4 predicted a binding energy of -15.73kcal/mol which was the lowest free binding energy out of the three compounds. When docked, using vina, to 1MS8 the compound had a predicted free binding energy of -13.8 kcal/mol, and AD4 predicted it to be -16.76 kcal/mol.

Bisiodiospyrin				
Docking Methods	Rank	1S0J	Rank	1MS8
Vina (kcal/mol)	3	-12.3	2	-13.8
AD4 (kcal/mol)	1	-15.73	2	-16.76

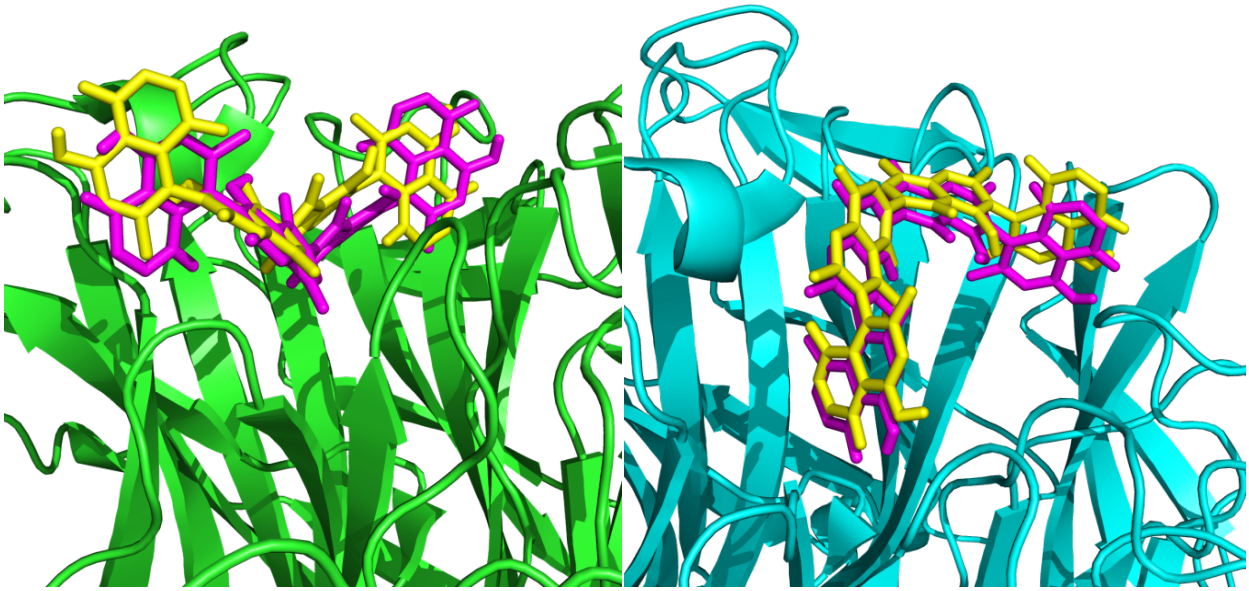


Figure 5: *(left) Bisisodiospyrin* docked to 1S0J. *(Right)* same compound docked to 1MS8. The yellow compound is vina's prediction, the pink is AD4 prediction.

Discussion

Taiwan Pharmaceutical Databank (http://tpd.mc.ntu.edu.tw/) contains about +2,000 compounds. All of the compounds were extracted from local fauna, both terrestrial and marine. Tropical fauna has an immense biodiversity and harbors the potential to treat current maladies. These compounds were docked against Trans-sialidase's structure in order to determine compounds that could function as potential competitive inhibitor, and in effect, inhibit T. Cruzi's ability to continue infecting and developing within its host. Trans-sialidase (TcTS) has been linked to several features that allow its success. For instance, the protein has been linked to promote host cell invasion, and impede the action of lytic antibodies (Mendonca-Previato, 2010) which allows the parasite to manifest itself in its host for decades. The protein's main function, however, is to obtain sialic acid from host cells, due to its inability of synthesizing the monosaccharide itself. The absence of this enzyme in mammals and the seemingly importance it has for the protozoan makes this protein a very attractive drug target.

Therefore, compounds were obtained from the Databank and docked to two structures from the PDB, 1S08 and 1MS8. Both these structures were selected due to the fact that the docking programs, vina and AD4, were able reproduce the crystral structure interactions with ligands. By docking the compounds from the databank in the same way, a ranking of compounds was determined, and the top three were highlighted. One of the promising compounds that continuously ranked high based on its free binding energy was *Dihalenaquinolide A* (SN: 71184833541). Its estimated energies were between -13.6 kcal/mol and -15.23 kcal/mol when docked to 1S0J, and -12.7 to -16.86 when docked to 1MS8. This compound

which is fairly non-polar due to its ten aromatic rings has a structure significantly different from Sialic Acid. It has a mass that is more than twice of sialic acid, and contains no alcohol groups. However, the static structure could pronate in aqueous solution and develop more alcohol groups that mimic sialic acid functional groups even closer. Furthermore, it was determined that the compound interacts with key residues, such as Asp59, Glu230, and Tyr342, which have been deemed as key for TcTS processes (Mendonca-Previato, 2010). It's also interesting to note that the compound is derived from a marine sponge called *petrosia elasticia*. Sponges are known to have antimicrobial properties (Laport, 2009), so this might be a promising indicator that this compound is a viable candidate.

Another promising compound was *(+)-Ovigeridimerin* (SN: 91185766573), with binding energies between -12.6 kcal/mol to -13.50 kcal/mol to 1S0J, and -14.4 to -15.92 kcal/mol to 1MS8. The compound is extracted from the trunk bark of *hernandia nymphaeifolia*, a tree widely distributed in tropical climates that's not only limited to Taiwan, though this compound was collected on the green island in Taiwan. The chemical composition of this compound is also different from sialic acid. The compound is non-polar like sialic acid, however, its mass is more than twice of sialic acid, and it contains 12 aromatic rings, which have no alcohol functional groups. The compound interacts with the same essential residues as *Dihalenaquinolide A*, which indicates it could be a promising drug target.

The third compound with consistent low binding energies was *Bisisodiospyrin* (SN: 281010096476) and it's from the stem of *diospyros maritime*. Its binding energies were around -12.3 kcal/mol to around -15.73 kcal/mol then docked to 1S0J, and -13.8 to -16.76 kcal/mol when docked to 1MS8. It was determined that it had interactions with essential residues. The compounds structure is different from sialic acid as well, with an atomic mass of 746 amu, which is almost 2.5 times greater than sialic acid. The functional groups on the compound are both alcohols and ketones, which can be pronated into alcohols, and resemble sialic acid functional groups as well.

Even though some compounds might show promising results, it is important to consider that they are merely predicted values. Vina is a popular docking program due to its speed and relative accuracy. However, its scoring function doesn't account for the electrostatic interactions between residues, and ligand, which can cause the free binding energy to be inaccurate. Perhaps the difference in AD4 docking predictions and vina can be attributed to the disregard of electrostatic interactions, since AD4 takes them into account. It's also worth mentioning that the ranking of compounds were slightly different when AD4 was used to dock the compound, but in general the ranking remained the same. However, during the preparation phase of the pdbqt files, water molecules were removed from the structure, even though a significant amount of interactions between residues and ligand are de facto via hydrogen bonding. These water molecules, despite their importance in stabilizing the compound, were removed to allow the ligand full mobility to interact with only residues. Nevertheless, this doesn't diminish the importance of water in the binding pocket and therefore the compounds have to be further studied to determine if they really interact in a promising way.

The highlighted compounds all interacted with key residues for catalysis, namely Asp59, Glu230, and Tyr342. In edition other significant residues are, Tyr119, the Arg triad group (Arg35, Arg245, Arg314), and Asp96, which all three compounds also interact with, except when *Bisisodiospyrin* was docked to 1MS8, it lacked interaction to Asp96, and Glu230. The mode of interaction between sialic acid and TcTs is thought to be a classical ping-pong mechanism, where the sialic acid interacts with the enzyme by initially displacing Tyr119 from binding site, and its carboxylate group interacts with the Arg triad group, while its acetamido group interacts with Asp96. Eventually, a covalent linkage is formed due to Tyr342 interaction, assisted by Glu230, and then the sialyl-enzyme intermediate becomes deprotonated by Asp59, which functions as an acid/base catalyst (Mendonca-Previato, 2010). The mechanism requires, mainly two functional groups from the sialic acid, a carboxylate, and an acetamido group. The compounds identified from the virtual screening lack both functional groups. However, it was verified by running a perl script that there are interactions between the compounds and the protein structure. In fact, even though the compounds lack similarity in chemical structure and composition to sialic acid, it cannot be ruled out that the compounds could function as a competitive inhibitor to TcTS. This could occur via interacting with the protein and altering its essential shape to catalyze sialic acid. This in effect would

prevent the protein to function as expected, and T. Cruzi would not be able to infect nor establish a long time presence in the host. Additionally, it cannot be ruled out either that despite the fact that the compounds might have a potential to inhibit TcTS and its activity, they might have further implications elsewhere in the host, and it is essential to verify if any off targets exist. This could be checked using SMAP, a component that wasn't studied in this research.

In general, natural products are novel in their drug design, and can often be used either as drugs to treat maladies, or function as a template for further synthetic drug design. In the past natural products accounted for big percentages of approved drugs by the FDA (David J Newman, 2012). Even though natural products are so lucrative, they can at times be difficult to obtain or synthesize, which can inhibit their attractiveness as a putative drug. However, currently 8 to 10 million people worldwide are infected with Chagas Disease, and therefore any potential drug candidates would be an attractive solution for both pharmaceuticals and people who are currently battling this detrimental disease.

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