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In silico screening of antitrypanosomal natural products against Trypanosoma cruzi proteins

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IN SILICO SCREENING OF ANTITRYPANOSOMAL NATURAL PRODUCTS AGAINST *TRYPANOSOMA CRUZI* PROTEINS

by

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ABSTRACT
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Name of Candidate: Stephanie Felker McCulley
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Chagas disease is a debilitating condition caused by the parasite *Trypanosoma cruzi* and is endemic to many countries in Latin America. Current treatments for parasite infection are not very effective and cause a host of potentially life-threatening side effects, so need for alternative treatments is dire. Eighty natural products from South and Central America have been found to have trypanocidal activity, but the targets of these agents is yet unknown. The purpose of this investigation was to find the binding affinity of these agents to known *T. cruzi* drug targets *in silico* and compare these results to those of human analogs. Forty-five antitrypanosomal agents were found to have selective binding with parasite proteins, but only five showed pharmaceutical promise with no notable docking with the human analogs investigated, the oxygenated hydrocarbons 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyhepatadec-16-yne, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, and (*E*)-1,2,4-trihydroxynonadec-6-ene, along with the alkaloid *N*-methyltetrahydropalmitine. Hydrogen bonding of these agents with drug targets was investigated, as well as the nature of hydrophobic interactions.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Purpose of Study

Chagas Disease, also known as American Trypanosomiasis, is caused by the parasite *Trypanosoma cruzi* and affects millions of people in Latin America, North America, Europe, and Japan [Bern et al., 2011]. There are several current treatments for the disease including nifurtimox and benznidazole, both of which have adverse effects, often leading to discontinuation, and neither is FDA-approved. These drugs have relatively low efficacy and do not affect progression of the disease in several organ systems [Bern 2011]. There is a treatment gap that needs to be assessed, and natural products solution may be able fulfill the efficacy and safety requirements for an acceptable treatment in the United States.

Virtual molecular docking is a relatively easy and inexpensive way to screen small molecules for potential pharmaceutical purposes. With current databases and cheminformatic software available protein-ligand docking screenings have become a more popular method of drug discovery in recent years. These methods, in combination with natural products drug discovery, can lead to innovative and efficient systems of drug discovery [Koutsoukas, 2011]. Dozens of potential drug targets have been identified in *T. cruzi*, and these protein structures have previously been determined via X-ray crystallography and are available from the Protein Data Bank. Over one hundred different natural products from several different continents have been found to have activity against *T. cruzi* [Izumi 2011]. This work includes the virtual screening of 80 different potential antitrypanosomal agents with structures from 19 different *T. cruzi* protein targets and their respective human analogs.

1.2 Virtual Screening

Virtual screening is a useful and inexpensive method of drug discovery and is gaining more momentum as chemical databases are becoming more substantial and new X-ray crystallographic structures are being added to the Protein Data Bank every day. Using these structures working binding sites of proteins can be more accurately predicted, and as readily available computational

power grows exponentially, computational screening is becoming faster and more sophisticated [Kirchmair et al. 2009]. With the wide availability of personal computers and powerful software packages, this type of screening is more accessible, less expensive, and faster than many current laboratory techniques. Available molecular docking software programs include DOCK, FlexX, GLIDE, ICM, PhDOCK, Surflex [Cross, 2009], Molegro Virtual Docker and AutoDock [Shityakov and Dandekar, 2010].

1.2.1 Common Strategies in Virtual Screening

To perform a molecular docking screen, a molecular target needs to be chosen with an appropriate set of ligands. This target can be a protein or another type of biologically relevant macromolecule such as large ribonucleic acids with active sites for ligand binding. Crystallographic structures of these targets are readily available using the Protein Data Bank along with the respective journal articles noting structure features including major domains and active sites. The ligand compound set can be created using molecular modeling software such as SPARTAN [SPARTAN, 2010] or ChemSketch [*ACD/Chem*, 2006], as it was in this work, or these structures can be found using electronic ligand databases. Databases such as PubChem [Wang, 2009] and ASINEX [Guiles, 2012] can provide access to myriad compounds and the accompanying information required for virtual screening.

1.2.2 Conformation Search Algorithms

Computationally analyzing a protein-ligand relationship would be daunting without particular search algorithms in place. To conduct such simulations with no guidance would lead to endless computing while the program tediously ran through each of the millions of different conformations in which a protein and ligand can participate over a given binding site. Given this hurdle, certain algorithms can be exploited to give “best” docking conformation in the shortest amount of time. These processes typically utilize several types of algorithms including Monte Carlo, fast shape matching, incremental constitution, distance geometry, simulated annealing, tabu search, genetic programming, and evolutionary programming methods [Herberlé and

Azevedo, 2011]. Molegro Virtual Docker utilizes the MolDock Optimizer search algorithm, which is based on a guided differential evolution algorithm. In this particular method, a randomly selected base set of solutions, referred to as "parents," is exposed to modification. In the case of guided differential evolution, this modification is the addition of a weighted difference of the previous generation. After modification the parent offspring mutate into an "offspring" state, and if these offspring are "more fit" or score higher in the program, they will in turn become parents to a new generation of offspring after mutation. If the parent solution scores higher than their subsequent offspring, the parents move on to the next generation without modification. In this method, possible pose candidates vary quite widely at initiation, but as the algorithm is carried out, these poses grow more and more similar as the simulation finishes. This is a particularly effective method when compared to a systematic full space search which would entail the time-consuming comparisons of randomly generated poses [Molegro, 2011].

1.2.3 Scoring Functions

In coordination with the computational algorithms, protein-ligand docking calculations require the ability to rank the resulting poses based on theoretical binding affinity. This is done by analyzing various physicochemical properties of the interaction such as the ligand's position in the binding pocket, hydrogen bonding, and hydrophobic interactions to "score" each pose. Scoring functions can exploit many different methods to rank the results. These methods initially relied heavily on force-field-derived approximations, which take into account electrostatic, hydrophobic, van der Waals, and hydrogen binding interactions but neglect effects like π-stacking that require a less static molecular model than force-field methods allow. More recently knowledge-based scoring functions have been incorporated, which take into consideration experimental binding data to give more effective energy approximations [Hsieh et al., 2012]. Empirical or regression methods incorporate a weighted binding energy equation alongside empirically-derived binding information from an X-ray crystallographic training set [Ferrara et al., 2004]. The scoring function utilized by Molegro is MolDock score, which is force-field-

based, and in conjunction with the guided differential evolution algorithm is considered one of the more accurate theoretical docking protocols, especially when using a re-rank procedure (which was exploited in this work) that takes the best scoring solutions of multiple runs and "re-ranks" them based on and additional scoring function [Thomsen and Christensen, 2006] involving a weighted linear combination of additional energy terms. The more negative the re-rank score, the better the protein-ligand interaction is considered [Molegro 2011].

1.2.4 Compound Construction and Conformer Energy Calculation

In coordination with proper protein crystal structures, one must also obtain or somehow generate structures for the small molecules of interest. Protein crystal structures can be readily downloaded from an online source such as Protein Data Bank, and similar resources are available for small molecules such as *ChemBank*, DrugBank, PubChem, and ZINC [Seiler et al., 2008]. Because of the diversity inherent in small molecules, there may be a necessity to build the molecule specifically for a particular investigation, as it was in this study. There are dozens of different molecular modeling software packages available including the Hyperchem, Gaussian, MOPAC, and Spartan, software packages, the last of which was used for this investigation.

As with molecular docking, there are thousands of different geometrical conformations each ligand can take, and there are many different methods one can use to find the lowest energy conformer of a particular small molecule. Molecular mechanics models such as SYBYL and MMFF force field methods calculate the energy of a conformer as a sum of the energies from bond distances as well as torsion and bond angles in conjunction with non-bonding interactions such as van der Waals and Coulombic interactions. The drawback of these models is that although they vary in the complexity of their parameters (MMFF is much more complex than SYBYL), molecular mechanics models are still fairly simple. Quantum chemical models are estimates based around the Schrödinger equation adapted for the incorporation of many electrons in a molecular setting. The Hartree-Fock approximation modifies the Schrödinger equation by using a "self-consistent-field" procedure where the base equation used is:

$$f(i)\chi(\boldsymbol{x_i}) = \boldsymbol{\varepsilon\chi}(\boldsymbol{x_i})$$

where χ are the spin orbitals, x_i are the spin and spatial coordinates of an electron, ε is the orbital energy and f(i) is the Fock operator given by:

$$f(i) = -\frac{1}{2}\nabla_i^2 + v^{eff}$$

where v^{eff} is the effective potential involved with a particular electron and ∇ is the del operator. A Gaussian basis set is used in conjunction with the Hartree-Fock approximation [Hehre, 2003].

1.2.5 Successes of Virtual Screening

The number of structures available through proteomic and genomic resources has provided the computational chemistry community with a large foundation for virtual screening. Due to the rapid and high-output nature of molecular virtual screening, a great number of target-ligand interactions have been investigated in the name of drug discovery. This structure-based computer-aided drug design allows for a protein with a known 3D structure involved in a particular disease or disorder to undergo a barrage of potential ligands, computationally, and after lowest-energy conformations of this binding nature of the target-ligand complex, the narrowed possible leads can then undergo binding assays in a laboratory environment, and if successful, they can progress to further testing and clinical trials. Pharmaceutical discoveries made using these methods include the influenza treatment zanamivir, neuramidase inhibitor, which is of particular note because this enzyme is not only essential to influenza replication, but is conserved in clinically relevant strains of the virus.

Even when the structure of the drug target is unknown, there are several success stories of ligand-based drug design, where a particular inhibitor of a protein is known, and screening of similar molecules is conducted to find other novel ligands. The Human Immunodeficiency Virus type 1 (HIV-1) integrase was examined in such a way with a known inhibitor, caffeic acid phenethyl ester as a template. Four potent inhibitors with an IC_{50} of less than 30 μM were found using this method. Another method using quantitative structure-activity relationship (QSAR)

models involves regression models that predict biological activities with various chemical properties. One QSAR study involved developing an acetylcholinesterase inhibitor from a lead from a random screening, benzylpiperazine. Derivatives calculated were developed into the compound donepizil hydrochloride, marketed as Aricept, for the treatment of Alzheimer's disease. Although few computational leads have been developed into marketable therapeutic drugs, with more computational and software developments being made every year, the future of molecular virtual screening looks promising [Ooms, 2000].

1.2.6 Limitations of Virtual Screening

Although computer-aided drug design has a number of success stories, there are still limitations of *in silico* methods. One of the major drawbacks of this method is the nature of the structural data. Protein structures are largely obtained using X-ray crystallographic methods, and the resulting structures are protein conformation under crystallographic conditions, which may vary widely from the bioactive protein structure found *in vivo*. These protein structures are also rigid, ignoring the flexibility of the protein found in its natural state. The same can be said for ligand flexibility, as the lowest-energy conformer may not be the molecular state found in biological conditions, and that molecule flexibility is difficult to take into consideration *in silico*. Solvation of the target and ligand in the biological environment stands as another obstacle because to incorporate hundreds of water molecules into a simulation is a great computational feat, adding hours, if not days, to the time spent on each individual simulation. This factor is becoming less of a problem as calculation time speeds up with every new generation of processor, but as it stands today, desolvating the simulation is common place. In addition, algorithms and scoring functions used in docking software vary widely, so lead scores can be considered significant using one program, but may not be considered so in others. Great consideration must be taken to choose the most appropriate docking tool, and with cost and computing power as influencing variables, results may vary in accuracy [Klebe, 2006].

That being said, the influence of these factors is being diminished as the relatively new field of computational chemistry matures and new technology is being developed. Induced fit docking, which accounts for some protein-ligand flexibility, continues to be refined, and the incorporation of solvating water molecules into docking simulations is becoming more common place [Borrelli et al., 2009; Sulea et al., 2012].

1.3 Natural Products as Source of Drugs

Natural products remain to be a major source of pharmaceutical small molecules with over half of the small molecule new chemical entities from January of 1981 to June of 2006 being natural products or natural product derivatives [Newmann and Cragg, 2007]. Although, with the advent of current biotechnological techniques in synthetic drug development and genetic sequencing, the interest in natural products has declined, compounds from natural sources remain to be an inspiration in synthetic pharmaceutical development and the limitations of natural product drug discovery have yet to be reached with millions of species left unanalyzed. New biosynthetic and genetic technologies can be used in conjunction with natural products drug discovery to lead to novel pharmaceutical agents and treatments [Walsh and Fischbach, 2010]. Recent drugs derived from natural products include Prialt, a synthetic derivative of the cone snail toxin ω-conotoxin MVIIA, which is a treatment for chronic pain [Molinski et al., 2009] and galantamine, a treatment for Alzheimer's disease, from *Leucojum aestivum* [Halpin et al, 2010]. As of 2008, 225 natural products and natural product derivatives were in development [Harvey, 2008].

1.3.1 Antitrypanosomal Natural Products from Latin America

Human trypanomiasis caused by *Trypanosoma cruzi* is a long-neglected disease, but research into this debilitating condition is starting to gather steam. A review of current known antitrypanosomal natural products was published with over 130 plant-derived compounds that showed activity against *T. cruzi*. This included various alkaloids, terpenoids, phenolics, and other compounds from plants all around the world, with a majority of the compounds from Central and

South American sources [Izumi et al, 2011]. A complete list of the structures and plant sources of the compounds examined in this source can be found in Table 2.1.

1.3.2 Antitrypanosomal Alkaloids

Alkaloids are nitrogen-containing compounds that are usually heterocyclic, and this class contains a significant amount of bioactive chemicals [IUPAC, 1997]. Alkaloids are a very common class of pharmaceutical agents and roughly 50% of plant-derived natural products are alkaloid in nature [Cordell et al., 2001). Alkaloids make up a large portion of compounds from Latin American sources that show activity against *T. cruzi*. From the Lauraceae *Ocotea lancifolia,* eleven compounds have had antitrypanosomal activity: caaverine, coclaurine, corytuberine, domesticine, glazovine, isoboldine, laurotetanine, *N*-methylcoclaurine, nordomesticine, norisoboldine, and pallidine. Antitrypanosomal agents found in *Almeidea rubra* in the Rutaccae family are 4-methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one, arborinine, kokusagine, *N*-methyl-1-hydroxy-3-methoxyacridone, *rel*-(7*R*,8*R*)-8-[(*E*)-3-hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate, and skimmianine. Five antitrypanosomal alkaloids have been isolated from the Annonnaceae *Dugetia furfuracea*: Dicenitrinone, dugetine, dugetine-β-*N*-oxide, *N*-methylglaucine, and *N*-methyltetrahydropalmitine. 5-Methoxycanthin-6-one and canthin-6-one were the only two alkaloids from the Rutaccae *Zanthoxylum chiloperone*, and only one alkaloid with activity against *T. cruzi from the* Solanaccae *Saracha punctata* was identified, sarachine [Izumi 2011].

1.3.3 Antitrypanosomal Terpenoids

Terpenes currently stand as the most common type of natural products, and are present in all domains of life as a chemical signal and a natural defense (Gershenzon and Dudavera, 2007). Terpenoids are compounds that consist of repeating five-carbon isoprene units, and may have oxygen-containing substituents. Division in this class is made by the number of isoprene units present [IUPAC, 1997]. Terpenoids studied in this work were of the di-, tri-, and sesquiterpene varieties. From the Fabaceae *Myrospermum frutescens*, 18-acetoxy-13-15-diene-cassanoic acid,

18-hydroxycassan-13-15-diene, 6β,13β-dihydroxy-18-acetoxy-cassan-14(17),15-diene, 6β-18-dihydroxycasssan-13,15-diene, and 6β-hydroxy-18-acetoxycassan-13,15-diene, all diterpenes, have previously shown anti-trypanosomal activity. *Lychnophora pohlii*, an Asteraceae, produces four trypanocidal terpenes, 15-deoxygoyazenolide, goyazenolide, centratherin, and lynchopholide, all sesquiterpenes. *Arrabidaea triplinervia* of Bignoniaceae has only two known trypanocidal terpenes, both being triterpenes: oleanolic acid and ursolic acid. The terpenes from the Asteraceae *Gaillardia megapotamica* are the sesquiterpenes helenalin and mexicanin. *Bertholletia excelsa* (Lecythidaceae), *Mikania hoehnei* (Asteraceae), *Mikania stipulacea* (Asteraceae), *Pterodon pubescens* (Fabaceae), and *Salvia gilliessi* (Lamiaceae) only contribute one trypanocidal terpene each: betulinic acid (triterpene), 8β-hydroxyzaluzanin D (sesquiterpene), ent-9α-Hydroxy-15β-E-cinnamoyloxy-16-kauren-19-oic acid (diterpene), geranygeraniol (diterpene), and 5-*epi*-icetexone (diterpene), respectively [Izumi 2011].

1.3.4 Antitrypanosomal Phenolics

Phenolic compounds are simply those that contain a hydroxyl group attached to a benzene ring, which include flavonoids (compounds with flavones as a structural base) and lignans (compounds with two coupled *p*-propylphenols) [IUPAC, 1997]. These compounds are commonly found in plants and are known for their medical benefits such as antioxidant activity in low doses [Tanaka et al., 1993]. *Lychnophora pohlii*, an Asteraceae, contains ten known phenolics with activity against *T. cruzi*: the flavonoids ganglin, ganglin 3-methyl ether, luteolin, pinobanksin, pinobanksin 3-acetate, pinocembrin, quercetin 3-methyl ether, and tectochrysin, along with two miscellaneous phenolics 4,5-di-*E*-*O*-caffeyoquinic acid and caffeic acid. The Lauraceae *Nectandra megapotamica* has seven lignans examined for anti-trypanosomal activity: aristolignan, calopipitin, galgravin, ganschisandrine, machilin G, nectandrin A, and nectandrin B. *Piper regnellii*, of the Piperaceae family contributed four lignans to screening: conocarpan, eupomatenoid-3, eupomatenoid-5, and eupomatenoid-6, while another Piperaceae, *Piper solmsianum* contributed three lignans, grandisin, *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-

3,4,5,5′-tetramethoxy-7,7′-epoxylignan, and *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan. *Arrabidaea triplinervia* (Bignoniaceae), *Baccharis retusa* (Asteraceae), *Notholaena nivea* (Pteridaceae), and *Oxalis erythrorrhiza* (Oxalidaceae) contributed one phenolic each to this study, alpinitine (flavonoid), 5,6,7-trihydroxy-4'-methoxyflavone (flavonoid), isonoholaenic acid (miscellaneous phenolic), and 3-heptadecyl-5-methoyphenol (miscellaneous phenolic), respectively.

1.3.5 Antitrypanosomal Oxygenated Hydrocarbons

Compounds that did not fit into the alkaloid, terpenoid, or phenolic classes were categorized as "oxygenated hydrocarbons" and these compounds made up a very small percent of the compounds analyzed. Almost all of these compounds are from the Lauraceae *Persea americana*: 1,2,4-trihydroxyheptadecane, 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyheptadec-16-yne, 1-acetoxy-2,4-dihydroxyheptadec-16-ene, 1-acetoxy-2,4-dihydroxyheptadec-16-yne, 4-acetoxy-1,2-dihydroxyhepta-dec-16-ene, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, and *E*-1,2,4-trihydroxynonadec-6-ene. Omly one compound was studied from the Oxalidaceae *Oxalis erythrorrhiza*, embelin.

1.4 Chagas Disease

Trypanosoma cruzi is the parasitic agent responsible for Chagas disease (American trypanosomiasis), a debilitating condition endemic to areas of Central and South America. The disease can be easily identified and treated with moderate success in the acute stage (up to 8-12 weeks after time of infection), but, as acutely infected individuals are frequently asymptomatic, the parasite often goes undiagnosed and the infection enters the chronic stage. If left untreated the infection is life-threatening, and chronic *T. cruzi* infection can cause cardiomyopathy, aneurisms, and gastrodiskinesia. The parasite can be transmitted through a Triatominae insect vector, orally (via contaminated food), and congenitally as well as through organ transplantation and blood transfusion. Although Chagas disease is endemic to many countries in Latin America, there has been a recent globalization of the parasite through immigration and goods transport. Currently

between eight and ten million people are infected with the parasite, and over 300,000 infected individuals are living in the United States, where there are also frequent cases of animal infection in the southern lower half of the country [Bern et al., 2011]. Although only 20-30% of individuals who have acquired the parasite will progress to the chronic stage, Chagas disease still takes 14,000 lives a year. Preventative measures are present in most endemic areas including the use of pesticides and insect repellent to prevent transmission via the Triatominae vector, but in rural and marginalized areas this disease is still a major problem. Re-emergence in previously controlled demographics is common and is only getting worse with pesticide resistance and lessening political action [Degaldo et al., 2011].

1.4.1 Life Cycle of *Trypanosoma cruzi* and Clinical Manifestation

The *Trypanosoma cruzi* life cycle begins in the gut of the insect vector, Triatominae, also known as "kissing bugs" which are native to South and Central American as well as the southern half of the United States. The protozoa replicate as epimastigotes in the gut of the insect and move to the rectum where the infective stage of the parasite, trypomastigotes, are released from the insect in feces. The parasite can then enter a mammalian host if these feces are rubbed into mucous membranes or broken skin, often the result of the insect taking a blood meal from the host. The parasites are then transformed to amastigotes, an intracellular stage where replication takes place until they burst from the host cells, and convert again to the infective trypomastigote stage. These trypomastigotes can then infect other parts of the mammalian host via the blood stream, or can be taken back up by the Triatomine in a blood meal, where epimastigote stage again becomes prominent and replication can occur once more in the gut of the insect (Figure 1.1) [Minning et al., 2009]. During the acute stages of this disease, within the first six to eight weeks of mammalian infection, symptoms include fever, fatigue, and edema, and the infection can be diagnosed and treated. After this stage, the disease progresses to the less easily treated chronic stage, which can be life threatening [Nóbrega et al., 2009].

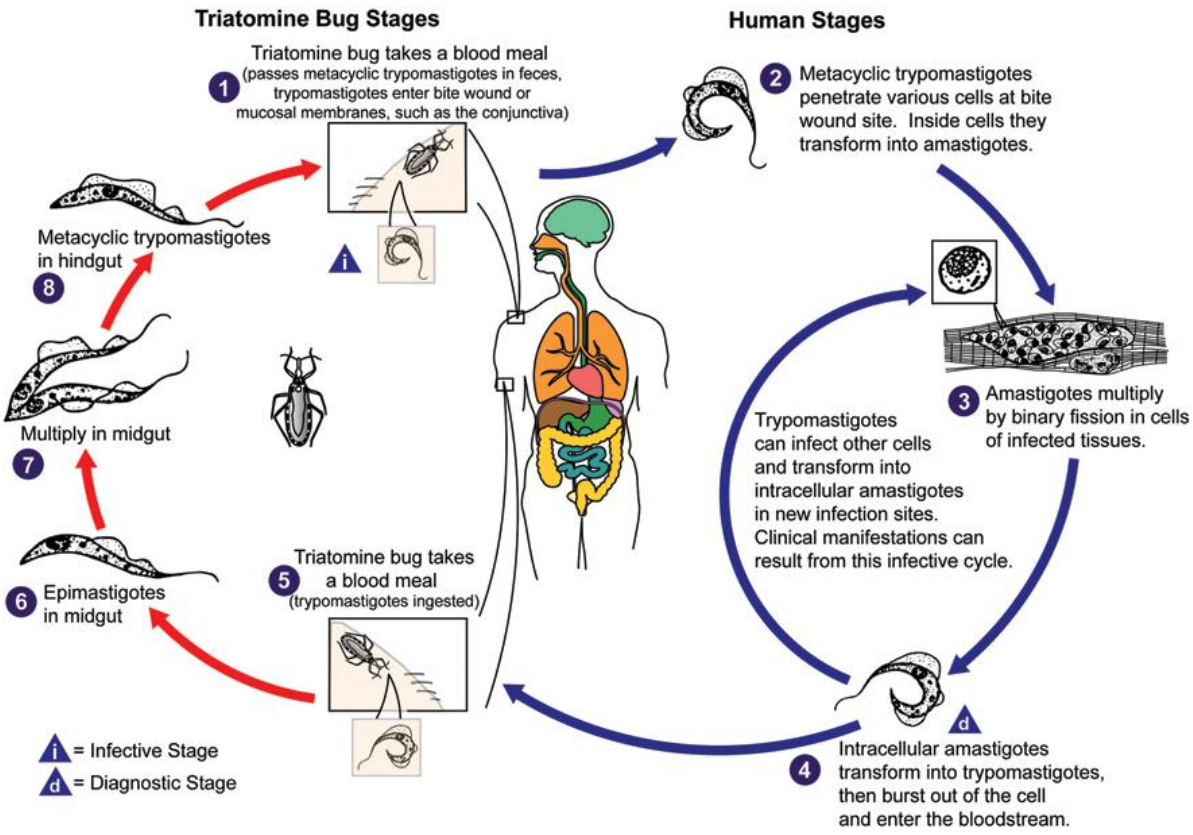


Figure 1.1 *Trypanosoma cruzi* life cycle [Center for Disease Control, 2011]; Used with permission

1.4.2 Control and Chemotherapy

Chagas prevention is a multi-fold process involving the elimination of the triatomine insect vector, establishing more effective disease surveillance, and early detection. The use of pesticides on the insect vector presents a difficult obstacle because with pesticide resistance, the disease vector is becoming more difficult to control. This in conjunction with the fact that the insect will quickly re-inhabit treated areas after pesticide use is discontinued makes the use of current pesticides ineffective. Because endemic areas are often rural and marginalized, taking census of the disease proves too costly, and currently Chagas disease is not monitored like malaria and tuberculosis in some countries. Without an appropriate model of disease burden it becomes increasingly difficult to distribute resources and advocate disease awareness in effected regions. Early

diagnosis of a *T. cruzi* infection is spotty and many currently used diagnostic tests often give inconclusive results and frequent false negatives. The most difficult hurdle in Chagas disease elimination is that current pharmacological treatment is often ineffective and sometimes dangerous when treating those with chronic stage trypanomiasis [Reithinger et al., 2009].

Two drugs are currently being used as treatment for Chagas disease, nifurtimox and benznidazole, but both of these treatments have side effects that commonly lead to treatment discontinuation. Approximately half of patients undergoing benznidazole treatment encounter adverse events including rash, dysphagia, and nervous system issues such as headaches and peripheral polyneuritis. Treatment discontinuation is required in 9-29% percent of affected patients. Nifurtimox is no better in this regard, where in chronic cases adverse events occur in 31.0-53.8% of children and 43.0-97.5% of adults. Side effects are similar to that of benznidazole, but serious adverse events such as inflammation of heart tissue and anaphylaxis have been reported, and treatment discontinuation is very common [Pérez-Molina et al., 2012]. Because of the low tolerability of these treatments, it is necessary to continue the search for an adequate therapy for this debilitating disease.

1.4.3 *Trypanosoma cruzi* Drug Targets

Dozens of proteins from the *T. cruzi* proteome have been identified [Schmidt et al., 2012], and many of these proteins have undergone analysis and protein structures have been determined and modeled *in silico*. Proteins considered as potential drug targets were considered in this work, including arginine kinase [Fernandez et al., 2007], cruzain [Eakin et al., 1992], cyclophilin [Búa et al., 2001], dihydrofolate reductase [Zuccato et al., 1998], dihydroorotate dehydrogenase [Annoura et al., 2005],

deoxyuridyltriphosphatase (dUTPase) [Harkiolaki et al., 2004], farnesyl diphosphate synthase [Gabelli et al., 2006], Fe-superoxide dismutase [Bachega et al., 2009], glucokinase [Cordeiro et al., 2007], glyceraldehydes-3-phosphate dehydrogenase [Pavão et al., 2002], histidyl-tRNA synthetase [Merritt et al., 2010], hypoxanthine phosphoribosyl transferase [Eakin et al., 1997], lipoamide dehydrogenase [Werner et al., 2008], old yellow enzyme [Okamoto et al., 2011], pteridine reductase 2 [Schormann et al., 2005], pyruvate kinase [Morgan et al., 2011], ribose 5-phosphate isomerase type B [Stern et al., 2011], sterol 14α-demethylase [Lepesheva et al., 2010], *trans*-sialidase [Mucci et al., 2006], triosephosphate isomerase [Olivares-Illana et al., 2007], trypanothione reductase [Bond et al., 1999], and tyrosine aminotransferase [Blankenfeldt et al., 1999]. In this study, the docking interaction of these drug targets from *Trypanosoma cruzi* and known antitrypanosomal agents from Latin American plants were examined to illuminate the activity of these agents in the *T. cruzi* cellular system. From this information we hope to identify specific protein-ligand interactions that can be used for an effective chemotherapy for Chagas Disease.

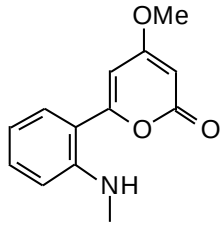
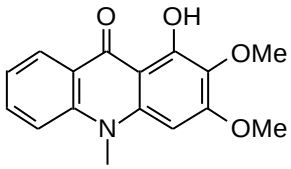
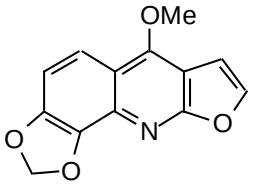
CHAPTER 2

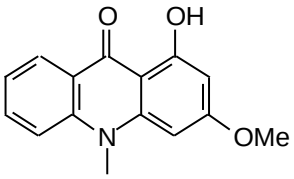
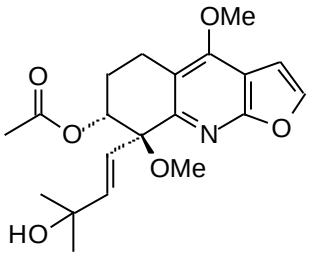
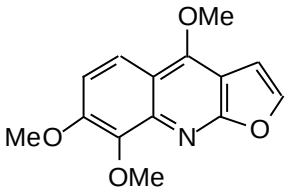
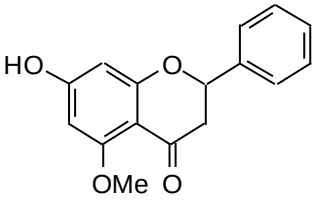
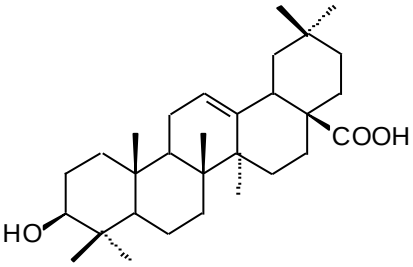
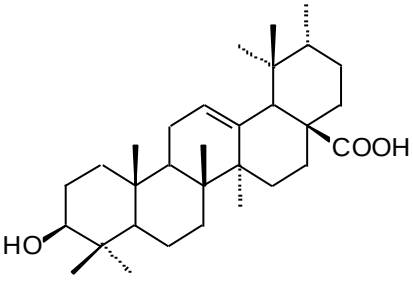
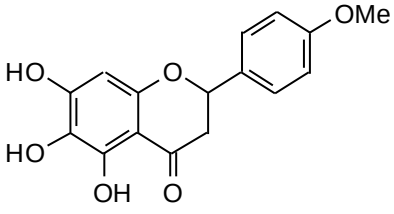
MATERIALS AND METHODS

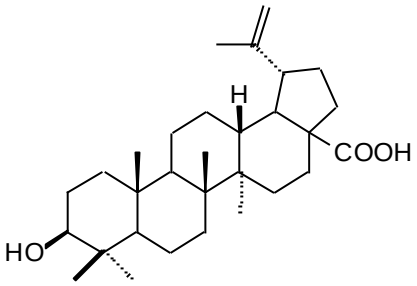
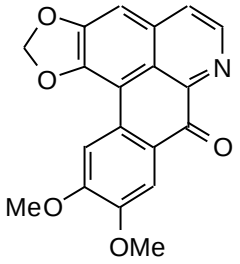
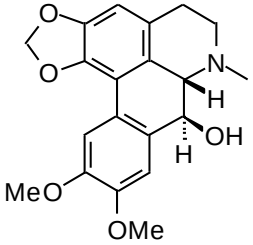
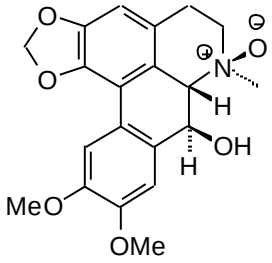
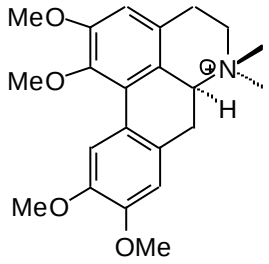
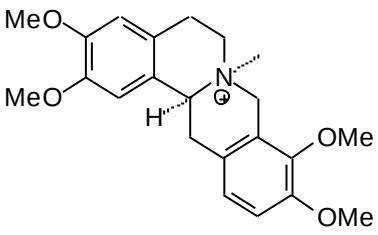
2.1 Compound Dataset

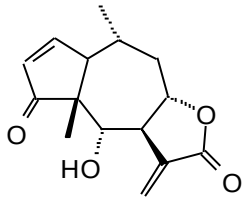
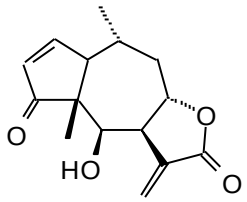
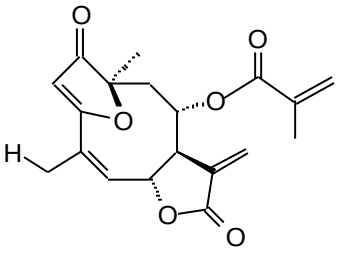
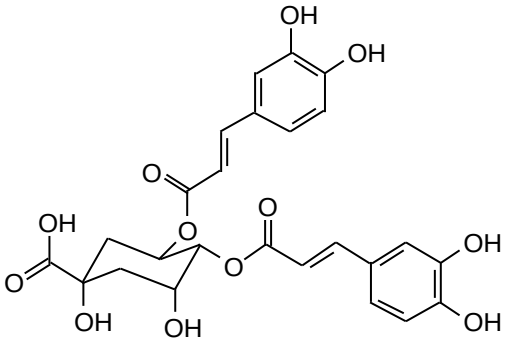
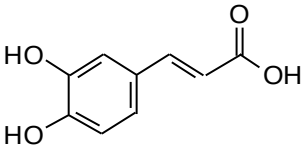
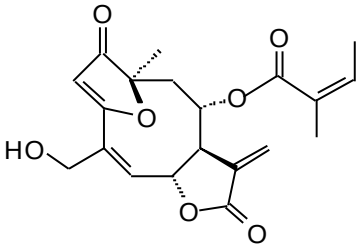
Natural products with trypanocidal properties (Table 2.1) were found from the literature review "Natural Products and Chagas Disease: a review of plant compounds studied for activity against *Trypanosoma cruzi*," published in February 2011 in *Natural Product Reports* [Izumi et al.]. This work included alkaloids, terpenoids, phenolics, and various miscellaneous oxygenated hydrocarbons. These compounds were constructed using Spartan '10, where the lowest energy conformer was found in the ground state using the Hartree-Fock 6-31G* method (as outlined in Section 1.2.4) and saved as a pdb file for import into Molegro Virtual Docker. To predict possible covalent bonding between proteins and ligands the LUMOs of α-β unsaturated carbonyls were examined in SPARTAN, and those with large LUMOs were noted.

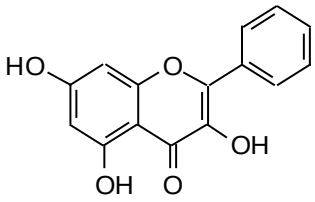
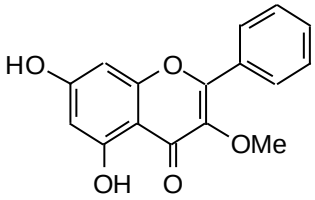
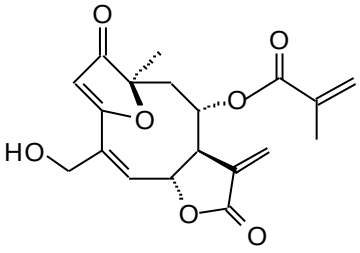
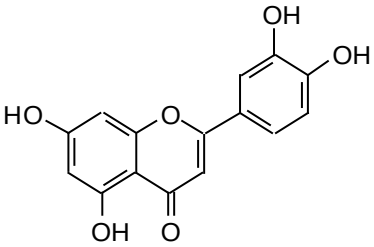
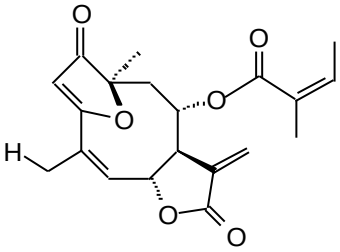
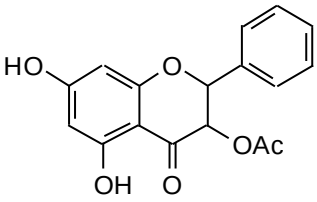
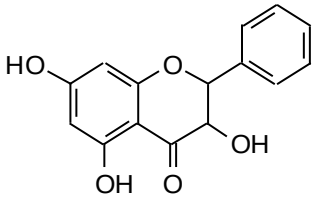
Table 2.1 Antitrypanosomal compound data set

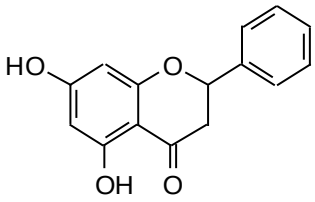
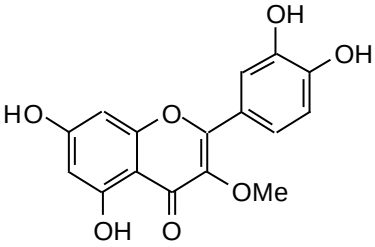
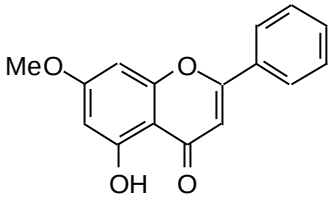
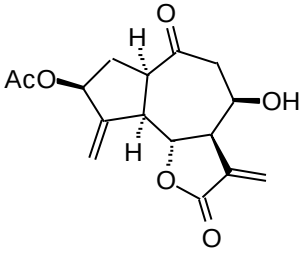
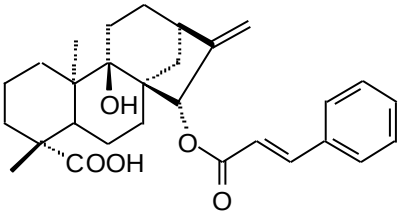
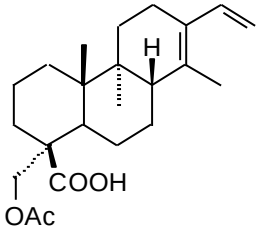
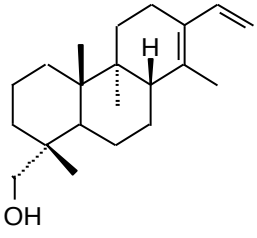
Compound	Natural Source	Compound Classification	Structure
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	*Almeida rubra*	Alkaloid	
Arborinine	*Almeida rubra*	Alkaloid	
Kokusagine	*Almeida rubra*	Alkaloid	

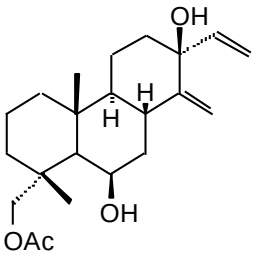
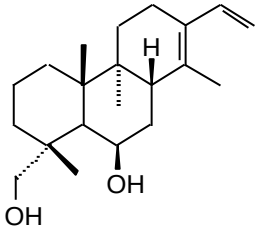
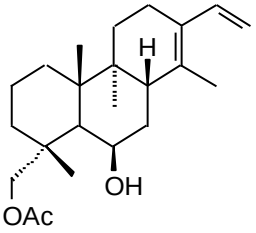
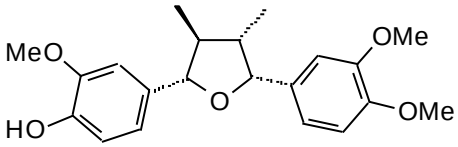
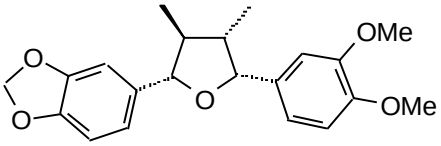
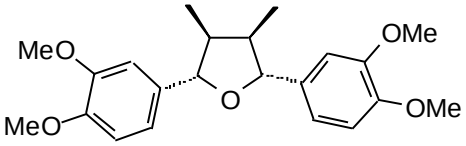
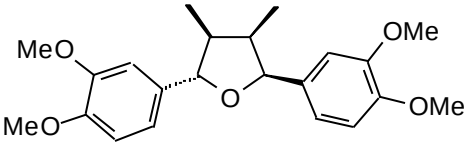
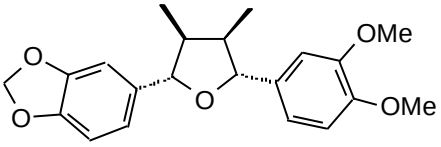
Compound	Natural Source	Compound Classification	Structure
N-Methyl-1-hydroxy-3-methoxyacridone	*Almeida rubra*	Alkaloid	
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	*Almeida rubra*	Alkaloid	
Skimmianine	*Almeida rubra*	Alkaloid	
Alpinitine	*Arrabidaea triplinervia*	Flavonoid	
Oleanolic acid	*Arrabidaea triplinervia*	Triterpene	
Ursolic acid	*Arrabidaea triplinervia*	Triterpene	
5,6,7-Trihydroxy4′-methoxyflavone	*Baccharis retusa*	Flavonoid	

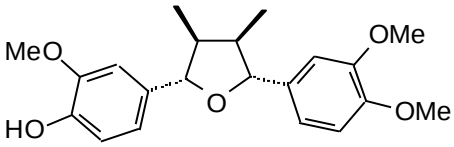
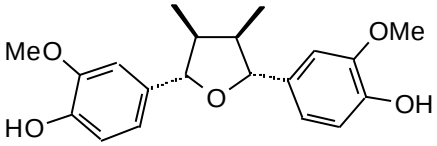
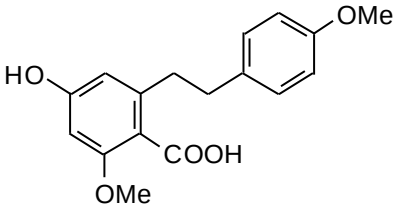
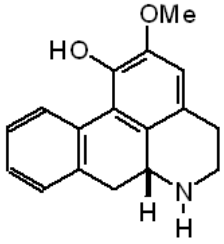
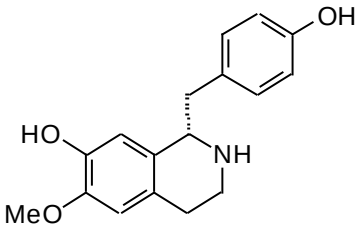
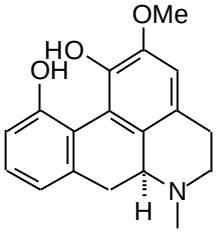
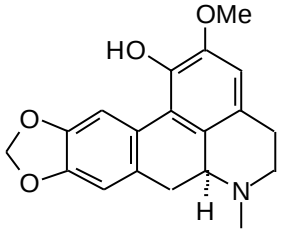
Compound	Natural Source	Compound Classification	Structure
Betulinic acid	*Bertholletia excelsa*	Triterpene	
Dicenitrinone	*Duguetia furfuracea*	Alkaloid	
Dugetine	*Duguetia furfuracea*	Alkaloid	
Dugetine-β-*N*-oxide	*Duguetia furfuracea*	Alkaloid	
N-Methylglaucine	*Duguetia furfuracea*	Alkaloid	
N-Methyltetrahydro-palmitine	*Duguetia furfuracea*	Alkaloid	

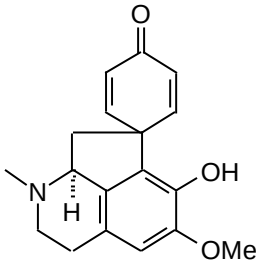
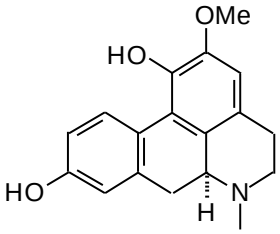
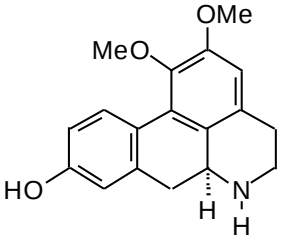
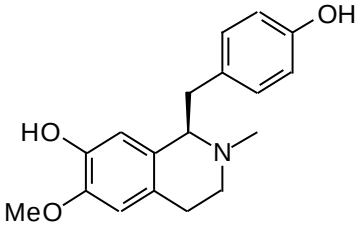
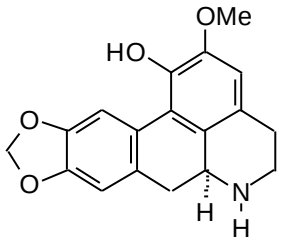
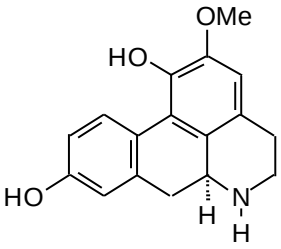
Compound	Natural Source	Compound Classification	Structure
Helenalin	*Gaillardia megapotamica*	Sesquiterpene	
Mexicanin	*Gaillardia megapotamica*	Sesquiterpene	
15-Deoxygoyazenolide	*Lychnophora pohlii*	Sesquiterpene	
4,5-di-E-O-Caffeyoquinic acid	*Lychnophora pohlii*	Misc	
Caffeic Acid	*Lychnophora pohlii*	Misc	
Centratherin	*Lychnophora pohlii*	Sesquiterpene	

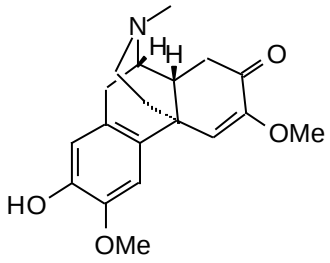
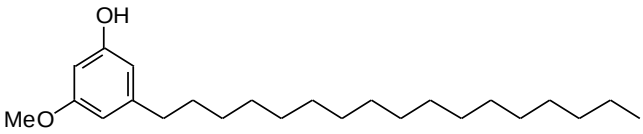
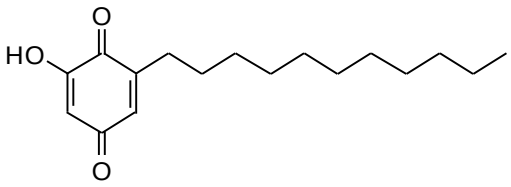
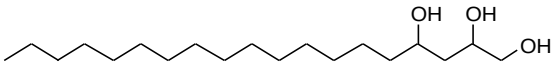
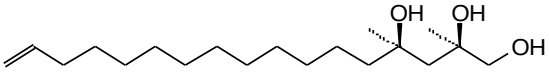
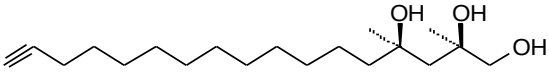
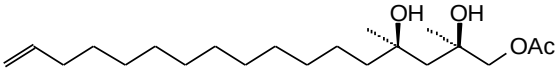
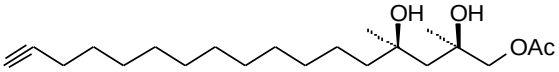
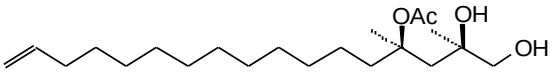
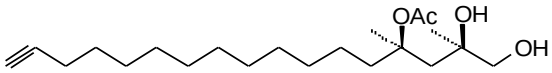
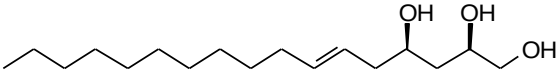
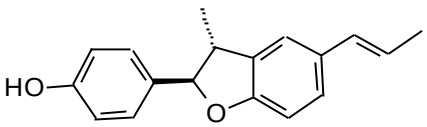
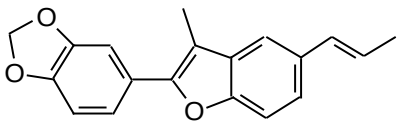
Compound	Natural Source	Compound Classification	Structure
Ganglin	*Lychnophora pohlii*	Flavonoid	
Ganglin-3-methyl ether	*Lychnophora pohlii*	Flavonoid	
Goyazenolide	*Lychnophora pohlii*	Sesquiterpene	
Luteolin	*Lychnophora pohlii*	Flavonoid	
Lynchopholide	*Lychnophora pohlii*	Sesquiterpene	
Pinobanksin 3-acetate	*Lychnophora pohlii*	Flavonoid	
Pinobanksin	*Lychnophora pohlii*	Flavonoid	

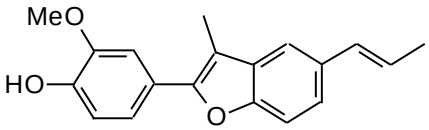
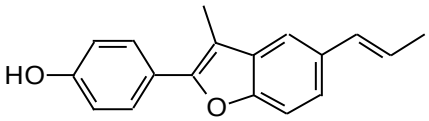
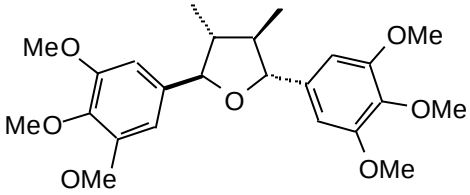
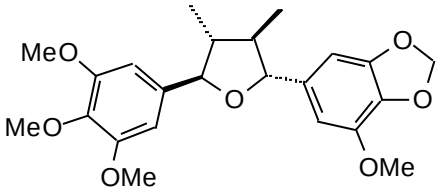
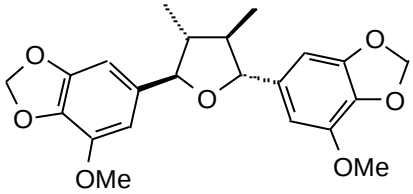
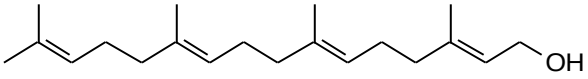
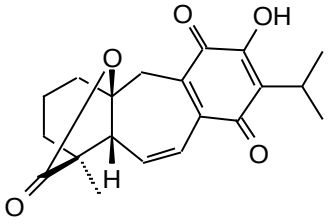
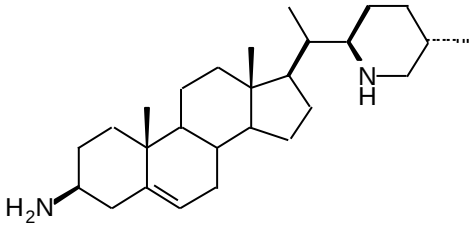
Compound	Natural Source	Compound Classification	Structure
Pinocembrin	*Lychnophora pohlii*	Flavonoid	
Quercetin-3-methyl ether	*Lychnophora pohlii*	Flavonoid	
Tectochrysin	*Lychnophora pohlii*	Flavonoid	
8β-Hydroxyzaluzanin D	*Mikania hoehnei*	Sesquiterpene	
ent-9α-Hydroxy-15β-(*E*)-cinnamoyloxy-16-kauren-19-oic acid	*Mikania stipulacea*	Diterpene	
18-Acetoxy-13,15-diene-cassanoic acid	*Myrospermum frutescens*	Diterpene	
18-Hydroxycassan-13,15-diene	*Myrospermum frutescens*	Diterpene	

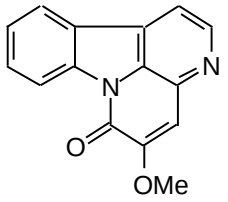
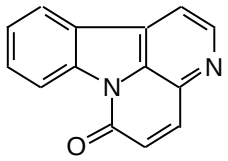
Compound	Natural Source	Compound Classification	Structure
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	*Myrospermum frutescens*	Diterpene	
6β-18-Dihydroxycasssan-13,15-diene	*Myrospermum frutescens*	Diterpene	
6β-Hydroxy-18-acetoxycassan-13,15-diene	*Myrospermum frutescens*	Diterpene	
Aristolignan	*Nectandra megapotamica*	Lignan	
Calopiptin	*Nectandra megapotamica*	Lignan	
Galgravin	*Nectandra megapotamica*	Lignan	
Ganschisandrine	*Nectandra megapotamica*	Lignan	
Machilin G	*Nectandra megapotamica*	Lignan	

Compound	Natural Source	Compound Classification	Structure
Nectandrin A	*Nectandra megapotamica*	Lignan	
Nectandrin B	*Nectandra megapotamica*	Lignan	
Isonoholaenic acid	*Notholaena nivea*	Misc	
Caaverine	*Ocotea lancifolia*	Alkaloid	
Coclaurine	*Ocotea lancifolia*	Alkaloid	
Corytuberine	*Ocotea lancifolia*	Alkaloid	
Domesticine	*Ocotea lancifolia*	Alkaloid	

Compound	Natural Source	Compound Classification	Structure
Glazovine	*Ocotea lancifolia*	Alkaloid	
Isoboldine	*Ocotea lancifolia*	Alkaloid	
Laurotetanine	*Ocotea lancifolia*	Alkaloid	
N-Methylcoclaurine	*Ocotea lancifolia*	Alkaloid	
Nordomesticine	*Ocotea lancifolia*	Alkaloid	
Norisoboldine	*Ocotea lancifolia*	Alkaloid	

Compound	Natural Source	Compound Classification	Structure
Pallidine	*Ocotea lancifolia*	Alkaloid	
3-Heptadecyl-5-methoxyphenol	*Oxalis erythrorrhiza*	Misc	
Embelin	*Oxalis erythrorrhiza*	Misc	
1,2,4-Trihydroxynonadecane	*Persea americana*	Oxygenated Hydrocarbon	
1,2,4-Trihydroxyheptadec-16-ene	*Persea americana*	Oxygenated Hydrocarbon	
1,2,4-Trihydroxyheptadec-16-yne	*Persea americana*	Oxygenated Hydrocarbon	
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	*Persea americana*	Oxygenated Hydrocarbon	
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	*Persea americana*	Oxygenated Hydrocarbon	
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	*Persea americana*	Oxygenated Hydrocarbon	
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	*Persea americana*	Oxygenated Hydrocarbon	
(*E*)-1,2,4-Trihydroxynonadec-6-ene	*Persea americana*	Oxygenated Hydrocarbon	
Conocarpan	*Piper regnellii*	Lignan	
Eupomatenoid-3	*Piper regnellii*	Lignan	

Compound	Natural Source	Compound Classification	Structure
Eupomatenoid-5	*Piper regnellii*	Lignan	
Eupomatenoid-6	*Piper regnellii*	Lignan	
Grandisin	*Piper solmsianum*	Lignan	
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	*Piper solmsianum*	Lignan	
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	*Piper solmsianum*	Lignan	
Geranygeraniol	*Pterodon pubescens*	Diterpene	
5-*epi*-Icetexone	*Salvia gilliessi*	Diterpene	
Sarachine	*Saracha punctata*	Alkaloid	

Compound	Natural Source	Compound Classification	Structure
5-Methoxycanthin-6-one	*Zanthoxylum chiloperone*	Alkaloid	
Canthin-6-one	*Zanthoxylum chiloperone*	Alkaloid	

2.2 Computational Method

Protein-ligand docking simulations were conducted using Molegro Virtual Docker, which uses the computational protocol outlined in Sections 1.2.2 and 1.2.3. In addition, solvent molecules and co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds with each of the proteins were undertaken with a sphere large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. If a co-crystallized inhibitor or substrate was present in the structure, then that site was chosen as the binding site. Standard protonation states of the proteins based on neutral pH were used in the docking studies. Assignments of charges on each protein were based on standard templates as part of the Molegro Virtual Docker program; no other charges were set. Flexible ligand models were used in the docking and subsequent optimization scheme. As a test of docking accuracy and for docking energy comparison, co-crystallized ligands were re-docked into the protein structures. Different orientations of the ligands were searched and ranked based on their re-rank scores. The maximum number of iterations for the docking algorithm was set to 1500, with a maximum population size of 50, and 30 runs per ligand. The RMSD threshold for multiple poses was set to 1.00 Å. The different poses generated were ranked by re-rank scores, and the re-rank scores of the strongest docking poses are contained in Tables 3.1-3.9**.** Re-rank scores that were two standard deviations below (more

negative) the average for each ligand were considered selective. If the average re-rank score for the ligand was more negative than -90.00, then the ligand was considered unselective.

2.3 Protein Structure Data Set

Protein crystal structures were obtained from the Protein Data Bank (Table 2.2) and human analogs of *T. cruzi* proteins were run against the same ligand subset (Table 2.3).

Table 2.2: *Trypanosoma cruzi* protein structures

T. cruzi Protein	Structure	Reference
Arginine Kinase	2J1Q	Fernandez et al., 2007
Cruzain	1AIM, 2AIM	Gillmor et al., 1997
	1F2A, 1F2B, 1F2C, 1F29	Brinen et al., 2000
	1ME3, 1ME4	Huang et al., 2003
	1U9Q	Choe et al., 2005
	2OZ2	Kerr et al., 2009
	3HD3	Bryant et al., 2009
	3I06	Mott et al., 2010
	3IUT	Brak et al., 2010
	3KKU	Ferriera et al., 2010
	3LXS	Chen et al., 2010
Cyclophilin	1XQ7	Caruthers and Hol, 2004
Dihydrofolate Reductase	2H2Q, 3CL9, 3CLB	Schormann et al., 2008
	3HBB	Senkovich et al., 2009
	3INV, 3IRM, 3IRN, 3IRO	Chitnumsub et al., 2010
	3KJS	Schormann et al., 2010
Dihydroorotate Dehydrogenase	2DJX, 2DJL, 2E6A	Inaoka et al., 2007
	2E6D, 2E68	Inaoka et al., 2008
	3C3N	Pinheiro et al., 2008
dUTPase	1OGK	Harkiolaki et al., 2004
Farnesyl Diphosphate Synthase	1YHL	Gabelli et al., 2006
	3IBA, 3ICK, 3ICZ, 3ICM, 3ICN, 3ID0	Huang et al., 2010
Fe-Superoxide Dismutase	2GPC	Bachega et al., 2009
Glucokinase	2Q2R	Cordeiro et al., 2007
Glyceraldehydes-3-Phosphate Dehydrogenase	1K3T	Pavão et al., 2002
	1ML3	Castilho et al., 2003
	1QXS	Ladame et al., 2003

T. cruzi Protein	Structure	Reference
	3IDS	Balliano et al., 2009
Histidyl-tRNA Synthetase	3HRK, 3LC0	Merritt et al., 2010
Hypoxanthine Phosphoribosyl Transferase	1IOI, 1I13, 1I14, 1I0L	Canyuk et al., 2001
	1P19	Canyuk et al., 2004
	1TC1, 1TC2	Focia et al., 1998
Lipoamide Dehydrogenase	2QAE	Werner et al., 2008
Old Yellow Enzyme	3ATY, 3ATZ	Okamoto et al., 2011
Pteridine Reductase 2	1MXF, 1MXH	Schormann et al., 2005
Pyruvate Kinase	3QV9	Morgan et al., 2011
Ribose 5-Phosphate Isomerase Type B	3K7S, 3K8C	Stern et al., 2011
Sterol 14α-Demethylase	2WX2	Chen et al., 2010
	3K1O, 3KHM, 3KSW	Lepesheva et al., 2010
trans-Sialidase	1MS0, 1MS1, 1MS8, 1MS9	Buschiazzo et al., 2002
	1S0I, 1S0J, 2AH2	Amaya et al., 2004
	3B69	Buchini et al., 2008
Triosephosphate Isomerase	2OMA	Olivares-Illana et al., 2007
Trypanothione Reductase	1NDA	Lantwin et al., 1994
	1AOG	Zhang et al., 1996
	1BZL	Bond et al., 1999
	1GXF	Saravanamuthu et al., 2004
Tyrosine Aminotransferase	1BW0	Blankenfeldt et al., 1999

Table 2.3: Human analogs of *Trypanosoma cruzi* structures investigated.

T. Cruzi Protein	*H. sapiens* Analog	*H. sapiens* Protein Structure	Reference
Arginine Kinase	No Analog	N/A	N/A
Cruzain	Cathepsin L	3H8B	Shenoy et al., 2009
Cyclophilin	Cyclophillin	2ESL	Walker et al., 2011
Dihydrofolate reductase	Dihydrofolate reductase	1DHF	Davies et al., 1990
Dihydroorotate Dehydrogenase	Dihydroorotate Dehydrogenase	1D3G	Liu et al., 2000
DUTPase	DUTPase	2HQU	Varga et al., 2007

T. Cruzi Protein	*H. sapiens* Analog	*H. sapiens* Protein Structure	Reference
Farnesyl Diphosphate Synthase	Farnesyl Diphosphate Synthase	1ZW5	Kavanagh et al., 2006
Fe-Superoxide Dismutase	Cu-Zn Superoxide Dismutase	2WYZ	Antonyuk et al., 2010
Glucokinase	Glucokinase	1V4S	Kamata et al., 2004
Glyceraldehyde-3-Phosphate Dehydrogenase	Glyceraldehyde-3-Phosphate Dehydrogenase (Liver and Muscle)	1ZNQ(L), 3GPD (M)	Ismail and Park, 2009; Mercer et al., 1976
Histidyl-tRNA Synthetase	No Structure	N/A	N/A
Hypoxanthine Phosphoribosyl Transferase	Hypoxanthine Phosphoribosyl Transferase	1HMP	Eads et al., 1994
Lipoamide Dehydrogenase	Dihydrolipooyl dehydrogenase	1ZMD	Brautigam et al., 2005
Old Yellow Enzyme	Biliverdin β-Reductase	1HDO	Pereira et al., 2001
Pteridine Reductase 2	Dihydrofolate reductase	1DHF	Davies et al., 1990
Pyruvate Kinase	Pyruvate Kinase	1T5A	Dombrauckas et al., 2005
Ribose 5-Phosphate Isomerase Type B	No Structure	N/A	N/A
Sterol 14-α Demethylase	Lanosterol 14-αDemethylase	3LD6	Strushkevich et al., 2010
trans-Sialidase	No Analog	N/A	N/A
Triosephosphate Isomerase	Triosephosphate Isomerase	1HTI	Mande et al., 1994
Trypanothione Reductase	Glutathione Reductase	1BWC	Gallwitz et al., 1999
Tyrosine Aminotransferase	Tyrosine Aminotransferase	3DYD	Karlberg et al., 2011

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Docking Results

Table 3.1 contains the selective interactions between the *T. cruzi* proteins and the ligands investigated. For extended tables with all docking interactions, please see the Appendix Tables A.1-A.9.

Table 3.1: Selective re-rank scores of *T. cruzi* proteins and investigated ligands. NS denotes no selective interaction between ligand and protein crystal structure.

Trypanothione Reductase				
Compound Classification	Ligand	Re-Rank Score		
		1AOG	1GXF	1NDA
Alkaloids	4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	NS	-93.7866	-94.1743
	Kokusagine	NS		-100.187
	Skimmianine	-99.0244	-97.2128	-98.8348
	Coclaurine	NS	-106.085	-108.534
	Corytuberine	NS	NS	-101.879
	Nordomesticine	-102.456	NS	-105
	Norisoboldine	NS	NS	-102.75
	Pallidine	NS	-104.611	NS
	5-Methoxycanthin-6-one	NS	NS	-91.6831
	Caaverine	NS	NS	-92.57
Flavonoids	Alpinitine	NS	NS	-104.092
	5,6,7-Trihydroxy-4'-methoxyflavone	NS	-112.648	-121.782
	Ganglin	NS	-111.125	-113.999
	Luteolin	NS	-113.517	-113.689
	Pinobanksin	NS	-113.517	-113.689
	Pinocembrin	NS	-107.046	-110.058
	Tectochrysin	NS	-113.293	NS
Sesquiterpenes	Helenalin	-97.3887	NS	-101.805
Diterpenes	Geranygeraniol	NS	-125.806	-129.542
	5-*epi*-Icetexone	-120.405	NS	NS
Lignans	Ganschisandrine	NS	NS	-125.11
	Eupomatenoid-6	NS	-110.162	NS

		1AOG	1GXF	1NDA
Oxygenated Hydrocarbons	1,2,4-Trihrooxyheptadecane	NS	-114.425	-130.127
	1,2,4-Trihydrooxyheptadec-16-ene	NS	-115.518	-113.925
	1,2,4-Trihydrooxyheptadec-16-yne	NS	-123.675	-115.612
	1-Acetoxy-2,4-dihydroxyheptadec-16-ene	NS	-137.731	-132.752
	1-Acetoxy-2,4-dihydroxyheptadec-16-yne	NS	-132.203	-125.863
	4-Acetoxy-1,2-dihydroxyheptadec-16-ene	NS	-121.372	-129.599
	4-Acetoxy-1,2-dihydroxyheptadec-16-yne	NS	-126.804	NS
	(*E*)-1,2,4-Trihydroxynonadec-6-ene	NS	-128.103	-124.468
Miscellaneous	Isonoholaenic acid	NS	NS	-113.274
	Embelin	-98.4642	NS	NS

Lipoamide Dehydrogenase

Compound Classification	Ligand	Re-Rank Score
		2QAE
Alkaloids	4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-98.3786
	Kokusagine	-107.736
	Skimmianine	-104.17
Flavonoids	Alpinitine	-97.8752
	Ganglin	-104.688
	Pinobanksin 3-acetate	-118.187
	Pinobanksin	-101.697
Diterpenes	5-*epi*-Icetexone	-118.293

Cruzain

Compound Classification	Ligand	Re-Rank Score
		3HD3
Alkaloids	*rel*-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-113.52
Miscellaneous	Isonoholaenic acid	-113.234

Dihydrofolate Reductase

Compound Classification	Ligand	Re-Rank Score		
		2H2Q	3CLB	3HBB
Alkaloids	Skimmianine	NS	-97.9989	-97.5006

		2H2Q	3CLB	3HBB
Diterpenes	6β-Hydroxy-18-acetoxycassan-13,15-diene	NS	-100.274	-99.5084
Lignans	Nectandrin B	-123.745	NS	NS
Miscellaneous	Embelin	-102.149	NS	NS

Dihydroorotate Dehydrogenase

Compound Classification	Ligand	Re-Rank Score		
		2E6A	2E68	3C3N
Alkaloids	Dicenitrinone	NS	NS	-108.51
	Dugetine-β-*N*-oxide	NS	NS	-108.12
	N-Methylglaucine	NS	NS	-108.848
Diterpenes	6β-Hydroxy-18-acetoxycassan-13,15-diene	-99.2055	-99.7656	NS

Glucokinase

Compound Classification	Ligand	Re-Rank Score
		2Q2R
Alkaloids	*N*-Methylglaucine	-112.392
	Caaverine	-94.60
	Pallidine	-102.082

Pteridine Reductase 2

Compound Classification	Ligand	Re-Rank Score	
		1MXF	1MXH
Alkaloids	Caaverine	-97.62	-95.75
	Nordomesticine	NS	-108.235
	Norisoboldine	NS	-101.72
	Sarachine	NS	-114.116
	5-Methoxycanthin-6-one	-95.3177	NS
	Canthin-6-one	-85.7344	NS

trans-sialidase

Compound Classification	Ligand	Re-Rank Score
		1MS8
Flavonoids	Pinobanksin 3-acetate	-107.842

Farnesyl Diphosphate Synthase

Compound Classification	Ligand	Re-Rank Score	
		3ICK	3ICZ
Flavonoids	Pinobanksin 3-acetate	NS	-106.119
Sesquiterpenes	8β-Hydroxyzaluzanin D	-103.824	NS

		3ICK	3ICZ	
Oxygenated Hydrocarbons	1-Acetoxy-2,4-dihydroxyheptadec-16-ene	NS	-120.25	
Miscellaneous	3-Heptadecyl-5-methoyphenol	-108.22	NS	

Histidyl-tRNA Synthetase			
Compound Classification	Ligand	Re-Rank Score	
		3HRK	
Oxygenated Hydrocarbons	4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-120.81	
Miscellaneous	Embelin	-97.7679	

3.2 Comparison to the human analogs

To further assess pharmacological viability, ligands were run against human analogs as well (Tables 3.10a-e). Interactions that returned re-rank scores below -90.00 have been highlighted in red. Because these are relatively strong binding scores, the associated ligands may bind to human proteins instead of the *T. cruzi* analogs, suggesting that these would not be appropriate antitrypanosomal agents in the human system. These compounds may be appropriate, given the appropriate environmental impact studies, in domestic or environmental sprays to rid the insect vector of the *T. cruzi* parasite. Rows shaded in green denote ligands that had no re-rank scores below -90.00 with any human analog, and may bind selectively to the *T. cruzi* proteins in the human system. Interestingly these ligands are mostly miscellaneous oxygenated hydrocarbons, 1,2,4-trihydrooxyheptadec-16-ene, 1,2,4-trihydrooxyheptadec-16-yne, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, (*E*)-1,2,4-trihydroxynonadec-6-ene, along with the alkaloid, *N*-methyltetrahydropalmitine.

Table 3.2a: Lowest-energy docked poses (re-rank scores) of *T. cruzi*-selective ligands with *H. sapiens* protein analogs

	Glutathione Reductase		Dihydroorotate Dehydrogenase	Dihydrofolate Reductase	Biliverdin B-Reductase
Ligand	1BWC-A	1BWC -B	1D3G	1DHF	1HDO
1,2,4-Trihrooxyheptadecane	-81.50	-78.48	-73.26	-69.36	-70.05
1,2,4-Trihydrooxyheptadec-16-ene	-81.32	-77.05	-75.12	-52.89	-72.96

Ligand	Glutathione Reductase		Dihydroorotate Dehydrogenase	Dihydrofolate Reductase	Biliverdin B-Reductase
	1BWC-A	1BWC -B	1D3G	1DHF	1HDO
1,2,4-Trihydrooxyheptadec-16-yne	-82.31	-85.22	-76.71	-62.84	-77.61
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-79.43	-78.54	-91.23	-62.62	-76.73
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-91.90	-93.37	-75.47	-73.76	-97.42
3-Heptadecyl-5-methoyphenol	-83.20	-83.13	-92.42	-70.93	-82.29
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-90.06	-56.38	-71.85	-67.51	-58.28
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-72.02	-73.16	-77.65	-64.14	-68.31
5-epi-Icetexone	-79.93	-78.33	-107.50	-66.69	-68.11
5-Methoxycanthin-6-one	-78.62	-89.64	-94.08	-65.23	-75.51
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-73.16	-85.48	-87.08	-63.35	-81.69
5,6,7-Trihydroxy4'-methoxyflavone	-103.92	-101.42	-104.78	-75.20	-80.27
6β-Hydroxy-18-acetoxycassan-13,15-diene	-91.80	-65.91	-84.37	-72.76	-61.79
8β-Hydroxyzaluzanin D	-91.47	-87.44	-99.01	-72.57	-95.57
Alpinitine	-87.27	-86.47	-100.53	-70.12	-90.01
Caaverine	-75.94	-86.00	-95.12	-69.03	-84.30
Canthin-6-one	-73.53	-73.18	-90.02	-58.62	-70.79
Coclaurine	-104.00	-107.35	-99.70	-70.58	-86.49
Corytuberine	-83.71	-54.14	-90.28	-72.11	-67.22
Dicenitrinone	-96.20	-61.14	-104.29	-74.22	-78.93
Dugetine-β-N-oxide	-81.30	-60.74	-106.60	-74.80	-75.36
E-1,2,4-Trihydroxynonadec-6-ene	-89.73	-80.25	-81.94	-56.83	-74.24
Embelin	-95.52	-81.79	-89.04	-72.66	-80.27
Eupomatenoid-6	-106.57	-107.89	-107.27	-82.13	-79.44
Ganglin	-92.32	-101.96	-101.79	-73.94	-90.42
Ganschisandrine	-103.59	-107.98	-111.71	-76.83	-78.06
Geranygeraniol	-97.08	-97.98	-96.44	-81.90	-97.25
Helenalin	-95.65	-97.99	-73.38	-70.17	-81.50
Isonoholaenic acid	-105.08	-103.14	-106.35	-71.78	-90.91
Kokusagine	-94.58	-93.86	-90.13	-66.83	-71.79
Luteolin	-100.83	-103.54	-106.48	-76.78	-97.23
Nectandrin B	-101.88	-110.94	-115.64	-88.34	-105.69
N-Methylglaucine	-86.16	-52.95	-94.54	-77.86	-59.11
N-Methyltetrahydropalmitine	-73.08	-66.28	-65.34	-77.72	-65.72

Ligand	Glutathione Reductase		Dihydroorotate Dehydrogenase	Dihydrofolate Reductase	Biliverdin B-Reductase
	1BWC-A	1BWC -B	1D3G	1DHF	1HDO
Nordomesticine	-94.81	-57.92	-84.06	-74.47	-82.22
Norisoboldine	-86.44	-58.54	-98.53	-70.24	-75.43
Pallidine	-93.12	-70.08	-56.01	-75.70	-67.27
Pinobanksin	-89.15	-100.18	-96.20	-72.85	-65.57
Pinobanksin 3-acetate	-96.03	-108.40	-98.54	-79.65	-91.19
Pinocembrin	-77.17	-100.86	-95.51	-69.39	-62.22
rel-(7R,8R)-8-[(E)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-88.81	-89.72	-94.53	-85.27	-80.85
Sarachine	-94.21	-82.83	-76.06	-90.46	-82.83
Skimmianine	-82.02	-86.28	-96.44	-63.42	-69.61
Tectochrysin	-83.20	-98.64	-98.83	-74.90	-67.79

Table 3.10b: Lowest-energy docked poses (re-rank scores) of *T. cruzi*-selective ligands with *H. sapiens* protein analogs

Ligand	Hypoxanthyl Ribotransferase		Triosephos-phate Isomerase	Pyruvate Kinase	
	1HMP-A	1HMP - B	1HTI	1T5A - A	1T5A – B
1,2,4-Trihrooxyheptadecane	-47.18	-48.55	-50.12	-54.13	-90.37
1,2,4-Trihydrooxyheptadec-16-ene	-52.44	-61.04	-48.66	-48.87	-81.01
1,2,4-Trihydrooxyheptadec-16-yne	-53.73	-36.50	-69.81	-58.72	-76.48
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-43.22	-49.43	-44.66	-45.45	-72.07
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-50.41	-51.69	-61.26	-45.66	-82.58
3-Heptadecyl-5-methoyphenol	-59.41	-45.31	-46.81	-58.18	-89.70
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-48.53	-29.71	-56.42	-42.90	-56.21
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-58.54	-53.74	-47.37	-54.95	-78.60
5-epi-Icetexone	-75.83	-51.00	-97.50	-64.31	-80.39
5-Methoxycanthin-6-one	-62.99	-58.31	-78.44	-68.33	-79.18
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-67.16	-69.98	-88.89	-64.72	-100.51

Ligand	Hypoxanthyl Ribotransferase		Triosephos-phate Isomerase	Pyruvate Kinase	
	1HMP-A	1HMP - B	1HTI	1T5A - A	1T5A – B
5,6,7-Trihydroxy4'-methoxyflavone	-94.65	-89.18	-84.68	-63.39	-96.63
6β-Hydroxy-18-acetoxycassan-13,15-diene	-77.79	-59.85	-67.95	-60.80	-77.48
8β-Hydroxyzaluzanin D	-74.18	-67.57	-68.87	-73.91	-116.68
Alpinitine	-76.62	-75.92	-86.16	-67.20	-91.24
Caaverine	-49.20	-71.00	-75.65	-65.55	-53.21
Canthin-6-one	-62.35	-60.63	-76.22	-58.74	-82.22
Coclaurine	-77.50	-76.02	-86.71	-67.17	-96.70
Corytuberine	-69.17	-68.99	-75.84	-58.24	-64.56
Dicenitrinone	-68.91	-53.81	-84.98	-62.52	-48.24
Dugetine-β-N-oxide	-73.00	-56.64	-77.81	-69.49	-50.16
E-1,2,4-Trihydroxynonadec-6-ene	-61.68	-57.21	-65.60	-67.35	-80.93
Embelin	-72.96	-66.86	-67.55	-57.31	-99.92
Eupomatenoid-6	-79.12	-68.02	-81.26	-69.43	-113.38
Ganglin	-74.37	-78.51	-88.36	-67.85	-95.35
Ganschisandrine	-77.62	-81.38	-33.21	-66.19	-63.51
Geranygeraniol	-76.02	-58.42	-74.93	-70.60	-105.41
Helenalin	-73.37	-72.90	-69.46	-68.42	-95.11
Isonoholaenic acid	-76.35	-76.00	-75.33	-69.49	-93.90
Kokusagine	-63.88	-67.38	-83.78	-57.87	-73.45
Luteolin	-95.07	-89.28	-88.45	-62.21	-102.09
Nectandrin B	-99.82	-97.53	-81.42	-67.26	-108.41
N-Methylglaucine	-55.01	-54.91	-87.18	-54.66	-54.47
N-Methyltetrahydropalmitine	-58.68	-49.96	-74.70	-65.99	-63.21
Nordomesticine	-66.14	-68.37	-83.97	-69.19	-62.77
Norisoboldine	-76.98	-68.79	-82.60	-64.61	-59.23
Pallidine	-72.37	-50.38	-83.37	-60.35	-122.61
Pinobanksin	-74.61	-76.50	-80.31	-65.48	-97.61
Pinobanksin 3-acetate	-83.73	-77.99	-94.70	-64.22	-112.93
Pinocembrin	-73.93	-74.75	-78.79	-65.72	-84.75
rel-(7R,8R)-8-[(E)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-91.72	-65.65	-98.57	-82.71	-92.34
Sarachine	-68.49	-85.01	-53.83	-64.60	-37.20
Skimmianine	-64.35	-62.17	-71.22	-61.49	-73.50
Tectochrysin	-76.17	-73.44	-83.96	-71.71	-95.82

Table 3.10c: Lowest-energy docked poses (re-rank scores) of *T. cruzi*-selective ligands with *H. sapiens* protein analogs

Ligand	Glucokinase		Dihydrolipooyl dehydrogenase	Glycer-aldehyde 3-Phosphate Liver	Glycer-aldehyde 3-Phosphate Muscle
	1V4S - A	1V4S - B	1ZMD	1ZNQ	3GPD
1,2,4-Trihrooxyheptadecane	-71.43	-55.16	-63.64	-51.78	-56.04
1,2,4-Trihydrooxyheptadec-16-ene	-74.31	-75.91	-78.54	-53.41	-56.32
1,2,4-Trihydrooxyheptadec-16-yne	-63.28	-66.30	-67.10	-56.34	-67.87
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-68.34	-47.85	-85.34	-47.85	-61.17
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-99.66	-55.55	-78.88	-53.18	-62.20
3-Heptadecyl-5-methoyphenol	-84.39	-80.39	-86.89	-62.16	-56.41
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-72.15	-72.53	-90.46	-37.80	-59.14
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-67.23	-48.12	-75.13	-47.99	-57.29
5-epi-Icetexone	-66.91	1.83	-109.76	-81.53	-74.59
5-Methoxycanthin-6-one	-54.65	-57.49	-93.74	-74.43	-71.05
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-82.97	-60.44	-93.11	-69.48	-74.45
5,6,7-Trihydroxy4'-methoxyflavone	-96.65	-39.29	-104.97	-82.85	-81.32
6β-Hydroxy-18-acetoxycassan-13,15-diene	-75.26	40.35	-60.11	-71.23	-76.76
8β-Hydroxyzaluzanin D	-67.47	-77.55	-76.92	-69.08	-77.90
Alpinitine	-88.76	-59.73	-97.57	-82.32	-69.45
Caaverine	-53.61	-62.01	-76.25	-69.70	-75.59
Canthin-6-one	-64.66	-50.74	-79.71	-68.35	-69.63
Coclaurine	-92.78	-70.57	-104.75	-79.93	-86.33
Corytuberine	-54.83	0.29	-60.54	-68.09	-81.24
Dicenitrinone	-60.02	-14.20	-67.23	-73.00	-79.67
Dugetine-β-N-oxide	-69.11	38.97	-82.52	-77.17	-82.73
E-1,2,4-Trihydroxynonadec-6-ene	-82.03	-83.86	-75.66	-50.56	-48.16
Embelin	-81.77	-72.73	-91.14	-67.80	-67.54
Eupomatenoid-6	-102.84	3.44	-102.45	-82.49	-89.71
Ganglin	-92.54	-51.37	-106.94	-78.86	-71.25
Ganschisandrine	-105.03	23.01	-109.89	-81.12	-69.40
Geranygeraniol	-100.09	-94.96	-98.30	-64.76	-86.00

Ligand	Glucokinase		Dihydrolipooyl dehydrogenase	Glycer-aldehyde 3-Phosphate Liver	Glycer-aldehyde 3-Phosphate Muscle
	1V4S - A	1V4S - B	1ZMD	1ZNQ	3GPD
Helenalin	-74.60	-64.36	-79.27	-64.01	-75.30
Isonoholaenic acid	-95.72	-75.51	-108.16	-78.20	-87.55
Kokusagine	-82.49	-58.74	-106.57	-76.63	-70.19
Luteolin	-101.68	-78.87	-110.01	-85.00	-80.73
Nectandrin B	-89.05	-30.31	-94.86	-83.78	-87.34
N-Methylglaucine	-51.77	79.38	-68.70	-75.41	-72.45
N-Methyltetrahydropalmitine	-57.10	85.43	-77.06	-73.96	-71.98
Nordomesticine	-73.08	-49.29	-86.20	-76.74	-79.94
Norisoboldine	-57.91	-28.20	-94.17	-72.51	-85.66
Pallidine	-68.59	-65.31	-73.69	-66.94	-83.05
Pinobanksin	-91.49	-45.46	-105.13	-79.90	-69.50
Pinobanksin 3-acetate	-73.11	-55.63	-116.22	-78.19	-93.98
Pinocembrin	-92.63	-46.49	-101.54	-80.37	-71.84
rel-(7R,8R)-8-[(E)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-95.50	40.26	-87.43	-61.76	-87.37
Sarachine	-96.18	24.73	-95.25	-73.37	-86.10
Skimmianine	-77.06	-54.26	-97.06	-61.97	-67.50
Tectochrysin	-98.14	-69.73	-105.19	-83.72	-72.58

Table 3.10d: Lowest-energy docked poses (re-rank scores) of *T. cruzi*-selective ligands with *H. sapiens* protein analogs

Ligand	Cyclophilin	dUTPase	Superoxide Dismutase	Tyrosine Aminotransferase
	2ESL	2HQU	2WYZ	3DYD
1,2,4-Trihrooxyheptadecane	-60.12	-80.77	-25.94	-42.96
1,2,4-Trihydrooxyheptadec-16-ene	-54.74	-87.13	-25.43	-37.52
1,2,4-Trihydrooxyheptadec-16-yne	-61.44	-75.66	-31.73	-43.12
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-62.13	-32.30	-28.82	-52.78
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-51.55	-68.64	-28.25	-40.84
3-Heptadecyl-5-methoyphenol	-57.60	-84.71	-46.23	-49.20
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-47.13	-63.74	-23.56	-34.30

Ligand	Cyclophilin 2ESL	dUTPase 2HQU	Superoxide Dismutase 2WYZ	Tyrosine Aminotransferase 3DYD
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-44.04	-70.26	-13.76	-41.50
5-epi-Icetexone	-78.46	-107.20	-51.53	-47.19
5-Methoxycanthin-6-one	-69.27	-84.93	-40.14	-55.59
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-72.44	-84.66	-50.44	-57.48
5,6,7-Trihydroxy4'-methoxyflavone	-76.50	-91.38	-52.47	-66.27
6β-Hydroxy-18-acetoxycassan-13,15-diene	-71.87	-52.35	-41.41	-68.98
8β-Hydroxyzaluzanin D	-61.95	-103.80	-52.98	-56.06
Alpinitine	-83.60	-94.08	-51.14	-55.79
Caaverine	-72.40	-77.96	-69.50	-60.73
Canthin-6-one	-67.68	-82.61	-34.54	-50.45
Coclaurine	-69.48	-96.15	-49.64	-58.14
Corytuberine	-72.75	-50.31	-41.80	-53.62
Dicenitrinone	-72.66	-57.16	-38.93	-45.97
Dugetine-β-N-oxide	-73.55	-54.89	-38.88	-51.86
E-1,2,4-Trihydroxynonadec-6-ene	-56.17	-84.59	-36.91	-42.24
Embelin	-71.24	-86.00	-48.88	-58.60
Eupomatenoid-6	-83.67	-94.46	-56.07	-67.95
Ganglin	-82.47	-97.76	-54.85	-57.45
Ganschisandrine	-77.63	-48.90	-43.70	-65.20
Geranygeraniol	-74.67	-89.48	-53.00	-65.16
Helenalin	-87.00	-91.40	-49.90	-48.14
Isonoholaenic acid	-80.27	-98.43	-47.08	-61.88
Kokusagine	-71.69	-91.77	-43.09	-67.38
Luteolin	-77.95	-101.41	-62.77	-64.83
Nectandrin B	-87.68	-59.34	-59.67	-76.11
N-Methylglaucine	-71.57	-49.51	-33.01	-58.89
N-Methyltetrahydropalmitine	-70.95	-57.06	-36.11	-45.29
Nordomesticine	-78.83	-59.01	-40.99	-53.78
Norisoboldine	-82.49	-62.07	-39.52	-51.80
Pallidine	-71.03	-52.45	-47.33	-49.93
Pinobanksin	-79.93	-99.34	-44.77	-63.58
Pinobanksin 3-acetate	-80.89	-94.77	-54.36	-67.09
Pinocembrin	-76.47	-95.72	-52.19	-53.22

Ligand	Cyclophilin	dUTPase	Superoxide Dismutase	Tyrosine Aminotransferase
	2ESL	2HQU	2WYZ	3DYD
rel-(7R,8R)-8-[(E)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-75.06	-90.39	-39.89	-67.54
Sarachine	-77.92	-62.18	-53.54	-61.12
Skimmianine	-71.31	-86.58	-40.30	-50.28
Tectochrysin	-62.46	-95.42	-56.69	-60.47

Table 3.10e: Lowest-energy docked poses (re-rank scores) of *T. cruzi*-selective ligands with *H. sapiens* protein analogs

Ligand	Farnesyl PP Synthase	Cathepsin	Lanosterol 14A demethylase	
	1ZW5	3H8B	3DL6 - A	3DL6 - B
1,2,4-Trihrooxyheptadecane	-67.02	-51.18	-35.88	-64.49
1,2,4-Trihydrooxyheptadec-16-ene	-59.03	-58.14	-46.65	-63.21
1,2,4-Trihydrooxyheptadec-16-yne	-66.87	-55.76	-35.87	-78.16
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-67.81	-57.38	-39.28	-69.52
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-88.77	-52.53	-41.24	-60.14
3-Heptadecyl-5-methoyphenol	-82.69	-57.58	-45.27	-67.19
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-67.70	-48.29	-34.15	-52.51
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-64.85	-52.74	-38.41	-71.89
5-epi-Icetexone	-69.36	-65.10	-74.57	-77.37
5-Methoxycanthin-6-one	-61.92	-60.82	-56.29	-81.01
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-67.46	-65.12	-51.54	-67.30
5,6,7-Trihydroxy4'-methoxyflavone	-87.72	-83.33	-62.79	-83.70
6β-Hydroxy-18-acetoxycassan-13,15-diene	-72.56	-62.53	-66.27	-75.11
8β-Hydroxyzaluzanin D	-86.16	-72.09	-80.35	-88.58
Alpinitine	-89.09	-76.33	-55.99	-75.05
Caaverine	-75.57	-70.65	-64.17	-84.24
Canthin-6-one	-64.51	-56.41	-50.77	-73.79
Coclaurine	-80.95	-84.04	-63.59	-76.67

Ligand	Farnesyl PP Synthase	Cathepsin	Lanosterol 14A demethylase	
	1ZW5	3H8B	3DL6 - A	3DL6 - B
Corytuberine	-78.31	-61.16	-60.52	-75.41
Dicenitrinone	-81.21	-64.80	-68.00	-81.49
Dugetine-β-N-oxide	-81.03	-66.12	-59.81	-78.14
E-1,2,4-Trihydroxynonadec-6-ene	-76.43	-66.96	-35.74	-70.75
Embelin	-82.95	-60.74	-50.77	-73.78
Eupomatenoid-6	-85.85	-85.46	-67.46	-82.59
Ganglin	-97.71	-68.84	-60.29	-79.67
Ganschisandrine	-83.59	-78.97	-62.57	-79.64
Geranygeraniol	-83.28	-81.89	-67.91	-75.40
Helenalin	-75.92	-63.91	-65.67	-87.37
Isonoholaenic acid	-83.28	-73.98	-63.22	-77.83
Kokusagine	-86.42	-77.60	-58.36	-81.35
Luteolin	-100.22	-75.45	-66.82	-87.85
Nectandrin B	-104.77	-88.16	-68.79	-89.79
N-Methylglaucine	-82.23	-55.56	-63.10	-72.53
N-Methyltetrahydropalmitine	-57.96	-63.33	-65.81	-79.43
Nordomesticine	-81.61	-69.55	-68.73	-84.83
Norisoboldine	-78.36	-65.96	-66.68	-80.64
Pallidine	-85.09	-71.20	-59.96	-69.61
Pinobanksin	-94.99	-67.52	-61.11	-74.17
Pinobanksin 3-acetate	-81.33	-69.86	-61.46	-85.84
Pinocembrin	-90.09	-66.41	-57.77	-72.15
rel-(7R,8R)-8-[(E)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-82.94	-69.27	-68.34	-85.49
Sarachine	-88.52	-62.20	-72.36	-85.50
Skimmianine	-69.58	-61.62	-49.17	-65.71
Tectochrysin	-97.21	-64.97	-57.88	-76.95

3.3 Notable ligands with *T. cruzi* proteins and hydrogen bond interactions

The five ligands that participated in selective binding with *Trypanosoma cruzi* proteins but had no notable interactions with human proteins were 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyheptadec-16-yne, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, (*E*)-1,2,4-trihydroxynonadec-6-ene, and *N*-methyltetrahydropalmitine. 1,2,4-Trihydroxyheptadec-16-ene showed significant binding with two trypanothione reductase structures, 1GXF and 1NDA

(Figure 3.1). Only one hydrogen bond was present in the simulation between a hydroxyl group and Gly 128, as showing in Figure 3.1b.

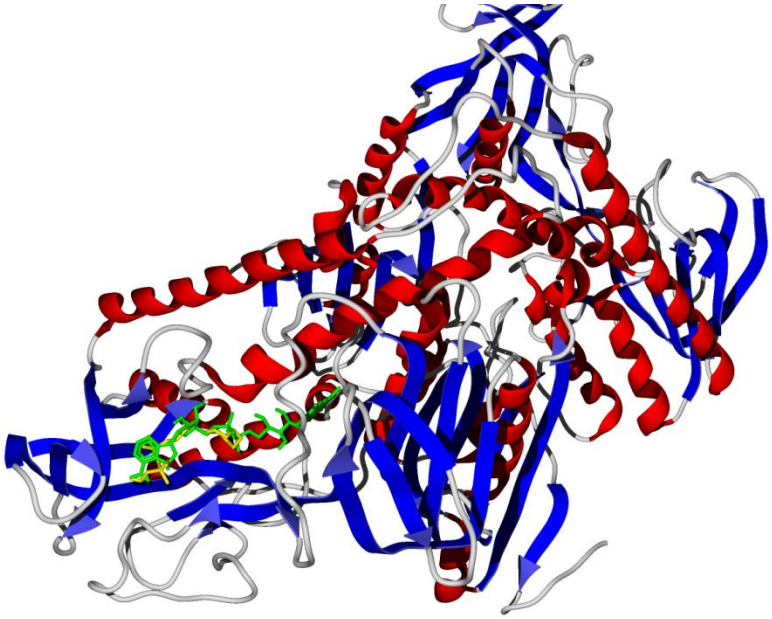


Figure 3.1a: 1,2,4-Trihydroxyheptadec-16-ene (yellow) docked with trypanothione reductase structure 1GXF. The co-crystallized cofactor flavin adenosine dinucleotide (FAD) is green.

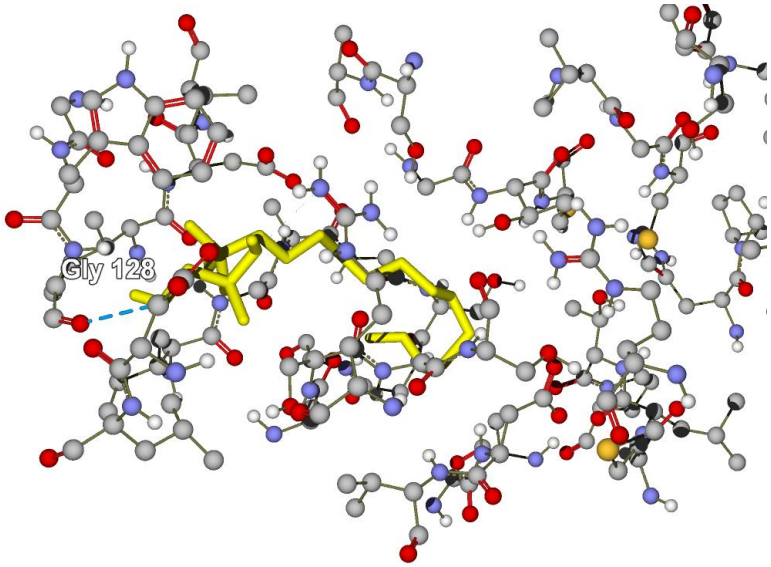


Figure 3.1b: A closer look at hydrogen bonding in the binding site of 1GXF. The dashed blue line indicates a hydrogen bond between a carbonyl oxygen on residue Gly 128 and a hydroxyl hydrogen in 1,2,4-trihydroxyheptadec-16-ene.

This antitrypanosomal agent also participated in selective binding with another trypanothione reductase structure 1NDA and was shown to participate in hydrogen bonds with residues Met 332 and Asp 326 (Figure 3.2).

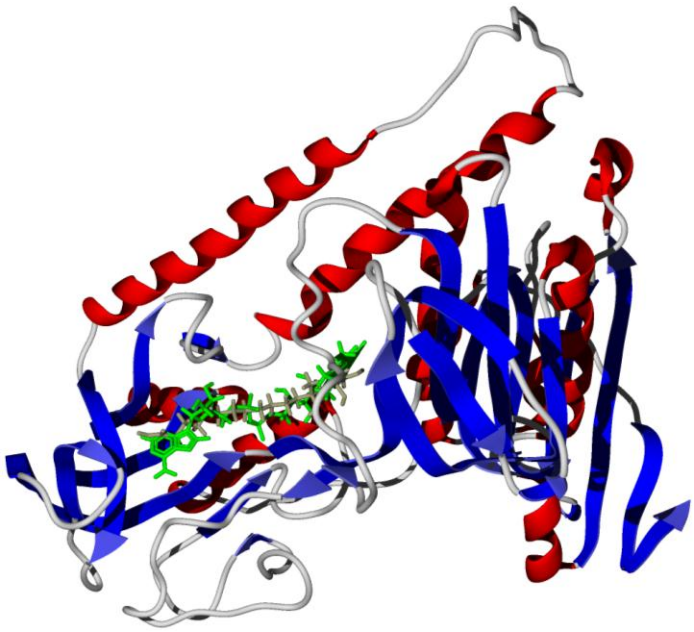


Figure 3.2a: 1,2,4-Trihydroxyheptadec-16-ene (gray) docked with trypanothione reductase structure 1NDA. The co-crystallized cofactor flavin adenosine dinucleotide (FAD) is green.

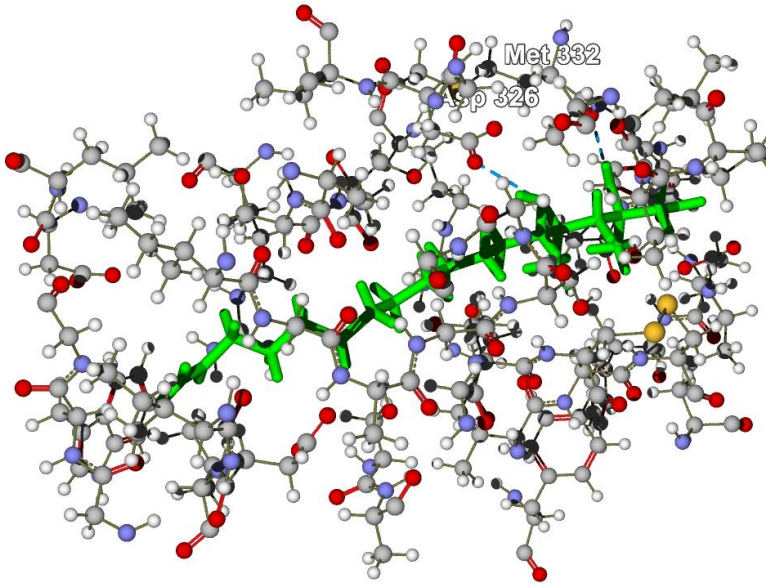


Figure 3.2b: A closer look at hydrogen bonding in the binding site of 1NDA. The dashed blue lines indicate hydrogen bond between residues Asp 326, Met 332, and 1,2,4-trihydroxyheptadec-16-ene.

1,2,4-Trihydroxyheptadec-16-yne only participated in selective binding with one of these trypanothione reductase structures, 1GXF, and two hydrogen bonds were noted in the simulation with Asp 294 and Arg 291 (Figure 3.3).

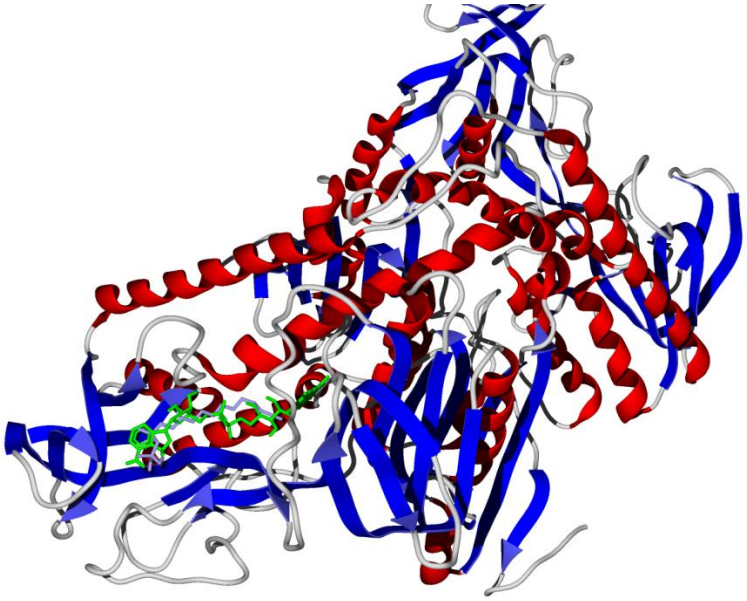


Figure 3.3a: 1,2,4-Trihydroxyheptadec-16-yne (light blue) docked with trypanothione reductase structure 1GXF. The co-crystallized cofactor flavin adenosine dinucleotide (FAD) is green.

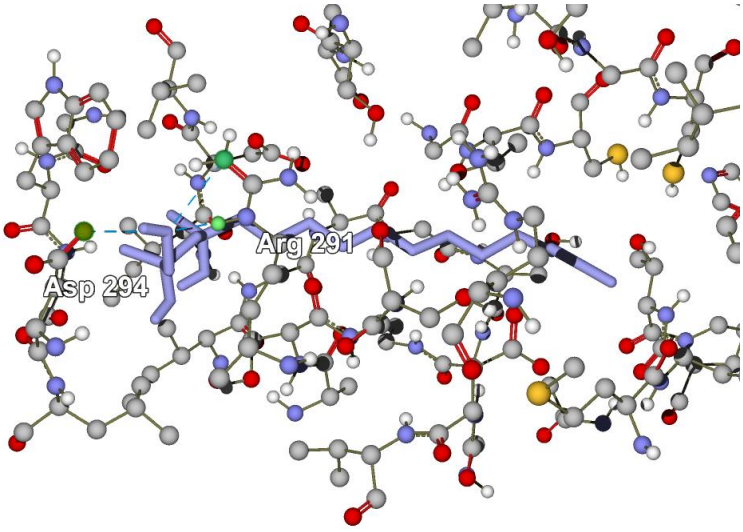


Figure 3.3b: A closer look at hydrogen bonding in the binding site of 1GXF. The dashed blue lines indicate hydrogen bonds between residues Asp 294, Arg 291, and 1,2,4-trihydroxyheptadec-16-yne.

4-Acetoxy-1,2-dihydroxyheptadec-16-yne was also involved in selective binding with the trypanothione reductase structure 1GXF, and the lowest energy docking pose participated in hydrogen binding with Gly 128 and Arg 291 (Figure 3.4).

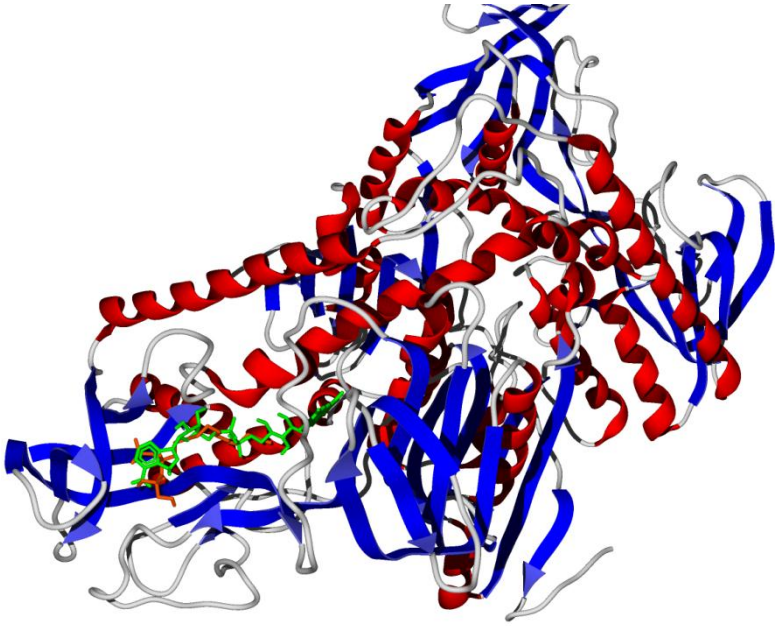


Figure 3.4a: 4-Acetoxy-1,2-dihydroxyheptadec-16-yne (orange) docked with trypanothione reductase structure 1GXF. The co-crystallized cofactor flavin adenosine dinucleotide (FAD) is green.

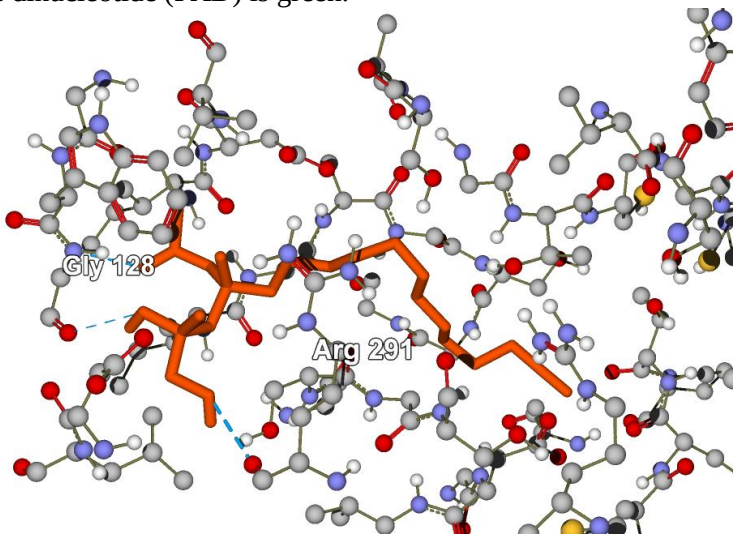


Figure 3.4b: A closer look at hydrogen bonding in the binding site of 1GXF. The dashed blue lines indicate hydrogen bond between residues Gly 128, Arg 291, and 4-acetoxy-1,2-dihydroxyheptadec-16-yne.

This compound also participated in selective binding with the histidyl-tRNA synthetase structure 3HRK. Hydrogen bonds between residues Glu 158, Arg 165, and Cys 365 contributed to the docked pose (Figure 3.5)

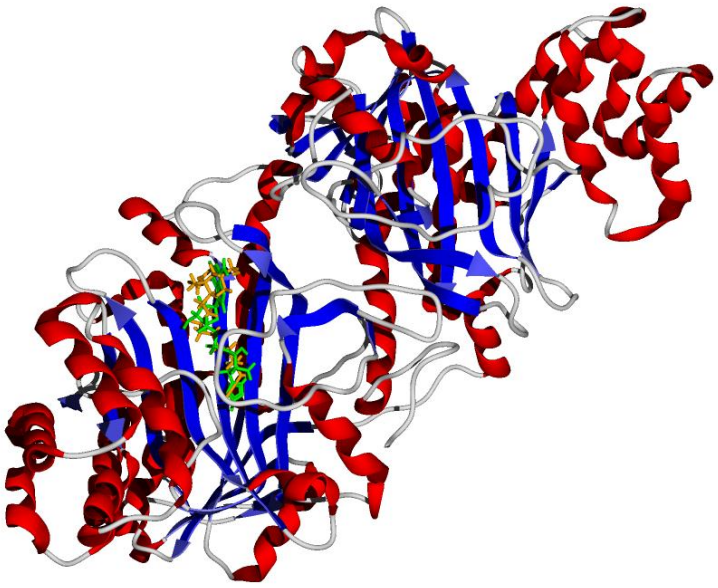


Figure 3.5a: 4-Acetoxy-1,2-dihydroxyheptadec-16-yne (orange) docked with histidyl-tRNA synthetase structure 3HRK. The co-crystallized ligand, histidyl-adenosine monophosphate, is green.

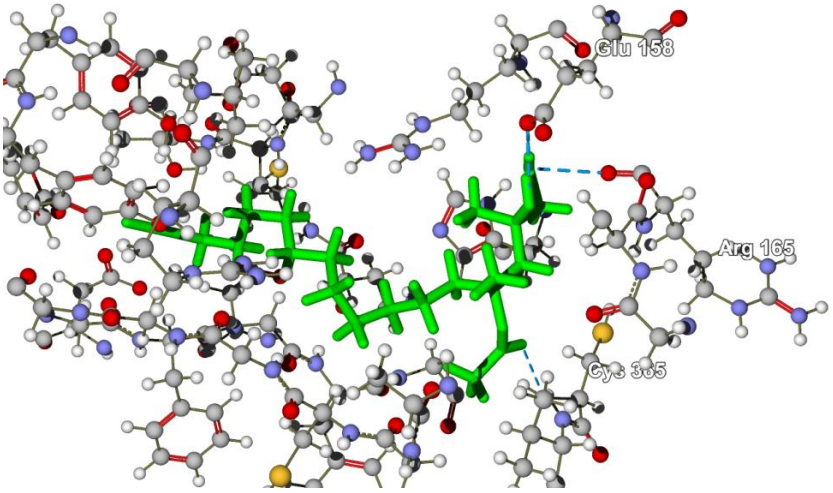


Figure 3.5b: A closer look at hydrogen bonding in the binding site of 3HRK. The dashed blue lines indicate hydrogen bonds Glu 158, Arg 165, Cys 365 and 4-acetoxy-1,2-dihydroxyheptadec-16-yne.

The last oxygenated hydrocarbon (*E*)-1,2,4-trihydroxynonadec-6-ene participated in selective binding with both previously noted trypanothione reductase structures 1GXF and 1NDA. With 1GXF, Gly 128, Asp 294, and Arg 291 all participated in hydrogen binding with the ligand (Figure 3.6), and in the simulation between (*E*)-1,2,4-trihydroxynonadec-6-ene and the other structure, 1NDA, Asp 326 and Met 332 were key residues for hydrogen binding (Figure 3.7).

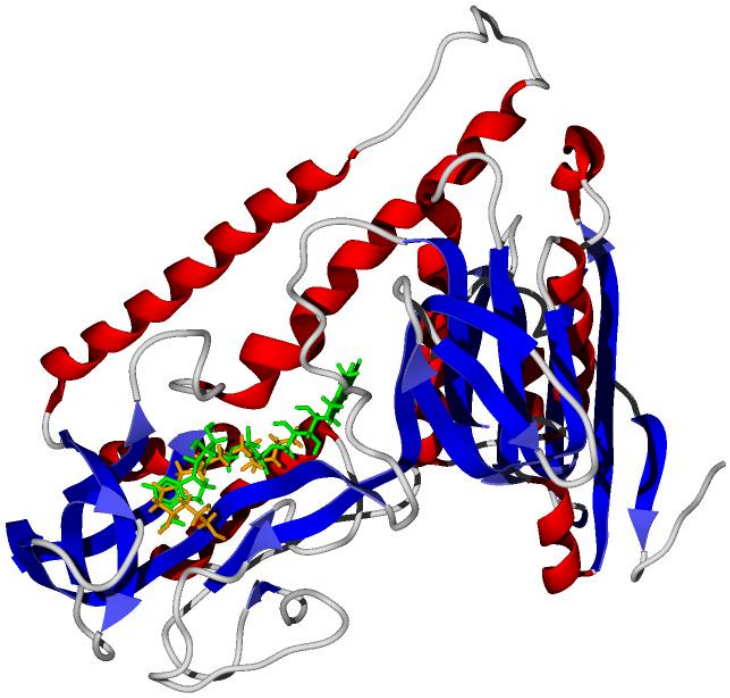


Figure 3.6a: (*E*)-1,2,4-Trihydroxynonadec-6-ene (orange) docked with trypanothione reductase structure 1GXF. The co-crystallized ligand, flavin adenosine dinucleotide (FAD), is green.

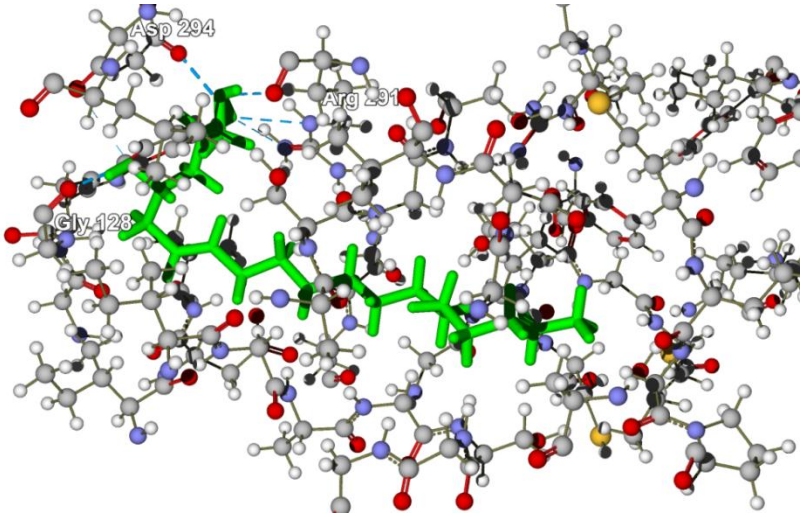


Figure 3.6b: A closer look at hydrogen bonding in the binding site of 1GXF. The dashed blue lines indicate hydrogen bonds Gly 128, Arg 291, Asp 294 and (*E*)-trihydroxynonadec-6-ene.

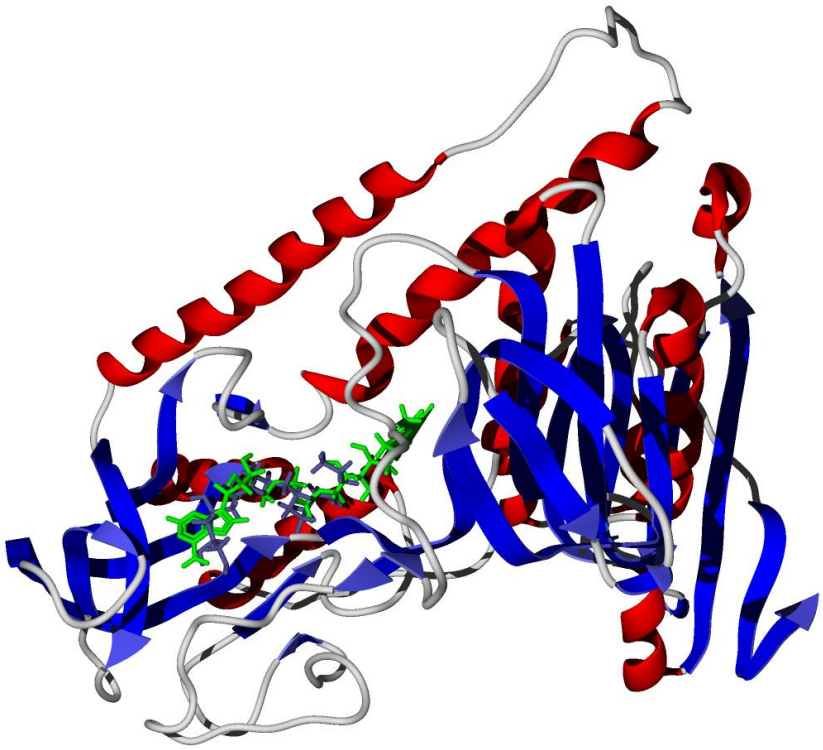


Figure 3.7a: (*E*)-1,2,4-Trihydroxynonadec-6-ene (blue) docked with trypanothione reductase structure 1NDA. The co-crystallized ligand, flavin adenosine dinucleotide (FAD), is green

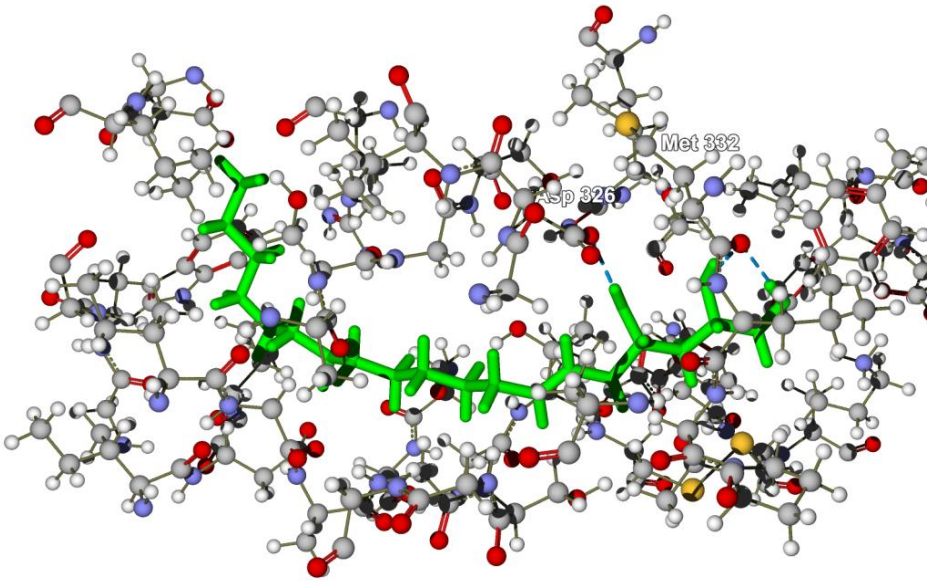


Figure 3.7b: A closer look at hydrogen bonding in the binding site of 1NDA. The dashed blue lines indicate a hydrogen bonds between Asp 326, Met 332, and (*E*)-1,2,4-trihydroxynonadec-6-ene.

The only alkaloid that showed selective binding to any *Trypanosoma cruzi* proteins and participated in no notable binding with human proteins was *N*-methyltetrahydropalmitine. It showed selective binding to the *T. cruzi* protein pteridine reductase 2 structure 1MXH with hydrogen bonds between the residues Asn 101 and Ser 103 (Figure 3.8).

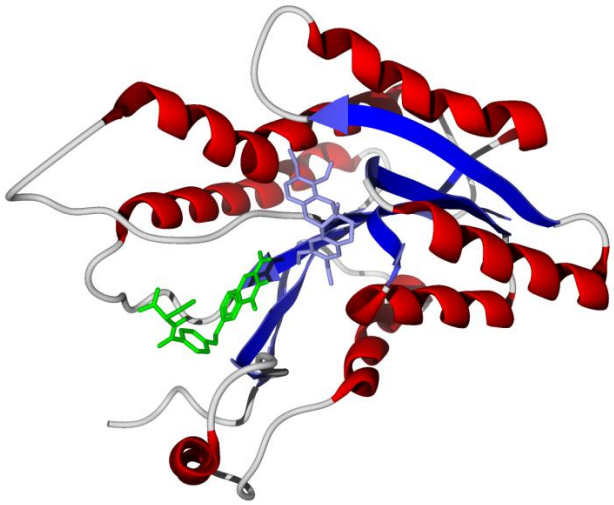


Figure 3.8a: *N*-methyltetrahydropalmitine (blue) docked with pteridine reductase 2 structure 1MXH. The co-crystallized ligand, dihydrofolic acid, is green.

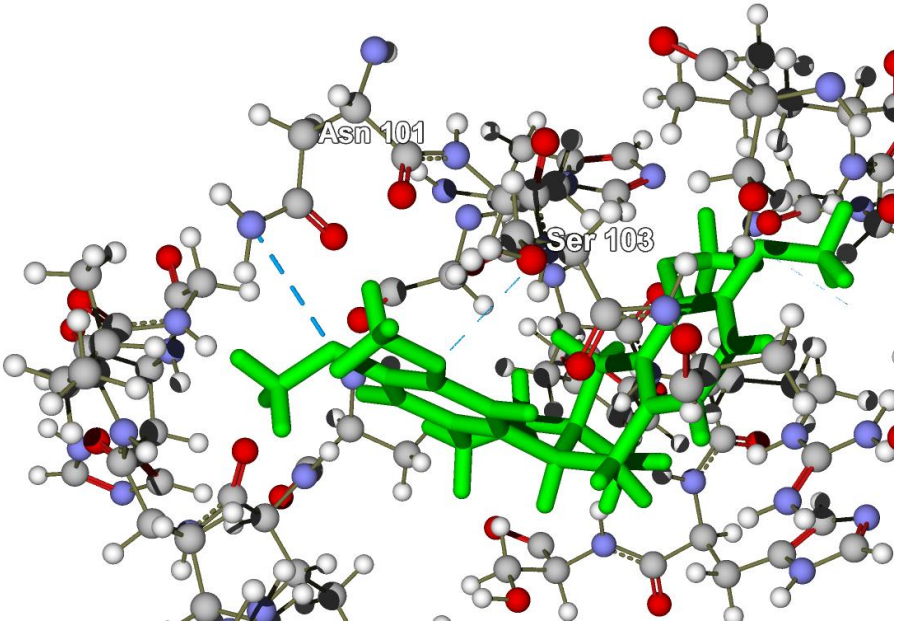


Figure 3.8b: A closer look at hydrogen bonding in the binding site of 1MXH. The dashed blue lines indicate hydrogen bonds between Asn 101, Ser 103, and *N*-methyltetrahydropalmitine.

There were some similarities in hydrogen bonding between trypanothione reductase structures 1NDA and 1GXF and the notable oxygenated hydrocarbons. Regarding structure 1GXF, two compounds participated in hydrogen binding with residue Asp 294 (1,2,4-trihydroxyheptadec-16-yne and (*E*)-1,2,4-trihydroxynonadec-6-ene), three ligands participated in hydrogen binding with residue Arg 291 (1,2,4-trihydroxyheptadec-16-yne and 4-acetoxy-1,2-dihydroxyheptadec-16-yne), and three ligands participated in hydrogen bonding with Gly 128 (1,2,4-trihydroxy heptadec-16-ene, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, and (*E*)-1,2,4-trihydroxynonadec-6-ene), and both ligands with notable docking with structure 1NDA participated in hydrogen binding with Met 332 and Asp326 (1,2,4-trihydroxy heptadec-16-ene and (*E*)-1,2,4-trihydroxynonadec-6-ene). Due to these common interactions, these residues may be key to protein-ligand interactions *in vivo*, and could potentially be studied further outside of a computational environment.

3.4 Hydrophobic interactions of oxygenated hydrocarbons and trypanothione reductase

Oxygenated hydrocarbons comprise the majority of the ligands that have selective docking with a *T. cruzi* compound, but have no notable docking with human analogs examined. All four of these compounds, 1,2,4-trihydrooxyheptadec-16-ene, 1,2,4-trihydrooxyheptadec-16-yne, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, and (*E*)-1,2,4-trihydroxynonadec-6-ene, showed selective binding with the trypanothione reductase structure 1GXF, so the nature of this interaction was investigated further. In Figure 3.9, the residues of the binding pocket of trypanothione reductase and the human analog, glutathione reductase, were colored by hydrophobicity, red being very hydrophilic, blue being very hydrophobic, and shades of purple being residues that fall somewhere in the middle of the spectrum. Aliphatic regions of the ligands are colored green and white, representing carbons and hydrogens respectively, and oxygens are colored orange.

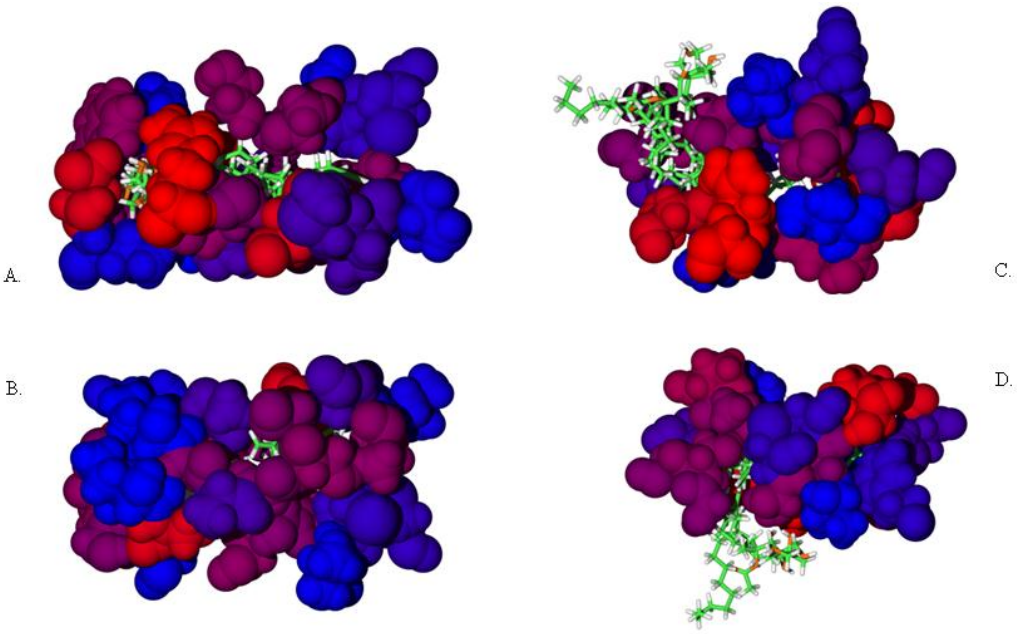


Figure 3.9: Hydrophobicity of the binding pockets of trypanothione reductase and the human analog, glutathione reductase. A and B are the front and back views of the binding pocket trypanothione reductase, and C and D are the front and back views of the binding pocket of glutathione reductase

The binding pocket of trypanothione reductase is more conducive to docking with these oxygenated hydrocarbons with hydrophilic residues on one end where the hydroxide groups can participate in hydrogen bonding, and a hydrophobic region where the aliphatic chains can fit without hydrophobic repulsion. The binding pocket of glutathione reductase has hydrophilic residues on both ends of the binding pocket resulting in a somewhat repulsive environment for hydrophobic interactions. This is a likely reason why there was selective binding in the *T. cruzi* protein but not the human analog.

3.5 Considering α-β unsaturated carbonyl conjugation

All α-β unsaturated carbonyls examined showed large LUMOs around the site of alkene-ketone conjugation. This group is vulnerable to attack, via Michael Addition, by adjacent nucleophilic amino acids (Cys, His, Lys, Arg, Met, Ser) which are common in the active sites of enzymes. One possible example of this phenomenon would be the docking between caffeic acid and the *T. cruzi* protein structure, 1AIM. The structure of caffeic acid and its large LUMO can be found in Figure 3.10.

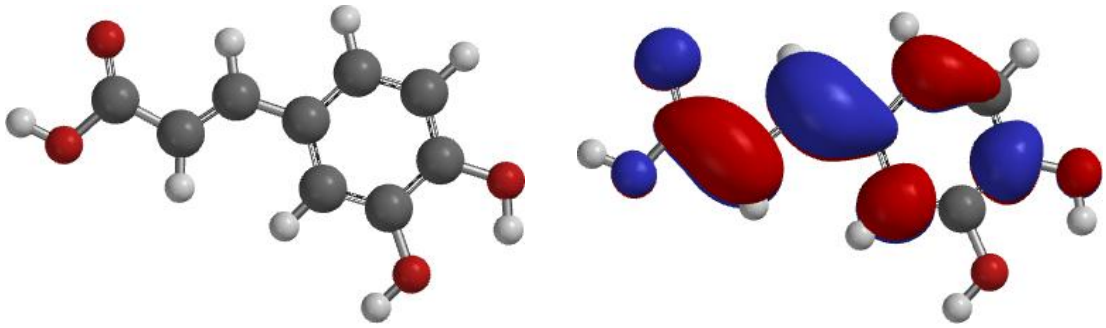


Figure 3.10: Ball and stick structure of caffeic acid and a map of its LUMO.

When the lowest energy conformer of caffeic acid is docked with 1AIM, a nucleophilic amino acid, Cys 25, and the β-carbon of the ligand are 3.42 Å apart (Figure 3.11).

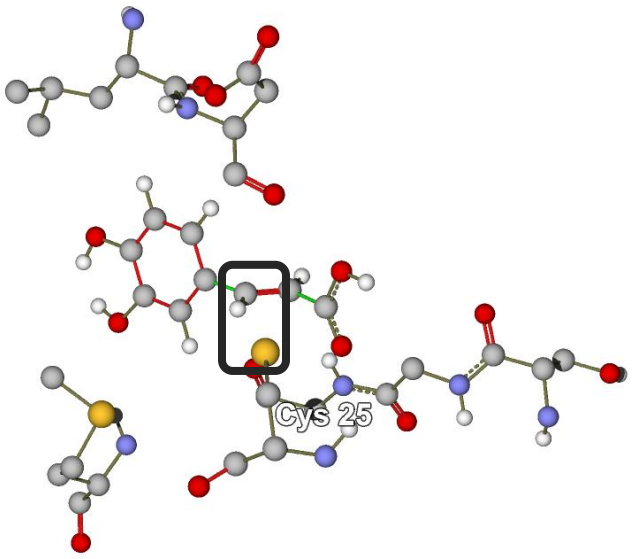


Figure 3.11: Nucleophilic interaction between cruzain and caffeic acid

This may lead to permanent or semi-permanent covalent bonding between cruzain and caffeic acid, thereby leading to possible anti-trypanosomal activity. This, however, may present a problem in the human system, as nucleophilic residues are very common in the binding pockets of enzymatic proteins. Fortunately, none of the five ligands this study showed may be suitable for pharmaceutical screening are α-β unsaturated carbonyls.

CHAPTER 4

CONCLUSION

This investigation has suggested several protein targets for phytochemical agents with known trypanocidal activity. Forty-five trypanosomal agents were shown to participate in selective binding with the *Trypanosoma cruzi* proteins analyzed. Although all of these agents would be appropriate for further investigations outside of the human system, only five of these did not show any notable binding with any of the human analogs examined. These agents were the oxygenated hydrocarbons 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyheptadec-16-yne, 4-acetoxy-1,2-dihydroxyheptadec-16-yne, and (*E*)-1,2,4-trihydrononadec-6-ene, and the alkaloid *N*-methyltetrahydropalmitine. All of the oxygenated hydrocarbons showed selectivity with various structures of the *T. cruzi* protein trypanothione reductase, a specifically trypanosomal protein to which the closet human analog is glutathione reductase. Key residues for hydrogen bonding in this protein were noted, and hydrophobic interactions may contribute to selective docking with hydrocarbon chains.

There are many limitations to these results, however. The protein structures available for this investigation may not be an inclusive representation of the true bioactive conformation in the *T. cruzi* system, and protein-ligand docking simulations do not take into account membrane permeability and target bioavailability. There are also probable *T. cruzi* protein targets that have yet to be isolated, and these unknown proteins may serve as the true target for these antitrypanosomal compounds. Finally, docking simulations for all human proteins were not conducted, and these compounds may be toxic in the human system as well. These factors being considered, protein-ligand selectivity *in silico* has been confirmed in this molecular docking study, and this evidence could be used as a foundation for *in vitro* or *in vivo* target binding analysis and the synthesis of further trypanocidal compounds. Future investigations may include computational work such as conducting simulations with more human proteins or proteins in the

Triatomine insect vector. This research could also be repeated as computational technology advances and as more protein structures and trypanocidal agents become available. These results could also serve as a foundation for environmental impact studies for any of the forty-five *T. cruzi*-selective ligands to become baits, sprays, or dusts to rid habituated areas of the parasite or its insect vector, thereby preventing the transmission and ridding the area of the disease-causing parasite.

APPENDIX

Extended Docking Results, Tables A.1-A.9. Values in red indicate selective binding and when a letter designation after the protein structure name is present, this notes multiple binding sites of interest in the crystal structure:

Table A.1a: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with cruzain structures.

Ligand	Cruzain							
	1AIM	1F2A	1F2B	1F2C	1ME3	1ME4	1U9Q	2AIM
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-64.16	-71.23	-62.78	-71.72	-70.06	-77.45	-81.78	-60.49
Arborinine	-63.26	-73.86	-74.27	-77.74	-72.95	-72.73	-70.69	-63.81
Kokusagine	-66.01	-73.75	-73.34	-81.12	-76.71	-69.83	-73.33	-60.66
N-Methyl-1-hydroxy-3-methoxyacridone	-63.40	-66.22	-65.17	-73.26	-66.45	-66.03	-65.77	-59.71
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-82.30	-45.06	-84.14	-92.30	-46.17	-56.00	-60.61	-83.52
Skimmianine	-69.79	-75.99	-76.46	-80.91	-78.79	-75.87	-74.21	-59.30
Dicenitrinone	-75.85	-80.85	-80.69	-86.58	-79.02	-77.81	-79.72	-71.22
Dugetine	-74.19	-73.31	-64.14	-65.96	-78.22	-76.97	-73.36	-68.95
Dugetine-β-*N*-oxide	-73.66	-64.07	-66.72	-64.61	-76.20	-75.18	-68.58	-67.58
N-Methylglaucine	-60.56	-80.84	-54.99	-58.57	-84.75	-86.02	-81.08	-64.60
N-Methyltetrahydropalmitine	-82.11	-76.82	-75.26	-79.05	-70.61	-79.81	-77.65	-42.99
Caaverine	-65.41	-65.42	-62.79	-68.28	-71.02	-70.65	-64.37	-55.75
Coclaurine	-77.01	-54.93	-71.25	-69.78	-74.58	-74.19	-76.91	-66.79
Corytuberine	-66.50	-80.69	-64.67	-72.23	-81.29	-78.34	-77.05	-62.02
Domesticine	-67.71	-40.01	-72.60	-73.00	-68.46	-62.20	-66.78	-62.17
Glazovine	-66.61	-69.91	-63.34	-57.11	-77.63	-77.41	-74.08	-70.62
Isoboldine	-61.86	-77.74	-65.74	-73.07	-80.66	-80.90	-78.27	-68.66
Laurotetanine	-70.95	-73.28	-71.49	-80.09	-78.38	-72.33	-73.46	-9.19
N-Methylcoclaurine	-65.34	-49.37	-69.18	-69.78	-63.10	-68.52	-65.13	-62.19
Nordomesticine	-72.81	-70.31	-72.33	-80.18	-76.54	-66.16	-74.75	-66.03

Ligand	Cruzain							
	1AIM	1F2A	1F2B	1F2C	1ME3	1ME4	1U9Q	2AIM
Norisoboldine	-69.23	-72.12	-70.66	-78.44	-75.83	-72.30	-70.99	-35.96
Pallidine	-54.50	-49.79	-63.62	-50.70	-64.36	-58.64	-61.04	-53.40
Sarachine	-57.51	-42.41	-54.05	-65.26	-60.25	-56.81	-57.57	-53.28
5-Methoxycanthin-6-one	-64.84	-67.86	-67.61	-74.25	-70.56	-74.76	-71.93	-60.78
Canthin-6-one	-55.43	-63.15	-63.45	-69.26	-64.16	-56.03	-61.81	-56.95
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-67.80	-69.99	-65.68	-49.82	-65.06	-73.28	-74.11	-39.96
18-Acetoxy-13-15-diene-cassanoic acid	-6.91	-36.41	-13.99	-60.90	-20.70	-4.88	-44.99	-67.88
18-Hydroxycassan-13,15-diene	-44.98	-53.61	-60.04	-13.44	-55.14	-63.34	-45.52	-53.03
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-42.67	-33.06	-64.21	-51.58	-35.38	-40.74	-52.31	-30.37
6β-18-Dihydroxycasssan-13,15-diene	-58.95	-46.98	-56.86	-10.64	-58.47	-60.43	-47.02	-61.36
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-73.18	-61.48	-71.20	-59.49	-66.23	-63.59	-68.47	-67.23
Geranygeraniol	-80.94	-85.74	-77.92	-82.96	-96.68	-86.39	-78.84	-74.04
5-epi-Icetexone	-55.56	-42.39	-58.23	-45.76	-65.04	-42.85	-60.02	-55.19
Alpinitine	-72.31	-71.96	-68.62	-76.88	-76.12	-76.55	-73.64	-62.52
5,6,7-Trihydroxy-4′-methoxyflavone	-73.66	-73.07	-69.92	-80.68	-71.25	-76.52	-77.45	-62.38
Ganglin	-67.76	-67.58	-64.43	-74.36	-70.93	-75.43	-74.42	-62.42
Ganglin-3-methyl ether	-40.42	-22.65	-64.97	-64.08	-74.96	-72.57	-70.96	-52.32
Luteolin	-64.42	-69.53	-59.89	-65.33	-70.90	-72.11	-36.02	-53.10
Pinobanksin 3-acetate	-64.53	-69.39	-60.27	-67.55	-72.00	-68.71	-70.73	-63.92
Pinobanksin	-70.23	-69.35	-67.46	-75.62	-70.45	-70.56	-66.68	-61.36
Pinocembrin	-67.55	-60.91	-61.82	-69.04	-69.58	-68.67	-62.22	-57.15
Quercetin-3-methyl ether	-38.51	-22.94	-64.82	-68.37	-74.39	-72.71	-75.43	-37.25
Tectochrysin	-64.72	-58.96	-59.20	-77.52	-78.73	-78.23	-61.46	-66.87
Aristolignan	-74.11	-87.76	-77.46	-79.36	-84.58	-86.68	-92.55	-36.28
Calopiptin	-85.39	-92.29	-86.55	-94.73	-80.61	-93.15	-87.97	-82.95
Galgravin	-73.31	-95.34	-71.87	-19.94	-96.87	-98.90	-96.23	-61.78
Ganschisandrine	-74.22	-38.13	-76.61	-86.56	-66.15	-47.93	-46.95	-78.33
Machilin G	-83.15	-92.80	-85.21	-94.71	-92.43	-84.25	-77.30	-65.28
Nectandrin A	-75.37	-94.72	-72.26	-18.73	-93.00	-93.83	-94.32	-75.50
Nectandrin B	-79.41	-89.90	-71.13	-90.20	-92.48	-84.00	-82.39	-75.81
Conocarpan	-63.34	-80.29	-62.23	-69.00	-78.12	-79.51	-81.57	-66.33

Ligand	Cruzain							
	1AIM	1F2A	1F2B	1F2C	1ME3	1ME4	1U9Q	2AIM
Eupomatenoid-3	-78.51	-79.91	-82.46	-89.03	-90.35	-90.82	-87.77	-68.46
Eupomatenoid-5	-80.00	-82.71	-76.28	-88.28	-72.48	-76.59	-87.18	-70.68
Eupomatenoid-6	-65.10	-75.20	-77.99	-85.79	-71.52	-79.97	-84.31	-63.54
Grandisin	-68.32	-82.03	-75.68	-68.79	-91.73	-90.94	-93.06	-76.03
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-79.25	-96.60	-77.39	-77.85	-92.85	-87.18	-59.16	-54.74
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-74.53	-95.88	-83.21	-71.15	-93.46	-97.25	-87.79	-47.99
4,5-di-(*E*)-O-Caffeyoquinic acid	-38.82	-120.83	-106.84	-113.45	-127.31	-126.53	-113.58	-123.44
Caffeic Acid	-60.49	-59.35	-56.05	-61.03	-63.08	-61.84	-60.64	-56.90
Isonoholaenic acid	-74.73	-81.31	-71.57	-79.92	-80.55	-84.50	-86.23	-73.93
3-Heptadecyl-5-methoyphenol	-51.18	-61.40	-45.52	-68.73	-58.99	-59.88	-51.59	-44.79
Embelin	-57.03	-66.36	-57.58	-73.13	-66.05	-68.72	-59.47	-65.88
1,2,4-Trihrooxyheptadecane	-74.08	-78.32	-70.30	-83.60	-78.99	-82.58	-77.74	-77.01
1,2,4-Trihydrooxyheptadec-16-ene	-65.95	-74.44	-66.31	-73.45	-72.31	-77.43	-66.03	-67.61
1,2,4-Trihydrooxyheptadec-16-yne	-73.54	-68.82	-75.29	-79.06	-80.22	-79.95	-81.93	-63.07
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-75.02	-59.74	-79.87	-77.44	-86.80	-76.66	-90.39	-70.19
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-81.43	-62.11	-72.45	-74.41	-80.75	-80.36	-74.33	-67.24
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-70.75	-73.94	-69.08	-76.88	-80.15	-78.55	-83.53	-67.77
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-76.41	-77.65	-65.62	-52.39	-81.40	-86.63	-78.38	-66.02
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-77.08	-79.42	-74.17	-76.13	-81.48	-85.64	-85.15	-67.03
Helenalin	-64.13	-57.33	-65.29	-64.63	-61.57	-55.57	-69.74	-68.34
Mexicanin	-59.32	-68.59	-68.23	-55.21	-75.73	-51.89	-66.99	-60.67
15-Deoxygoyazenolide	-78.66	-70.78	-80.87	-86.84	-72.48	-77.46	-73.66	-81.32
Centratherin	-87.69	-90.57	-82.74	-91.29	-87.59	-85.24	-86.89	-83.92
Goyazenolide	-82.02	-81.37	-81.81	-89.95	-71.61	-71.47	-84.05	-81.30
Lynchopholide	-84.31	-79.24	-80.66	-88.88	-73.91	-79.36	-78.38	-83.15
8β-Hydroxyzaluzanin D	-74.80	-75.04	-75.05	-76.81	-73.39	-73.38	-74.36	-72.05
Oleanolic acid	-40.78	-3.45	-12.07	-45.46	9.54	22.04	-46.57	-38.58

Ligand	Cruzain							
	1AIM	1F2A	1F2B	1F2C	1ME3	1ME4	1U9Q	2AIM
Ursolic acid	24.58	-23.23	11.88	16.43	-45.47	48.42	-45.05	-42.62
Betulinic acid	-5.16	-5.10	-8.48	2.36	-31.48	-30.34	-25.37	-51.56

Table A.1b: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with cruzain structures, continued.

Ligand	Cruzain							
	2OZ2	3HD3	3I06	3IUT	1F29	3KKU	3LXS-A	3LXS-C
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-71.13	-80.78	-73.23	-70.65	-66.38	-64.21	-67.67	-67.23
Arborinine	-77.77	-72.86	-79.89	-67.96	-73.80	-83.39	-77.64	-76.55
Kokusagine	-83.73	-83.77	-73.54	-71.48	-76.47	-77.28	-71.49	-72.83
N-Methyl-1-hydroxy-3-methoxyacridone	-73.88	-72.46	-76.24	-60.59	-66.31	-74.42	-74.54	-72.89
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-83.13	-113.52	-92.27	-69.37	-44.83	-67.97	-80.96	-81.93
Skimmianine	-74.06	-81.14	-70.74	-79.10	-79.21	-77.65	-72.73	-72.71
Dicenitrinone	-89.02	-74.33	-83.35	-77.68	-81.22	-81.90	-80.50	-81.37
Dugetine	-81.88	-76.88	-92.79	-77.91	-64.38	-89.45	-85.29	-75.78
Dugetine-β-*N*-oxide	-78.17	-85.46	-72.83	-76.81	-62.26	-70.24	-63.97	-72.36
N-Methylglaucine	-80.83	-93.51	-61.49	-77.01	-68.26	-68.24	-61.67	-64.17
N-Methyltetrahydropalmitine	-83.42	-86.67	-82.02	-68.72	-78.74	-82.95	-75.40	-73.24
Caaverine	-68.33	-78.04	-71.83	-63.74	-68.12	-67.95	-67.79	-67.73
Coclaurine	-70.19	-91.99	-80.32	-74.55	-70.24	-64.23	-79.51	-80.86
Corytuberine	-74.44	-79.58	-73.18	-71.73	-61.95	-66.44	-72.84	-70.80
Domesticine	-72.94	-88.62	-65.05	-18.44	-72.54	-72.68	-69.75	-66.24
Glazovine	-74.74	-91.35	-35.49	-64.45	-63.64	-29.56	-64.13	-62.55
Isoboldine	-74.62	-88.61	-52.81	-71.77	-73.35	-71.81	-70.28	-72.59
Laurotetanine	-76.25	-72.92	-74.05	-66.76	-72.37	-75.10	-76.67	-77.20
N-Methylcoclaurine	-76.12	-94.31	-66.35	-50.85	-39.30	-66.59	-70.51	-67.28
Nordomesticine	-78.71	-81.65	-74.59	-70.44	-74.15	-80.79	-79.56	-78.98
Norisoboldine	-74.73	-87.39	-73.33	-67.11	-74.10	-75.75	-71.80	-76.73

Ligand	Cruzain							
	2OZ2	3HD3	3I06	3IUT	1F29	3KKU	3LXS-A	3LXS-C
Pallidine	-73.80	-90.08	-64.24	-56.73	-66.92	-51.40	-63.73	-57.83
Sarachine	-88.75	-79.04	-64.22	-45.79	-52.34	-52.44	-72.20	-67.60
5-Methoxycanthin-6-one	-74.04	-61.24	-72.08	-68.70	-68.04	-75.81	-73.46	-76.27
Canthin-6-one	-70.95	-77.40	-72.28	-47.57	-62.68	-71.06	-68.71	-71.89
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-96.23	-83.16	-66.79	-74.14	-67.45	-77.12	-79.83	-25.97
18-Acetoxy-13-15-diene-cassanoic acid	-81.38	-85.76	-67.43	-67.63	-15.05	-8.89	-68.56	-60.96
18-Hydroxycassan-13,15-diene	-68.01	-69.13	-53.82	-66.11	-57.75	-32.43	-14.56	-41.83
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-72.60	-57.65	-62.88	-39.30	-45.51	-64.99	-50.28	-4.58
6β-18-Dihydroxycasssan-13,15-diene	-69.69	-65.14	-4.44	-65.16	-54.89	-20.94	-7.42	-19.12
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-65.13	-78.04	-61.83	-65.41	-64.90	-67.85	-61.39	-59.32
Geranygeraniol	-95.44	-111.15	-81.29	-85.09	-94.62	-88.76	-80.61	-85.74
5-epi-Icetexone	-81.27	-79.83	-33.11	-66.04	-58.60	-57.83	-46.44	-61.66
Alpinitine	-75.65	-86.25	-77.27	-74.56	-66.92	-80.49	-72.06	-71.63
5,6,7-Trihydroxy-4′-methoxyflavone	-74.01	-92.49	-78.74	-77.12	-71.24	-76.63	-75.31	-76.65
Ganglin	-72.49	-84.65	-74.46	-75.12	-71.71	-80.08	-70.39	-79.70
Ganglin-3-methyl ether	-69.61	-90.34	-75.86	-78.54	-27.08	-75.14	-67.96	-69.40
Luteolin	-70.52	-100.15	-75.21	-70.23	-70.91	-74.00	-69.51	-72.05
Pinobanksin 3-acetate	-72.09	-89.30	-69.89	-63.85	-64.20	-64.68	-68.62	-66.70
Pinobanksin	-72.94	-83.52	-70.37	-70.59	-70.74	-78.49	-69.66	-68.28
Pinocembrin	-67.14	-87.85	-68.95	-68.16	-65.74	-72.75	-65.16	-64.43
Quercetin-3-methyl ether	-83.86	-96.99	-78.76	-73.77	-51.50	-81.79	-69.37	-76.64
Tectochrysin	-72.06	-93.52	-72.38	-77.52	-72.11	-75.59	-69.21	-68.43
Aristolignan	-84.42	-123.74	-53.04	-81.13	-72.06	-71.54	-87.96	-33.86
Calopiptin	-94.02	-116.05	-95.02	-94.32	-92.09	-103.08	-93.43	-88.76
Galgravin	-88.25	-112.44	-56.73	-89.70	-84.19	-31.70	-83.65	-69.19
Ganschisandrine	-89.69	-101.97	-95.34	-71.47	-77.29	-89.66	-83.36	-74.08
Machilin G	-97.83	-122.34	-87.64	-81.63	-91.00	-91.85	-98.28	-90.95
Nectandrin A	-92.06	-118.82	-81.65	-88.77	-33.45	-81.42	-92.49	-54.59
Nectandrin B	-93.45	-109.95	-48.71	-85.42	-74.18	-54.16	-87.39	-65.18
Conocarpan	-78.18	-97.57	-69.94	-74.64	-59.88	-43.17	-67.71	-58.73
Eupomatenoid-3	-98.01	-106.95	-84.00	-83.81	-85.31	-88.69	-84.83	-80.89

Ligand	Cruzain							
	2OZ2	3HD3	3I06	3IUT	1F29	3KKU	3LXS-A	3LXS-C
Eupomatenoid-5	-88.34	-108.33	-96.38	-78.16	-84.86	-89.35	-93.70	-90.59
Eupomatenoid-6	-86.30	-100.62	-86.67	-77.88	-80.49	-66.71	-83.03	-75.98
Grandisin	-88.59	-99.36	-66.13	-90.26	-85.47	-47.67	-78.78	-83.82
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-93.30	-105.05	-68.11	-98.97	-82.15	-93.31	-86.26	-82.20
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-105.94	-111.77	-84.74	-100.40	-73.40	-63.63	-82.97	-57.45
4,5-di-(*E*)-O-Caffeyoquinic acid	-121.31	-104.44	-106.62	-122.02	-116.75	-100.92	-116.24	-120.18
Caffeic Acid	-67.37	-74.48	-57.38	-59.25	-55.19	-56.87	-62.40	-64.17
Isonoholaenic acid	-79.10	-113.23	-71.29	-88.62	-76.86	-80.40	-71.70	-72.50
3-Heptadecyl-5-methoyphenol	-64.48	-92.74	-49.65	-59.84	-51.11	-54.94	-75.45	-57.83
Embelin	-65.16	-87.82	-64.20	-69.02	-61.08	-62.01	-59.14	-59.55
1,2,4-Trihrooxyheptadecane	-80.53	-91.18	-69.89	-76.58	-58.94	-80.88	-83.88	-82.81
1,2,4-Trihydrooxyheptadec-16-ene	-77.96	-91.62	-66.72	-69.90	-53.71	-74.67	-75.81	-73.32
1,2,4-Trihydrooxyheptadec-16-yne	-84.00	-88.78	-60.83	-67.91	-59.78	-70.96	-73.08	-76.81
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-80.71	-99.55	-63.91	-66.05	-74.03	-74.87	-82.02	-83.29
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-90.29	-92.69	-84.68	-74.46	-83.41	-75.54	-70.98	-81.93
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-81.64	-96.07	-74.60	-79.12	-71.60	-80.94	-70.77	-76.36
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-88.52	-94.21	-77.41	-60.49	-68.07	-77.62	-74.44	-69.67
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-81.08	-97.62	-83.42	-80.67	-76.85	-69.22	-84.63	-82.68
Helenalin	-66.34	-63.98	-60.13	-59.80	-64.68	-64.75	-64.72	-47.38
Mexicanin	-69.73	-70.51	-66.37	-50.83	-70.13	-63.52	-67.78	-62.19
15-Deoxygoyazenolide	-83.79	-90.70	-89.70	-71.08	-83.63	-90.83	-87.52	-82.70
Centratherin	-90.32	-86.46	-96.93	-77.78	-87.44	-96.01	-90.82	-90.34
Goyazenolide	-88.02	-101.13	-91.49	-81.52	-82.20	-91.25	-91.17	-89.93
Lynchopholide	-86.29	-80.96	-89.89	-74.62	-86.42	-92.79	-89.95	-88.88
8β-Hydroxyzaluzanin D	-73.98	-72.50	-66.18	-72.94	-76.34	-74.40	-82.04	-81.40
Oleanolic acid	-69.09	-62.61	-21.29	19.30	-23.82	-52.09	-53.08	-30.05

Ligand	Cruzain							
	2OZ2	3HD3	3I06	3IUT	1F29	3KKU	3LXS-A	3LXS-C
Ursolic acid	-56.03	41.57	-37.98	34.55	-32.13	-43.96	-25.11	-26.73
Betulinic acid	-68.53	-81.32	-13.55	-41.12	11.68	-7.08	1.93	14.28

Table A.2a: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with dihydrofolate reductase-thymidylate synthase structures.

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	2H2Q-A	2H2Q-B	3CL9-A	3CL9-B	3CLB	3HBB	3INV
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-64.88	-85.80	-67.88	-80.26	-89.15	-83.81	-69.11
Arborinine	-72.51	-83.82	-73.12	-88.83	-81.29	-81.46	-71.40
Kokusagine	-67.32	-98.39	-65.18	-84.06	-96.28	-96.74	-64.90
N-Methyl-1-hydroxy-3-methoxyacridone	-61.56	-76.64	-65.72	-84.17	-76.61	-77.82	-64.73
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-79.74	-102.24	-76.85	-106.92	-97.96	-96.18	-82.16
Skimmianine	-63.17	-86.17	-66.21	-85.74	-98.00	-97.50	-70.07
Dicenitrinone	-76.91	-93.28	-79.34	-97.08	-96.06	-94.44	-80.20
Dugetine	-75.22	-94.51	-70.98	-96.28	-102.51	-102.09	-75.99
Dugetine-β-*N*-oxide	-68.03	-96.79	-71.61	-98.24	-99.14	-100.51	-67.42
N-Methylglaucine	-74.11	-79.40	-68.47	-85.17	-83.98	-79.89	-68.52
N-Methyltetrahydropalmitine	-81.57	-103.05	-77.91	-101.39	-99.75	-88.82	-69.12
Caaverine	-64.28	-75.90	-65.89	-82.92	-78.23	-72.98	-82.04
Coclaurine	-81.53	-98.01	-73.97	-94.50	-88.69	-85.53	-78.11
Corytuberine	-69.60	-81.11	-66.78	-85.14	-85.06	-84.21	-68.10
Domesticine	-75.47	-79.93	-62.09	-87.26	-85.87	-96.94	-77.35
Glazovine	-73.57	-84.25	-72.53	-88.86	-84.02	-85.80	-74.91
Isoboldine	-72.07	-83.80	-67.74	-84.74	-91.82	-81.88	-70.43
Laurotetanine	-70.13	-76.50	-64.74	-80.53	-82.12	-80.04	-72.10
N-Methylcoclaurine	-75.32	-93.74	-70.74	-93.55	-84.96	-89.99	-73.62
Nordomesticine	-67.70	-100.79	-66.56	-83.01	-100.41	-75.16	-77.86
Norisoboldine	-67.81	-80.79	-66.70	-81.49	-91.76	-91.00	-74.94

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	2H2Q-A	2H2Q-B	3CL9-A	3CL9-B	3CLB	3HBB	3INV
Pallidine	-74.25	-84.24	-69.22	-80.58	-86.68	-81.88	-70.98
Sarachine	-73.11	-99.42	-69.90	-68.57	-75.37	-80.44	-76.28
5-Methoxycanthin-6-one	-59.91	-77.20	-69.28	-81.07	-80.90	-75.28	-72.28
Canthin-6-one	-57.76	-74.78	-62.71	-77.02	-73.02	-72.42	-65.14
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-81.91	-99.97	-92.46	-92.92	-59.32	-80.35	-91.78
18-Acetoxy-13-15-diene-cassanoic acid	-86.81	-102.25	-85.72	-91.71	-106.51	-103.90	-78.19
18-Hydroxycassan-13,15-diene	-79.29	-94.53	-67.57	-87.51	-89.04	-86.64	-67.75
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-84.90	-99.37	-84.33	-88.38	-87.49	-86.58	-78.18
6β-18-Dihydroxycasssan-13,15-diene	-81.69	-94.36	-74.40	-84.85	-90.38	-85.37	-72.61
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-73.09	-101.32	-65.85	-79.12	-100.27	-99.51	-69.13
Geranygeraniol	-90.72	-108.29	-84.36	-107.59	-110.63	-111.87	-93.98
5-epi-Icetexone	-78.87	-104.90	-74.98	-90.43	-105.83	-105.18	-72.27
Alpinitine	-70.09	-91.92	-71.67	-90.13	-93.17	-94.17	-67.22
5,6,7-Trihydroxy-4′-methoxyflavone	-74.32	-91.35	-72.23	-101.60	-85.83	-90.39	-66.14
Ganglin	-64.38	-82.38	-68.02	-88.68	-85.01	-84.45	-65.99
Ganglin-3-methyl ether	-66.78	-90.71	-68.62	-90.44	-87.67	-94.25	-70.11
Luteolin	-70.98	-94.76	-76.73	-99.70	-85.17	-89.51	-81.12
Pinobanksin 3-acetate	-65.88	-94.90	-69.39	-87.96	-72.82	-77.09	-65.11
Pinobanksin	-59.80	-82.54	-65.10	-86.39	-83.44	-84.56	-62.32
Pinocembrin	-63.78	-85.68	-70.58	-87.89	-85.64	-86.44	-58.74
Quercetin-3-methyl ether	-77.35	-100.53	-75.21	-102.01	-92.03	-93.49	-76.77
Tectochrysin	-69.11	-91.85	-65.76	-88.19	-92.31	-94.23	-66.13
Aristolignan	-82.96	-127.57	-77.60	-108.40	-123.58	-124.90	-87.70
Calopiptin	-85.61	-116.06	-79.06	-111.16	-111.63	-126.79	-95.64
Galgravin	-80.39	-125.90	-85.70	-97.59	-115.46	-118.81	-86.25
Ganschisandrine	-87.34	-114.71	-75.77	-103.48	-109.31	-114.02	-87.52
Machilin G	-81.16	-119.68	-90.05	-97.47	-124.16	-125.74	-96.94
Nectandrin A	-82.31	-103.48	-81.62	-96.68	-92.05	-117.63	-81.90
Nectandrin B	-78.39	-123.75	-81.74	-88.14	-107.08	-114.57	-82.65
Conocarpan	-73.83	-103.13	-71.75	-97.25	-99.76	-99.12	-89.70
Eupomatenoid-3	-78.20	-112.20	-90.57	-108.95	-108.45	-108.13	-99.97

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	2H2Q-A	2H2Q-B	3CL9-A	3CL9-B	3CLB	3HBB	3INV
Eupomatenoid-5	-86.76	-103.15	-90.03	-110.82	-103.46	-103.63	-80.55
Eupomatenoid-6	-75.94	-98.71	-81.17	-99.56	-95.34	-96.04	-83.97
Grandisin	-112.72	-118.12	-84.94	-98.47	-111.78	-106.63	-105.49
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-118.74	-121.44	-104.53	-111.59	-115.73	-114.89	-104.87
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-102.43	-126.41	-100.61	-109.32	-112.62	-117.36	-105.62
4,5-di-(*E*)-O-Caffeyoquinic acid	-118.88	-131.86	-116.84	-149.84	-131.35	-124.73	-124.95
Caffeic Acid	-58.38	-73.95	-60.49	-73.90	-71.01	-70.79	-68.00
Isonoholaenic acid	-85.18	-104.47	-79.32	-103.56	-98.62	-101.11	-90.77
3-Heptadecyl-5-methoyphenol	-58.45	-80.19	-55.41	-75.44	-89.22	-80.01	-51.24
Embelin	-54.62	-102.15	-49.10	-86.77	-83.64	-93.55	-63.69
1,2,4-Trihrooxyheptadecane	-77.23	-97.06	-69.32	-98.01	-96.08	-93.75	-76.12
1,2,4-Trihydrooxyheptadec-16-ene	-83.09	-108.67	-79.67	-88.63	-105.49	-103.44	-84.42
1,2,4-Trihydrooxyheptadec-16-yne	-82.30	-105.68	-86.58	-99.85	-101.07	-106.29	-77.41
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-76.79	-98.89	-79.61	-100.07	-103.37	-118.43	-72.88
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-77.97	-110.09	-78.32	-111.64	-103.84	-104.03	-72.46
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-75.96	-100.49	-72.47	-96.95	-89.35	-108.68	-81.96
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-80.82	-96.53	-76.77	-101.30	-95.97	-94.49	-81.15
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-76.93	-100.68	-81.54	-102.50	-106.83	-99.13	-82.91
Helenalin	-73.52	-82.62	-71.22	-84.09	-80.77	-80.47	-66.08
Mexicanin	-65.55	-82.01	-72.36	-78.45	-86.10	-84.76	-67.51
15-Deoxygoyazenolide	-80.75	-96.34	-82.07	-83.10	-85.21	-78.05	-88.02
Centratherin	-89.58	-98.43	-89.21	-101.78	-87.59	-86.12	-88.43
Goyazenolide	-86.65	-93.24	-88.42	-72.54	-97.85	-90.22	-87.45
Lynchopholide	-83.36	-92.04	-84.09	-79.39	-88.63	-65.26	-84.28
8β-Hydroxyzaluzanin D	-76.46	-77.02	-79.11	-84.65	-87.92	-84.83	-74.71
Oleanolic acid	-71.01	-70.46	-67.36	-62.62	-60.53	-68.98	-70.58

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	2H2Q-A	2H2Q-B	3CL9-A	3CL9-B	3CLB	3HBB	3INV
Ursolic acid	-69.90	-81.47	-62.49	-33.71	-44.07	-36.63	-68.43
Betulinic acid	-86.16	-89.10	-69.13	-92.22	-53.69	-66.83	-72.68

Table A.2b: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with dihydrofolate reductase-thymidylate synthase structures.

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	3IRN-A	3IRN-C	3IRO-A	3IRO-C	3KJS-A	3KJS-C	3IRM
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-75.59	-73.61	-80.88	-82.47	-81.31	-76.61	-82.97
Arborinine	-71.34	-72.83	-71.36	-70.57	-60.47	-63.10	-83.44
Kokusagine	-70.93	-72.06	-77.71	-71.66	-74.16	-73.23	-86.43
N-Methyl-1-hydroxy-3-methoxyacridone	-73.68	-72.98	-71.43	-66.46	-71.81	-61.41	-78.75
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-94.15	-92.32	-84.73	-89.23	-90.10	-82.26	-86.32
Skimmianine	-73.07	-68.41	-74.55	-79.66	-73.02	-67.13	-84.90
Dicenitrinone	-71.17	-73.38	-76.53	-75.62	-79.48	-81.47	-97.55
Dugetine	-83.60	-80.12	-82.48	-77.09	-84.10	-80.41	-88.59
Dugetine-β-*N*-oxide	-86.96	-85.65	-81.74	-76.04	-80.07	-78.65	-90.98
N-Methylglaucine	-70.94	-72.91	-64.21	-78.62	-77.09	-71.63	-86.83
N-Methyltetrahydropalmitine	-91.85	-76.69	-87.23	-80.54	-77.99	-72.23	-84.13
Caaverine	-71.00	-72.44	-76.95	-72.32	-72.84	-64.47	-69.92
Coclaurine	-73.92	-82.15	-82.96	-76.97	-79.19	-87.64	-89.96
Corytuberine	-72.67	-70.05	-77.01	-75.12	-70.82	-68.83	-77.76
Domesticine	-76.42	-76.64	-80.02	-79.61	-79.23	-80.94	-87.98
Glazovine	-68.47	-71.60	-79.54	-66.22	-71.20	-70.01	-78.40
Isoboldine	-75.44	-72.33	-78.44	-75.24	-80.08	-71.23	-76.03
Laurotetanine	-79.49	-70.95	-78.82	-69.97	-75.52	-69.84	-76.78
N-Methylcoclaurine	-83.07	-85.02	-95.07	-86.87	-93.88	-91.41	-86.12
Nordomesticine	-72.56	-73.42	-80.58	-76.56	-76.33	-66.32	-84.31
Norisoboldine	-78.42	-70.84	-86.51	-70.44	-76.45	-69.30	-85.23

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	3IRN-A	3IRN-C	3IRO-A	3IRO-C	3KJS-A	3KJS-C	3IRM
Pallidine	-91.41	-85.52	-75.80	-80.85	-73.74	-81.14	-83.84
Sarachine	-84.81	-85.48	-82.96	-84.59	-92.78	-92.04	-79.57
5-Methoxycanthin-6-one	-69.98	-74.89	-75.31	-73.83	-69.97	-67.94	-74.24
Canthin-6-one	-64.13	-67.22	-67.92	-63.46	-65.97	-61.17	-70.07
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-90.01	-94.88	-103.16	-102.75	-60.17	-110.00	-97.91
18-Acetoxy-13-15-diene-cassanoic acid	-90.09	-78.47	-86.17	-84.25	-71.64	-82.51	-97.29
18-Hydroxycassan-13,15-diene	-79.68	-72.08	-78.65	-77.25	-65.63	-61.80	-79.25
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-79.59	-86.13	-76.89	-85.22	-68.48	-70.32	-98.53
6β-18-Dihydroxycasssan-13,15-diene	-85.62	-77.90	-73.17	-83.21	-30.20	-71.63	-84.22
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-84.72	-73.78	-81.45	-79.09	-63.50	-75.20	-83.86
Geranygeraniol	-90.91	-86.23	-98.90	-92.88	-97.28	-101.37	-102.84
5-epi-Icetexone	-73.55	-81.86	-71.22	-79.16	-70.77	-67.99	-85.14
Alpinitine	-74.99	-77.24	-76.28	-73.93	-71.15	-71.31	-85.48
5,6,7-Trihydroxy-4′-methoxyflavone	-81.42	-75.74	-82.22	-76.34	-78.55	-75.67	-93.96
Ganglin	-75.26	-74.40	-73.00	-73.86	-73.50	-76.83	-87.80
Ganglin-3-methyl ether	-77.37	-77.23	-82.10	-75.35	-73.19	-80.89	-82.11
Luteolin	-79.90	-81.08	-85.84	-79.19	-83.39	-81.90	-88.01
Pinobanksin 3-acetate	-76.39	-79.50	-89.74	-82.35	-82.50	-87.04	-87.66
Pinobanksin	-73.63	-72.09	-72.23	-70.14	-70.91	-71.84	-82.28
Pinocembrin	-72.57	-70.44	-70.93	-72.99	-73.29	-72.01	-80.02
Quercetin-3-methyl ether	-84.19	-85.59	-97.84	-83.57	-83.61	-79.71	-90.93
Tectochrysin	-76.91	-77.98	-71.14	-77.33	-78.41	-80.51	-81.09
Aristolignan	-99.10	-92.40	-97.02	-97.78	-100.54	-93.01	-98.20
Calopiptin	-95.55	-94.00	-95.56	-93.97	-101.58	-99.64	-100.45
Galgravin	-98.25	-90.11	-96.68	-108.55	-95.13	-96.58	-103.16
Ganschisandrine	-85.22	-83.92	-90.42	-92.95	-87.27	-85.42	-101.00
Machilin G	-99.10	-95.22	-91.98	-101.98	-88.08	-88.97	-105.84
Nectandrin A	-95.74	-99.49	-91.24	-95.26	-92.76	-98.68	-98.44
Nectandrin B	-90.13	-101.42	-96.08	-88.08	-94.90	-94.34	-97.14
Conocarpan	-82.37	-80.75	-82.48	-84.09	-87.38	-92.73	-77.16
Eupomatenoid-3	-85.10	-85.07	-55.83	-74.83	-80.19	-81.75	-104.05

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	3IRN-A	3IRN-C	3IRO-A	3IRO-C	3KJS-A	3KJS-C	3IRM
Eupomatenoid-5	-85.38	-83.75	-81.86	-83.35	-76.21	-83.35	-108.16
Eupomatenoid-6	-76.45	-76.62	-62.76	-72.64	-69.03	-68.24	-102.87
Grandisin	-81.10	-83.87	-94.10	-79.60	-81.20	-76.03	-102.69
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-91.84	-91.94	-92.06	-90.58	-93.02	-89.46	-104.47
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-99.95	-98.32	-94.51	-84.20	-93.71	-89.46	-99.71
4,5-di-(*E*)-O-Caffeyoquinic acid	-115.31	-129.56	-123.41	-120.99	-130.66	-128.30	-126.28
Caffeic Acid	-75.20	-73.74	-80.44	-65.72	-74.85	-71.89	-76.85
Isonoholaenic acid	-85.90	-78.08	-96.57	-87.86	-98.43	-98.57	-91.78
3-Heptadecyl-5-methoyphenol	-79.84	-54.29	-64.02	-68.51	-58.38	-57.84	-80.14
Embelin	-59.31	-67.92	-70.78	-70.91	-59.45	-65.94	-92.04
1,2,4-Trihrooxyheptadecane	-88.17	-88.19	-82.98	-71.22	-85.45	-80.64	-92.37
1,2,4-Trihydrooxyheptadec-16-ene	-88.26	-80.95	-88.13	-82.99	-87.51	-74.71	-96.71
1,2,4-Trihydrooxyheptadec-16-yne	-73.40	-86.91	-87.33	-76.79	-95.50	-75.98	-101.42
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-80.68	-86.46	-83.85	-82.09	-96.93	-81.71	-86.72
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-75.38	-94.36	-83.76	-85.16	-73.09	-89.04	-100.68
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-74.90	-86.57	-96.96	-78.82	-74.17	-72.37	-82.64
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-85.81	-78.00	-70.65	-81.23	-74.51	-79.03	-85.36
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-88.67	-82.64	-88.00	-75.28	-80.68	-79.06	-96.10
Helenalin	-73.65	-73.01	-67.95	-69.12	-68.76	-68.00	-79.58
Mexicanin	-73.09	-72.72	-71.80	-69.12	-71.91	-68.37	-81.34
15-Deoxygoyazenolide	-83.75	-96.39	-88.64	-90.19	-79.26	-80.72	-93.22
Centratherin	-93.82	-104.03	-90.23	-93.96	-79.46	-91.63	-103.29
Goyazenolide	-96.32	-100.82	-91.48	-93.96	-80.86	-87.64	-99.62
Lynchopholide	-86.71	-94.58	-84.57	-91.12	-66.47	-85.30	-96.58
8β-Hydroxyzaluzanin D	-67.66	-76.22	-76.45	-81.82	-70.52	-67.81	-83.32
Oleanolic acid	-70.78	-79.60	-67.04	-72.95	-45.97	-69.95	-65.43

Ligand	Dihydrofolate Reductase-Thymidylate Synthase						
	3IRN-A	3IRN-C	3IRO-A	3IRO-C	3KJS-A	3KJS-C	3IRM
Ursolic acid	-49.98	-55.88	-50.10	-68.27	-21.37	-23.18	-60.14
Betulinic acid	-76.64	-75.11	-79.31	-92.87	-12.08	-70.83	-75.65

Table A.3: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with hypoxanthine phosphoribosyl transferase.

Ligand	Hypoxanthine Phosphoribosyl Transferase						
	1I0I	1I13	1I14	1I0L	1P19	1TC1	1TC2
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-66.94	-62.59	-64.48	-66.11	-68.30	-72.34	-70.01
Arborinine	-67.19	-59.77	-68.17	-61.95	-67.67	-65.26	-62.57
Kokusagine	-71.89	-75.12	-74.03	-64.78	-80.33	-73.33	-75.48
N-Methyl-1-hydroxy-3-methoxyacridone	-61.19	-62.57	-56.29	-59.97	-67.81	-60.29	-65.66
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-75.07	-79.15	-82.87	-67.07	-76.33	-74.94	-79.28
Skimmianine	-67.96	-72.98	-73.47	-64.83	-73.12	-60.69	-70.98
Dicenitrinone	-66.69	-71.56	-70.15	-68.60	-82.52	-75.16	-71.22
Dugetine	-68.19	-68.16	-64.72	-69.76	-85.89	-81.62	-63.12
Dugetine-β-*N*-oxide	-63.62	-67.35	-66.03	-68.63	-87.63	-79.14	-59.42
N-Methylglaucine	-64.04	-61.99	-60.44	-71.46	-76.90	-75.36	-63.74
N-Methyltetrahydropalmitine	-59.86	-63.53	-63.31	-66.62	-78.23	-66.44	-61.01
Caaverine	-64.32	-69.23	-66.93	-64.19	-66.93	-63.70	-62.76
Coclaurine	-82.46	-67.39	-74.44	-79.85	-78.60	-76.66	-75.89
Corytuberine	-64.08	-58.66	-61.63	-63.81	-70.87	-76.56	-69.16
Domesticine	-68.52	-68.35	-67.30	-75.69	-76.74	-82.42	-74.34
Glazovine	-68.22	-67.17	-71.25	-67.73	-74.81	-76.21	-65.65
Isoboldine	-67.35	-68.83	-61.38	-70.03	-82.00	-77.78	-72.65
Laurotetanine	-69.35	-66.91	-66.97	-67.83	-73.89	-72.81	-76.05
N-Methylcoclaurine	-77.64	-73.24	-75.51	-75.32	-79.46	-70.39	-79.78
Nordomesticine	-74.62	-68.62	-66.32	-75.35	-76.12	-74.14	-78.22
Norisoboldine	-69.37	-70.17	-69.08	-70.48	-78.13	-75.10	-75.85

Ligand	Hypoxanthine Phosphoribosyl Transferase						
	1I0I	1I13	1I14	1I0L	1P19	1TC1	1TC2
Pallidine	-66.48	-65.92	-65.89	-65.51	-71.75	-71.45	-65.39
Sarachine	-58.23	-62.27	-71.09	-56.42	-79.17	-80.30	-61.60
5-Methoxycanthin-6-one	-65.95	-64.13	-69.89	-67.58	-71.26	-62.09	-63.74
Canthin-6-one	-67.28	-63.46	-65.48	-63.84	-67.16	-53.35	-63.14
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-74.84	-78.89	-79.17	-87.05	-87.02	-71.03	-71.47
18-Acetoxy-13-15-diene-cassanoic acid	-67.13	-64.27	-73.91	-66.45	-73.16	-80.13	-67.78
18-Hydroxycassan-13,15-diene	-72.49	-75.68	-75.77	-68.00	-73.35	-69.51	-71.70
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-69.45	-66.97	-72.23	-62.92	-70.12	-80.43	-66.95
6β-18-Dihydroxycasssan-13,15-diene	-78.77	-77.85	-78.68	-68.44	-74.58	-67.71	-75.44
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-63.91	-70.55	-61.39	-64.77	-72.04	-68.15	-59.17
Geranygeraniol	-93.14	-82.44	-87.11	-84.58	-77.14	-81.81	-90.34
5-epi-Icetexone	-74.06	-72.33	-72.56	-75.75	-79.74	-61.40	-74.70
Alpinitine	-73.38	-69.77	-66.78	-65.92	-74.93	-79.52	-70.61
5,6,7-Trihydroxy-4′-methoxyflavone	-102.93	-97.66	-103.46	-99.79	-87.27	-81.64	-101.02
Ganglin	-87.27	-80.76	-83.55	-84.34	-78.77	-79.05	-82.41
Ganglin-3-methyl ether	-76.22	-80.65	-76.28	-68.23	-81.60	-79.87	-76.09
Luteolin	-93.60	-85.85	-94.12	-92.15	-92.58	-85.97	-93.59
Pinobanksin 3-acetate	-80.16	-82.18	-69.89	-83.47	-76.29	-61.14	-72.22
Pinobanksin	-84.41	-64.37	-82.54	-83.68	-76.57	-80.24	-82.23
Pinocembrin	-80.99	-76.08	-81.38	-78.55	-74.89	-78.24	-80.27
Quercetin-3-methyl ether	-87.29	-93.48	-87.06	-82.64	-97.09	-89.35	-90.07
Tectochrysin	-79.30	-71.98	-80.82	-72.09	-78.81	-79.04	-80.34
Aristolignan	-92.23	-83.13	-80.14	-81.74	-84.69	-95.28	-86.66
Calopiptin	-96.98	-85.34	-85.57	-79.54	-98.08	-90.17	-86.91
Galgravin	-83.36	-72.73	-81.40	-80.07	-79.78	-80.75	-83.95
Ganschisandrine	-80.64	-79.64	-68.31	-84.70	-90.97	-69.11	-72.12
Machilin G	-92.38	-83.90	-74.59	-91.73	-98.66	-91.51	-89.85
Nectandrin A	-78.75	-78.65	-93.22	-76.34	-85.25	-85.87	-82.37
Nectandrin B	-85.31	-95.64	-72.70	-89.16	-92.73	-93.66	-75.64
Conocarpan	-71.59	-68.47	-69.61	-68.91	-74.69	-82.78	-58.42
Eupomatenoid-3	-75.99	-80.74	-74.30	-80.73	-83.60	-84.72	-66.17

Ligand	Hypoxanthine Phosphoribosyl Transferase						
	1I0I	1I13	1I14	1I0L	1P19	1TC1	1TC2
Eupomatenoid-5	-77.72	-76.87	-72.69	-84.96	-88.18	-82.06	-85.05
Eupomatenoid-6	-72.12	-73.11	-71.17	-72.00	-84.78	-74.45	-72.42
Grandisin	-76.54	-74.60	-77.01	-86.00	-91.28	-55.51	-56.80
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-84.99	-90.55	-83.01	-91.02	-98.10	-89.00	-81.25
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-83.02	-94.97	-85.26	-82.39	-98.76	-83.82	-88.85
4,5-di-(*E*)-O-Caffeyoquinic acid	-101.84	-89.07	-112.89	-108.95	-124.96	-125.17	-104.59
Caffeic Acid	-68.52	-64.71	-64.82	-68.46	-69.67	-68.26	-63.59
Isonoholaenic acid	-82.78	-78.50	-74.45	-69.18	-78.93	-71.49	-71.54
3-Heptadecyl-5-methoyphenol	-55.82	-36.66	-57.07	-40.85	-63.35	-52.40	-53.48
Embelin	-69.09	-62.73	-69.43	-67.10	-74.18	-72.54	-66.48
1,2,4-Trihrooxyheptadecane	-83.68	-73.50	-76.16	-73.24	-87.11	-75.71	-71.06
1,2,4-Trihydrooxyheptadec-16-ene	-84.36	-72.84	-67.32	-77.22	-80.43	-85.69	-61.67
1,2,4-Trihydrooxyheptadec-16-yne	-79.08	-75.08	-69.05	-81.65	-85.55	-82.58	-66.62
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-78.92	-70.84	-83.60	-72.85	-77.34	-82.19	-75.17
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-71.98	-66.63	-75.88	-76.96	-77.40	-87.21	-68.08
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-62.59	-80.11	-62.41	-59.33	-82.59	-76.17	-73.26
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-79.51	-78.37	-71.16	-72.29	-98.50	-79.65	-63.79
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-74.14	-80.20	-75.55	-82.53	-91.85	-79.48	-78.82
Helenalin	-71.46	-68.73	-68.83	-67.24	-71.27	-63.28	-72.58
Mexicanin	-74.74	-69.35	-72.86	-63.84	-78.16	-65.66	-71.11
15-Deoxygoyazenolide	-84.59	-78.53	-82.99	-72.91	-72.97	-76.32	-80.30
Centratherin	-83.45	-83.14	-77.93	-72.83	-86.48	-87.24	-79.95
Goyazenolide	-81.62	-87.56	-78.67	-74.18	-87.19	-81.64	-81.41
Lynchopholide	-82.66	-74.49	-77.36	-75.38	-62.65	-78.90	-73.02
8β-Hydroxyzaluzanin D	-82.68	-73.11	-79.07	-70.29	-68.41	-66.45	-81.47
Oleanolic acid	-63.72	-61.54	-66.56	-57.08	-57.19	-67.51	-62.26

Ligand	Hypoxanthine Phosphoribosyl Transferase						
	1I0I	1I13	1I14	1I0L	1P19	1TC1	1TC2
Ursolic acid	-63.68	-62.25	-57.28	-57.32	-58.61	-63.30	-65.52
Betulinic acid	-55.89	-66.99	-69.91	-73.46	-75.79	-67.59	-69.49

Table A.4: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with *trans*-sialidase.

Ligand	*trans*-Sialidase							
	1MS0	1MS1	1MS8	1MS9	1S0I	1S0J	2AH2	3B69
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-72.24	-75.83	-82.84	-73.67	-67.67	-68.46	-86.37	-80.88
Arborinine	-81.68	-87.35	-80.69	-73.50	-70.15	-69.19	-77.35	-88.22
Kokusagine	-78.04	-84.76	-89.51	-74.86	-77.25	-77.57	-91.82	-88.05
N-Methyl-1-hydroxy-3-methoxyacridone	-76.26	-83.89	-81.57	-72.61	-73.45	-73.35	-67.84	-77.28
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-97.99	-104.91	-76.98	-87.87	-99.97	-90.56	-93.59	-101.04
Skimmianine	-81.55	-82.65	-87.91	-73.54	-71.84	-76.24	-89.82	-86.91
Dicenitrinone	-90.24	-92.59	-83.81	-82.64	-82.83	-80.74	-80.53	-89.41
Dugetine	-89.09	-91.98	-77.06	-86.73	-82.90	-80.12	-73.60	-89.55
Dugetine-β-*N*-oxide	-92.63	-98.81	-86.46	-80.97	-83.05	-82.76	-83.51	-91.83
N-Methylglaucine	-80.53	-91.20	-78.65	-84.32	-82.56	-81.15	-78.83	-83.59
N-Methyltetrahydropalmitine	-88.95	-103.39	-91.69	-80.56	-90.44	-88.11	-86.77	-89.30
Caaverine	-83.36	-74.95	-78.12	-74.34	-66.45	-66.22	-65.70	-77.37
Coclaurine	-85.00	-87.57	-86.06	-86.58	-77.48	-75.53	-84.99	-89.21
Corytuberine	-87.80	-89.31	-88.61	-83.54	-73.00	-74.70	-80.32	-83.00
Domesticine	-83.85	-94.80	-87.22	-86.61	-85.68	-86.38	-81.41	-87.23
Glazovine	-77.21	-79.09	-80.02	-74.65	-71.31	-79.50	-80.22	-77.32
Isoboldine	-84.53	-87.22	-91.67	-87.63	-85.48	-85.82	-67.24	-91.07
Laurotetanine	-87.89	-79.38	-91.92	-78.61	-73.06	-77.81	-90.52	-84.04
N-Methylcoclaurine	-86.90	-95.03	-102.31	-86.00	-83.64	-86.59	-96.78	-94.40
Nordomesticine	-79.40	-89.50	-94.30	-86.77	-84.09	-84.33	-78.64	-86.37
Norisoboldine	-81.62	-83.47	-82.91	-86.93	-81.30	-84.68	-93.21	-80.00

Ligand	*trans*-Sialidase							
	1MS0	1MS1	1MS8	1MS9	1S0I	1S0J	2AH2	3B69
Pallidine	-85.02	-86.04	-68.82	-76.06	-79.19	-82.49	-74.86	-78.63
Sarachine	-83.56	-83.95	-86.05	-71.33	-78.41	-83.65	-87.29	-81.12
5-Methoxycanthin-6-one	-81.22	-78.98	-75.68	-69.83	-73.49	-71.72	-74.08	-80.64
Canthin-6-one	-69.88	-73.83	-71.42	-63.04	-64.58	-62.83	-68.94	-68.21
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-101.18	-103.31	-83.30	-93.23	-94.42	-99.08	-89.10	-106.64
18-Acetoxy-13-15-diene-cassanoic acid	-85.43	-90.62	-85.61	-86.64	-83.86	-81.87	-73.61	-93.01
18-Hydroxycassan-13,15-diene	-83.72	-85.84	-70.79	-75.53	-72.16	-74.67	-75.72	-82.06
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-96.89	-97.32	-82.95	-84.99	-82.46	-92.24	-75.62	-96.98
6β-18-Dihydroxycasssan-13,15-diene	-84.89	-87.33	-73.34	-78.13	-76.88	-72.08	-77.24	-85.68
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-78.97	-74.48	-75.19	-78.67	-72.89	-72.84	-76.15	-73.55
Geranygeraniol	-91.53	-98.35	-98.84	-99.52	-85.44	-101.61	-97.39	-97.50
5-epi-Icetexone	-81.78	-89.83	-81.47	-78.81	-85.08	-85.77	-74.87	-87.35
Alpinitine	-82.15	-89.10	-95.59	-75.56	-81.42	-81.11	-93.35	-91.28
5,6,7-Trihydroxy-4′-methoxyflavone	-83.03	-91.14	-97.24	-81.77	-90.88	-94.47	-96.29	-91.38
Ganglin	-83.78	-88.59	-100.07	-74.20	-75.69	-79.28	-94.75	-86.31
Ganglin-3-methyl ether	-88.42	-95.83	-105.37	-78.65	-82.30	-84.57	-101.12	-97.06
Luteolin	-85.13	-90.69	-100.67	-80.87	-84.07	-86.88	-97.39	-90.03
Pinobanksin 3-acetate	-66.97	-88.49	-107.84	-81.28	-66.19	-65.43	-93.86	-76.52
Pinobanksin	-80.76	-86.84	-95.17	-79.76	-77.38	-79.21	-94.55	-89.12
Pinocembrin	-78.28	-83.88	-93.03	-76.83	-75.90	-76.29	-89.59	-86.30
Quercetin-3-methyl ether	-92.23	-100.82	-111.36	-86.97	-88.03	-89.28	-106.22	-100.54
Tectochrysin	-86.39	-89.25	-95.66	-79.65	-86.56	-84.81	-99.34	-92.24
Aristolignan	-96.02	-101.04	-113.00	-95.96	-90.89	-91.08	-111.82	-104.86
Calopiptin	-101.86	-107.04	-114.61	-89.81	-97.78	-101.15	-105.37	-103.40
Galgravin	-94.86	-101.55	-95.40	-100.75	-98.83	-95.91	-102.48	-113.35
Ganschisandrine	-91.54	-87.33	-87.24	-94.49	-91.55	-92.41	-84.80	-89.15
Machilin G	-99.57	-103.66	-105.27	-98.49	-103.31	-103.06	-100.35	-107.11
Nectandrin A	-97.91	-104.22	-102.40	-90.59	-94.43	-93.15	-103.61	-108.83
Nectandrin B	-100.14	-103.21	-101.51	-92.37	-92.72	-90.78	-98.57	-112.50
Conocarpan	-86.98	-89.50	-98.81	-82.62	-84.66	-83.02	-91.68	-89.28
Eupomatenoid-3	-85.69	-92.75	-92.54	-94.85	-89.43	-90.52	-93.18	-96.26

Ligand	*trans*-Sialidase							
	1MS0	1MS1	1MS8	1MS9	1S0I	1S0J	2AH2	3B69
Eupomatenoid-5	-93.86	-98.11	-91.32	-94.42	-90.18	-86.14	-91.31	-101.69
Eupomatenoid-6	-80.55	-87.54	-81.98	-86.70	-82.79	-84.28	-81.01	-87.21
Grandisin	-100.67	-103.90	-101.34	-100.85	-94.24	-90.92	-89.65	-102.81
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-102.26	-104.62	-97.66	-93.27	-105.68	-94.49	-96.72	-99.74
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-114.39	-106.95	-95.63	-98.34	-114.15	-112.33	-99.93	-108.61
4,5-di-(*E*)-O-Caffeyoquinic acid	-130.99	-133.70	-130.33	-133.27	-136.09	-130.17	-134.29	-151.09
Caffeic Acid	-69.51	-73.84	-74.29	-63.26	-69.32	-69.13	-72.20	-73.93
Isonoholaenic acid	-82.27	-89.84	-94.38	-84.94	-83.37	-92.86	-97.03	-89.95
3-Heptadecyl-5-methoyphenol	-60.02	-80.73	-79.60	-55.37	-56.80	-75.75	-75.35	-79.92
Embelin	-65.19	-69.30	-73.67	-77.96	-68.63	-67.65	-76.85	-75.45
1,2,4-Trihrooxyheptadecane	-92.74	-88.14	-92.04	-95.21	-76.61	-101.57	-88.76	-85.56
1,2,4-Trihydrooxyheptadec-16-ene	-83.68	-84.46	-84.43	-100.14	-83.27	-92.44	-91.77	-95.98
1,2,4-Trihydrooxyheptadec-16-yne	-87.74	-90.85	-89.51	-100.77	-80.34	-86.64	-88.81	-96.40
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-97.36	-93.76	-84.21	-84.36	-92.66	-97.70	-90.78	-89.55
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-86.10	-94.09	-87.02	-85.31	-80.01	-83.58	-89.81	-94.23
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-87.71	-92.62	-80.43	-88.42	-77.73	-82.93	-97.84	-98.60
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-88.63	-87.64	-81.83	-107.14	-86.51	-89.92	-91.91	-88.39
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-94.57	-92.37	-94.00	-94.99	-82.39	-85.40	-98.65	-83.10
Helenalin	-86.52	-79.95	-84.63	-72.82	-74.08	-70.26	-87.01	-77.43
Mexicanin	-77.21	-81.16	-77.23	-75.57	-71.91	-68.50	-74.62	-74.32
15-Deoxygoyazenolide	-92.12	-94.52	-91.73	-92.01	-93.34	-89.46	-96.63	-98.61
Centratherin	-99.66	-100.43	-96.00	-99.51	-93.37	-94.67	-105.07	-91.18
Goyazenolide	-96.62	-93.51	-88.37	-91.13	-94.49	-88.06	-109.29	-103.93
Lynchopholide	-93.21	-103.10	-86.64	-89.77	-94.28	-97.97	-99.73	-102.98
8β-Hydroxyzaluzanin D	-89.70	-96.77	-74.52	-79.08	-78.63	-82.05	-72.59	-92.10
Oleanolic acid	-66.46	-70.12	-65.72	-57.04	-64.13	-54.17	-53.26	-56.47
Ursolic acid	-36.00	-23.00	-56.15	-61.26	-65.23	-57.36	-49.47	-40.16

Ligand	*trans*-Sialidase							
	1MS0	1MS1	1MS8	1MS9	1S0I	1S0J	2AH2	3B69
Betulinic acid	-75.11	-81.94	-74.52	-81.82	-87.23	-85.14	-56.53	-80.14

Table A.5: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with arginine kinase and farnesyl diphosphate synthase.

Ligand	Arginine Kinase	Farnesyl Diphosphate Synthase						
	2J1Q	1YHL	3IBA	3ICK	3ICZ	3ICM	3ICN	3ID0
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-63.85	-77.99	-87.66	-81.11	-81.89	-83.91	-85.15	-78.41
Arborinine	-38.23	-72.03	-68.95	-75.01	-71.56	-70.49	-71.40	-77.79
Kokusagine	-62.06	-84.94	-79.86	-86.01	-80.37	-77.58	-69.41	-75.52
N-Methyl-1-hydroxy-3-methoxyacridone	-35.85	-68.61	-67.05	-78.18	-64.00	-75.34	-74.22	-76.58
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-54.46	-102.63	-90.65	-89.63	-87.69	-70.96	-103.93	-84.01
Skimmianine	-60.81	-79.16	-73.64	-83.41	-82.39	-76.04	-70.01	-77.72
Dicenitrinone	-41.43	-83.40	-83.30	-81.38	-85.34	-17.93	-64.07	-83.50
Dugetine	-39.82	-78.15	-90.04	-92.20	-82.30	-17.26	-69.67	-83.86
Dugetine-β-*N*-oxide	-42.42	-84.44	-82.37	-85.69	-88.24	-24.56	-72.32	-70.30
N-Methylglaucine	-5.69	-62.35	-76.41	-94.20	-94.54	-65.34	-45.88	-76.50
N-Methyltetrahydropalmitine	-53.61	-64.06	-75.71	-40.49	-91.42	-13.52	-8.25	-21.53
Caaverine	-46.05	-70.53	-71.09	-69.19	-74.32	-68.16	-74.23	-71.67
Coclaurine	-58.30	-87.52	-84.11	-85.28	-99.37	-98.54	-91.02	-83.53
Corytuberine	-47.73	-66.90	-83.24	-91.33	-90.68	-71.87	-12.00	-74.15
Domesticine	-47.20	-77.26	-85.94	-92.60	-88.87	-62.87	-57.02	-74.26
Glazovine	-22.90	-80.18	-94.04	-90.83	-96.79	-84.56	-81.27	-82.91
Isoboldine	-46.09	-73.74	-86.41	-93.77	-92.26	-65.78	-46.49	-76.12
Laurotetanine	-45.90	-65.01	-83.28	-79.14	-82.10	-9.12	-49.03	-76.74
N-Methylcoclaurine	-53.69	-81.35	-92.97	-92.41	-91.69	-96.45	-72.55	-88.14
Nordomesticine	-65.28	-79.64	-82.15	-85.94	-83.39	-88.97	-74.64	-77.04
Norisoboldine	-49.29	-74.73	-81.62	-84.11	-87.97	-74.91	-60.50	-81.01
Pallidine	-45.31	-85.71	-89.05	-90.34	-91.43	-76.52	-84.57	-87.93

Ligand	Arginine Kinase	Farnesyl Diphosphate Synthase						
	2J1Q	1YHL	3IBA	3ICK	3ICZ	3ICM	3ICN	3ID0
Sarachine	-56.64	-88.33	-84.77	-98.90	-86.54	-81.19	-79.92	-63.75
5-Methoxycanthin-6-one	-54.56	-78.95	-67.72	-78.78	-73.17	-67.31	-78.94	-66.30
Canthin-6-one	-42.47	-71.03	-58.19	-74.70	-67.64	-61.82	-66.25	-60.20
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-50.87	-80.62	-101.05	-92.82	-107.63	-103.96	-107.78	-83.65
18-Acetoxy-13-15-diene-cassanoic acid	17.67	-94.98	-88.34	-82.21	-98.80	-105.01	-75.47	-89.17
18-Hydroxycassan-13,15-diene	-50.41	-77.47	-76.69	-75.29	-87.05	-90.28	-66.88	-60.35
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-58.41	-94.72	-97.70	-83.63	-95.76	-73.27	-95.56	-94.03
6β-18-Dihydroxycasssan-13,15-diene	-26.81	-75.24	-81.99	-64.89	-83.28	-81.55	-66.89	-71.66
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-45.87	-88.49	-75.11	-77.40	-81.46	-76.55	-66.18	-52.32
Geranygeraniol	-33.71	-93.71	-96.46	-100.66	-105.66	-107.62	-112.24	-100.94
5-epi-Icetexone	24.59	-83.76	-84.31	-84.94	-82.91	-85.94	-96.80	-90.90
Alpinitine	-69.50	-76.37	-76.53	-81.69	-89.78	-84.89	-85.52	-86.76
5,6,7-Trihydroxy-4′-methoxyflavone	-55.05	-82.03	-79.50	-85.58	-94.44	-98.48	-90.04	-91.64
Ganglin	-72.54	-85.54	-91.57	-86.33	-97.49	-86.10	-81.50	-78.79
Ganglin-3-methyl ether	-65.50	-90.81	-82.86	-93.52	-81.02	-82.96	-77.92	-72.46
Luteolin	-64.36	-92.06	-89.76	-88.28	-103.81	-98.54	-92.40	-92.89
Pinobanksin 3-acetate	-56.10	-82.69	-88.51	-82.60	-106.12	-103.55	-99.83	-87.88
Pinobanksin	-69.88	-83.99	-90.86	-84.21	-96.85	-82.25	-82.29	-79.00
Pinocembrin	-66.58	-80.37	-88.10	-80.85	-98.16	-82.63	-82.47	-81.45
Quercetin-3-methyl ether	-66.64	-99.59	-84.14	-99.78	-110.48	-80.70	-87.50	-91.25
Tectochrysin	-71.27	-88.68	-87.84	-88.04	-100.48	-90.02	-84.15	-83.02
Aristolignan	-68.27	-96.43	-102.89	-99.57	-108.36	-93.47	-83.55	-95.00
Calopiptin	-60.75	-99.14	-109.17	-101.17	-112.06	-101.81	-72.78	-98.32
Galgravin	-60.82	-99.42	-101.25	-95.73	-111.59	-81.46	-70.04	-100.79
Ganschisandrine	-72.94	-91.56	-95.92	-97.42	-108.58	-109.74	-96.97	-105.86
Machilin G	-75.49	-106.17	-112.10	-103.86	-116.38	-95.06	-91.62	-105.16
Nectandrin A	-75.21	-100.73	-94.43	-102.98	-110.03	-86.76	-78.60	-105.80
Nectandrin B	-57.43	-92.95	-98.96	-100.12	-107.99	-89.20	-65.10	-97.51
Conocarpan	-58.43	-85.20	-86.52	-90.45	-99.56	-96.57	-86.62	-91.76
Eupomatenoid-3	-77.21	-92.97	-92.68	-98.91	-105.45	-110.68	-98.18	-101.97
Eupomatenoid-5	-73.87	-93.49	-89.25	-100.91	-109.72	-105.11	-97.52	-99.64

Ligand	Arginine Kinase	Farnesyl Diphosphate Synthase						
	2J1Q	1YHL	3IBA	3ICK	3ICZ	3ICM	3ICN	3ID0
Eupomatenoid-6	-72.59	-87.19	-87.04	-92.53	-103.68	-103.52	-94.46	-93.89
Grandisin	-52.33	-77.26	-93.13	-106.77	-97.82	-90.14	-79.74	-79.31
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-73.96	-93.25	-102.93	-106.54	-97.19	-78.51	-80.31	-88.80
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-82.73	-107.31	-109.01	-112.73	-108.73	-112.08	-82.94	-96.76
4,5-di-(*E*)-O-Caffeyoquinic acid	-92.84	-142.10	-152.35	-148.41	-155.68	-144.48	-150.85	-157.91
Caffeic Acid	-59.77	-72.01	-75.34	-73.07	-78.16	-75.77	-70.96	-65.07
Isonoholaenic acid	-58.00	-88.95	-88.07	-92.47	-103.07	-102.39	-100.71	-92.31
3-Heptadecyl-5-methoyphenol	-29.87	-70.29	-73.22	-108.22	-85.84	-76.03	-79.71	-87.45
Embelin	-60.49	-69.70	-73.39	-71.11	-77.41	-92.88	-70.91	-65.96
1,2,4-Trihrooxyheptadecane	-37.24	-91.67	-87.70	-101.44	-108.90	-91.75	-93.18	-89.59
1,2,4-Trihydrooxyheptadec-16-ene	-66.74	-92.65	-84.69	-100.56	-97.79	-100.64	-97.93	-91.79
1,2,4-Trihydrooxyheptadec-16-yne	-67.30	-87.77	-93.44	-94.36	-102.31	-99.18	-97.39	-92.94
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-40.46	-98.21	-103.75	-108.24	-120.25	-121.85	-89.69	-104.08
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-37.88	-92.27	-86.21	-99.14	-101.31	-109.76	-95.42	-105.86
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-53.49	-85.88	-96.71	-102.81	-98.06	-102.60	-86.99	-79.57
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-37.97	-95.45	-102.01	-97.85	-95.83	-107.39	-92.83	-98.17
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-66.38	-92.11	-96.62	-98.32	-102.53	-111.01	-96.40	-97.28
Helenalin	-46.12	-84.76	-89.60	-81.56	-81.83	-78.57	-79.02	-79.24
Mexicanin	-36.92	-83.93	-90.94	-84.04	-80.34	-72.44	-80.60	-78.54
15-Deoxygoyazenolide	-69.66	-79.52	-103.05	-100.06	-101.11	-13.15	-39.66	-82.89
Centratherin	-72.81	-89.59	-113.62	-111.03	-108.07	-27.35	-25.46	-38.62
Goyazenolide	-74.34	-85.76	-107.34	-106.28	-107.17	-33.26	-70.38	-56.24
Lynchopholide	-72.17	-78.36	-110.36	-104.80	-98.21	12.83	-21.72	-86.76
8β-Hydroxyzaluzanin D	-49.65	-95.69	-90.97	-103.82	-94.05	-87.41	-91.56	-87.95
Oleanolic acid	122.95	-68.12	-58.65	-37.00	0.13	83.31	5.34	-14.11
Ursolic acid	140.25	-51.21	-28.84	-13.99	56.42	64.16	73.03	24.13
Betulinic acid	-9.72	-67.20	-73.90	-78.31	-63.83	-20.30	-71.12	-66.63

Table A.6: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with dihydroorotate dehydrogenase and tyrosine aminotransferase.

Ligand	Dihydroorotate Dehydrogenase						Tyrosine Aminotrans-ferase
	2DJX	2DJL	2E6A	2E6D	2E68	3C3N	1BW0
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-77.59	-79.85	-76.43	-79.79	-76.57	-87.31	-78.88
Arborinine	-74.18	-76.93	-80.58	-80.96	-79.50	-91.72	-69.41
Kokusagine	-91.38	-92.78	-93.72	-92.30	-88.47	-86.17	-70.59
N-Methyl-1-hydroxy-3-methoxyacridone	-72.59	-77.09	-77.01	-76.16	-76.82	-85.30	-65.24
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-93.44	-91.90	-86.70	-86.14	-96.94	-85.08	-86.91
Skimmianine	-77.91	-89.28	-85.96	-88.54	-90.46	-87.89	-65.20
Dicenitrinone	-87.59	-95.97	-98.72	-97.05	-96.77	-108.51	-84.78
Dugetine	-92.08	-96.91	-100.14	-96.78	-98.56	-105.79	-87.02
Dugetine-β-*N*-oxide	-91.23	-98.86	-97.51	-100.34	-96.43	-108.12	-85.96
N-Methylglaucine	-87.77	-89.47	-93.07	-90.73	-88.92	-108.85	-77.91
N-Methyltetrahydropalmitine	-79.25	-46.62	-75.99	-54.65	-77.36	-57.97	-79.48
Caaverine	-72.32	-78.10	-77.93	-78.01	-76.75	-86.44	-61.27
Coclaurine	-99.69	-104.30	-104.86	-102.56	-102.58	-97.72	-79.42
Corytuberine	-79.78	-86.72	-86.42	-86.24	-85.28	-92.83	-75.05
Domesticine	-84.84	-94.19	-94.61	-94.33	-93.29	-105.03	-82.89
Glazovine	-75.58	-85.13	-83.00	-83.47	-84.12	-94.46	-78.60
Isoboldine	-82.63	-89.21	-90.81	-90.18	-88.25	-99.28	-77.76
Laurotetanine	-77.68	-85.85	-91.81	-91.16	-84.69	-97.66	-73.50
N-Methylcoclaurine	-84.07	-91.57	-90.12	-89.19	-90.03	-97.72	-78.29
Nordomesticine	-81.86	-88.55	-89.26	-89.88	-86.72	-99.96	-78.74
Norisoboldine	-79.84	-86.24	-88.32	-88.42	-84.68	-94.85	-72.37
Pallidine	-80.03	-60.72	-82.46	-77.19	-82.05	-78.95	-74.04
Sarachine	-91.23	-91.16	-90.74	-73.66	-93.08	-77.13	-74.80
5-Methoxycanthin-6-one	-65.71	-72.98	-74.36	-76.26	-74.23	-83.88	-68.46
Canthin-6-one	-60.30	-64.05	-65.00	-65.77	-60.61	-72.68	-55.74
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-121.48	-122.46	-109.38	-120.35	-112.68	-107.95	-101.40

Ligand	Dihydroorotate Dehydrogenase						Tyrosine Aminotrans-ferase
	2DJX	2DJL	2E6A	2E6D	2E68	3C3N	1BW0
18-Acetoxy-13-15-diene-cassanoic acid	-87.48	-87.67	-100.48	-85.56	-103.65	-36.10	-83.52
18-Hydroxycassan-13,15-diene	-79.56	-81.53	-83.08	-81.65	-86.62	-83.42	-70.47
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-102.17	-98.42	-103.66	-95.41	-108.48	-96.78	-75.67
6β-18-Dihydroxycasssan-13,15-diene	-81.43	-84.42	-87.98	-84.60	-85.74	-84.57	-75.77
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-81.35	-90.40	-99.21	-87.91	-99.77	-49.27	-65.43
Geranygeraniol	-110.68	-105.86	-104.26	-109.34	-112.57	-108.39	-86.29
5-epi-Icetexone	-92.68	-93.85	-94.81	-94.56	-93.96	-102.64	-83.40
Alpinitine	-76.63	-82.33	-83.75	-81.05	-83.74	-92.36	-74.99
5,6,7-Trihydroxy-4′-methoxyflavone	-83.89	-91.22	-91.36	-91.39	-90.33	-101.54	-78.68
Ganglin	-92.00	-81.03	-82.85	-83.37	-81.55	-91.87	-80.70
Ganglin-3-methyl ether	-94.36	-82.71	-83.68	-82.02	-80.88	-90.99	-81.33
Luteolin	-91.98	-90.86	-91.19	-91.82	-91.44	-99.12	-79.49
Pinobanksin 3-acetate	-89.23	-93.19	-95.62	-94.68	-92.18	-100.35	-73.63
Pinobanksin	-89.62	-86.24	-87.77	-87.22	-86.64	-92.78	-74.22
Pinocembrin	-86.81	-84.05	-85.46	-80.30	-83.68	-89.00	-73.78
Quercetin-3-methyl ether	-92.58	-89.66	-90.35	-89.31	-89.04	-103.22	-87.69
Tectochrysin	-88.74	-88.76	-91.01	-87.09	-88.60	-89.67	-74.52
Aristolignan	-102.64	-124.44	-119.70	-105.34	-120.49	-101.33	-89.26
Calopiptin	-113.64	-120.67	-121.97	-120.18	-121.91	-101.60	-92.95
Galgravin	-105.82	-105.44	-112.36	-106.94	-113.48	-118.05	-98.92
Ganschisandrine	-117.51	-106.45	-103.21	-86.13	-113.86	-81.94	-83.10
Machilin G	-99.55	-115.17	-123.61	-115.73	-123.93	-105.15	-96.34
Nectandrin A	-79.12	-107.39	-108.51	-104.98	-106.26	-113.31	-94.13
Nectandrin B	-100.84	-99.32	-110.63	-107.03	-99.36	-109.15	-93.88
Conocarpan	-101.17	-101.63	-102.01	-100.95	-100.66	-97.59	-78.17
Eupomatenoid-3	-99.17	-107.51	-109.18	-106.23	-109.51	-111.33	-89.52
Eupomatenoid-5	-96.94	-100.43	-105.83	-101.85	-102.73	-109.27	-84.30
Eupomatenoid-6	-92.48	-93.16	-95.28	-95.25	-96.72	-102.15	-83.89
Grandisin	-74.34	-64.58	-101.96	-42.50	-44.36	-84.43	-103.05
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-120.56	-116.85	-120.54	-86.71	-99.30	-116.65	-105.13

Ligand	Dihydroorotate Dehydrogenase						Tyrosine Aminotrans-ferase
	2DJX	2DJL	2E6A	2E6D	2E68	3C3N	1BW0
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-122.09	-132.50	-129.28	-122.36	-126.49	-102.11	-105.61
4,5-di-(*E*)-O-Caffeyoquinic acid	-142.91	-148.95	-153.72	-165.21	-154.41	-170.88	-122.55
Caffeic Acid	-84.01	-85.37	-85.36	-85.23	-84.99	-82.35	-60.30
Isonoholaenic acid	-105.99	-102.55	-105.71	-105.95	-104.66	-105.22	-81.48
3-Heptadecyl-5-methoyphenol	-72.20	-65.16	-72.53	-78.60	-80.95	-84.58	-54.83
Embelin	-81.25	-84.20	-81.54	-89.14	-84.61	-84.09	-53.64
1,2,4-Trihrooxyheptadecane	-105.07	-100.92	-96.78	-104.53	-104.66	-104.94	-80.76
1,2,4-Trihydrooxyheptadec-16-ene	-97.76	-94.65	-95.47	-97.46	-88.41	-91.48	-83.69
1,2,4-Trihydrooxyheptadec-16-yne	-103.00	-88.08	-95.48	-96.09	-92.19	-102.41	-75.61
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-93.24	-85.49	-105.39	-99.03	-109.75	-108.15	-77.68
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-94.84	-104.15	-92.52	-111.10	-114.30	-123.05	-82.28
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-92.56	-91.24	-104.12	-95.09	-100.87	-102.24	-76.05
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-100.18	-109.22	-97.93	-90.41	-104.91	-95.63	-82.30
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-97.58	-108.14	-103.86	-108.35	-93.24	-100.06	-87.95
Helenalin	-93.43	-90.42	-96.48	-88.74	-96.99	-82.52	-75.09
Mexicanin	-90.84	-87.44	-93.42	-85.66	-94.49	-79.70	-72.10
15-Deoxygoyazenolide	-94.68	-98.98	-100.20	-100.47	-94.12	-103.75	-91.89
Centratherin	-103.05	-79.47	-85.91	-78.47	-86.30	-112.04	-97.70
Goyazenolide	-101.29	-108.02	-110.99	-111.80	-110.93	-114.16	-98.43
Lynchopholide	-87.18	-102.77	-98.85	-79.76	-83.29	-105.81	-93.73
8β-Hydroxyzaluzanin D	-74.98	-82.89	-78.98	-85.45	-78.56	-91.04	-75.51
Oleanolic acid	-43.69	46.27	-10.31	63.43	-19.01	93.76	-60.39
Ursolic acid	0.57	78.36	47.42	86.38	63.15	147.51	-63.82
Betulinic acid	-38.29	-14.97	-38.58	-18.39	-10.27	55.02	-84.37

Table A.7: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with trypanothione reductase.

Ligand	Trypanothione Reductase					
	1NDA	1AOG	1BZL	1GXF-A	1GXF-B	1GXF-C
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-94.17	-77.08	-75.21	-71.68	-79.58	-93.79
Arborinine	-87.13	-93.17	-67.82	-80.41	-85.27	-87.61
Kokusagine	-100.19	-89.05	-83.07	-77.37	-80.92	-93.78
N-Methyl-1-hydroxy-3-methoxyacridone	-81.49	-83.10	-66.75	-63.00	-93.08	-84.19
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-96.21	-66.59	-96.63	-100.08	-79.10	-97.87
Skimmianine	-98.83	-99.02	-76.89	-68.57	-82.85	-97.21
Dicenitrinone	-97.57	-95.37	-82.02	-80.71	-87.35	-84.32
Dugetine	-92.27	-77.80	-87.47	-94.67	-75.45	-103.63
Dugetine-β-*N*-oxide	-94.02	-76.62	-83.85	-91.75	-83.72	-82.96
N-Methylglaucine	-105.65	-91.20	-78.26	-80.58	-60.86	-85.58
N-Methyltetrahydropalmitine	-88.04	-79.15	-84.79	-85.92	-69.29	-105.02
Caaverine	92.57	-79.60	-67.40	-75.87	-75.50	-83.06
Coclaurine	-108.53	-89.91	-88.25	-89.84	-89.32	-106.09
Corytuberine	-101.88	-79.99	-82.62	-75.73	-71.28	-78.65
Domesticine	-97.98	-99.34	-80.35	-67.53	-89.35	-89.62
Glazovine	-88.71	-76.37	-83.30	-80.62	-76.34	-82.48
Isoboldine	-96.84	-94.44	-82.19	-68.89	-92.51	-91.60
Laurotetanine	-82.64	-94.31	-89.12	-75.15	-89.76	-84.28
N-Methylcoclaurine	-106.47	-91.35	-84.56	-84.35	-89.44	-96.88
Nordomesticine	-105.00	-102.46	-76.78	-80.13	-93.08	-91.81
Norisoboldine	-102.75	-92.46	-85.76	-77.44	-87.93	-96.02
Pallidine	-94.73	-94.97	-81.14	-82.71	-83.86	-104.61
Sarachine	-100.83	-89.62	-86.38	-69.02	-74.82	-82.91
5-Methoxycanthin-6-one	-91.68	-85.51	-70.70	-71.76	-75.03	-82.51
Canthin-6-one	-80.60	-76.87	-65.41	-64.76	-67.98	-73.36
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-118.07	-71.96	-119.17	-105.24	-76.84	-67.41

Ligand	Trypanothione Reductase					
	1NDA	1AOG	1BZL	1GXF-A	1GXF-B	1GXF-C
18-Acetoxy-13-15-diene-cassanoic acid	-99.63	-78.57	-87.07	-84.17	-72.90	-88.26
18-Hydroxycassan-13,15-diene	-88.07	-92.55	-71.02	-72.89	-72.17	-83.97
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-110.26	-88.98	-88.39	-84.51	-61.62	-81.50
6β-18-Dihydroxycasssan-13,15-diene	-89.35	-76.32	-75.18	-76.15	-74.96	-86.19
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-93.31	-93.67	-78.38	-76.76	-67.39	-87.00
Geranygeraniol	-129.54	-116.52	-95.29	-94.29	-111.59	-125.81
5-epi-Icetexone	-106.53	-120.41	-81.35	-74.95	-54.81	-102.79
Alpinitine	-104.09	-93.55	-72.10	-77.37	-89.34	-91.02
5,6,7-Trihydroxy-4′-methoxyflavone	-121.78	-105.90	-84.68	-70.33	-96.58	-112.65
Ganglin	-114.00	-95.28	-78.28	-76.35	-89.07	-111.13
Ganglin-3-methyl ether	-104.66	-99.01	-76.84	-74.21	-91.78	-102.75
Luteolin	-113.69	-102.41	-84.28	-79.42	-95.57	-113.52
Pinobanksin 3-acetate	-98.36	-95.94	-93.65	-88.91	-90.46	-98.74
Pinobanksin	-113.24	-90.96	-81.87	-71.97	-85.05	-109.84
Pinocembrin	-110.06	-91.46	-82.31	-69.79	-84.42	-107.05
Quercetin-3-methyl ether	-108.15	-109.09	-89.14	-90.68	-94.96	-112.91
Tectochrysin	-100.52	-66.33	-80.25	-74.44	-89.23	-113.29
Aristolignan	-121.55	-87.17	-92.89	-81.42	-119.48	-119.28
Calopiptin	-131.05	-114.23	-83.95	-88.79	-102.39	-115.55
Galgravin	-120.74	-114.98	-96.37	-95.52	-97.27	-117.77
Ganschisandrine	-125.11	-120.16	-93.02	-93.26	-117.50	-118.77
Machilin G	-125.71	-124.25	-94.61	-93.85	-111.43	-125.70
Nectandrin A	-119.04	-110.73	-89.20	-89.97	-99.87	-122.76
Nectandrin B	-111.78	-106.20	-93.51	-97.35	-110.73	-112.71
Conocarpan	-99.36	-97.41	-86.39	-78.37	-96.52	-97.04
Eupomatenoid-3	-115.65	-111.46	-99.57	-93.45	-107.10	-112.64
Eupomatenoid-5	-113.33	-111.81	-92.54	-91.21	-101.99	-114.95
Eupomatenoid-6	-107.63	-104.19	-86.04	-78.59	-98.32	-110.16
Grandisin	-99.80	-83.04	-104.99	-103.17	-117.94	-118.35

Ligand	Trypanothione Reductase					
	1NDA	1AOG	1BZL	1GXF-A	1GXF-B	1GXF-C
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-143.54	-104.95	-91.56	-114.63	-101.82	-131.10
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-134.13	-116.83	-111.21	-113.15	-124.31	-131.24
4,5-di-(*E*)-O-Caffeyoquinic acid	-161.89	-143.82	-136.17	-122.85	-132.44	-159.49
Caffeic Acid	-82.49	-74.74	-76.06	-70.46	-74.59	-82.56
Isonoholaenic acid	-113.27	-107.27	-81.49	-83.62	-98.71	-111.20
3-Heptadecyl-5-methoyphenol	-87.26	-86.08	-81.35	-76.18	-58.52	-90.98
Embelin	-85.47	-98.46	-72.44	-83.31	-79.87	-90.57
1,2,4-Trihrooxyheptadecane	-130.13	-101.15	-80.10	-88.42	-106.29	-114.43
1,2,4-Trihydrooxyheptadec-16-ene	-113.93	-99.72	-87.99	-87.49	-83.58	-115.52
1,2,4-Trihydrooxyheptadec-16-yne	-115.61	-104.32	-83.21	-76.80	-86.55	-123.68
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-132.75	-86.12	-89.84	-96.90	-83.16	-137.73
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-125.86	-97.11	-88.25	-92.72	-96.85	-132.20
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-129.60	-95.36	-83.63	-87.08	-91.07	-121.37
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-105.19	-102.40	-95.32	-96.21	-88.81	-126.80
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-124.47	-105.05	-87.04	-89.70	-97.66	-128.10
Helenalin	-101.81	-97.39	-67.88	-76.82	-73.54	-97.52
Mexicanin	-92.95	-88.97	-81.01	-74.60	-82.17	-88.05
15-Deoxygoyazenolide	-89.62	-75.73	-95.96	-102.60	-87.07	-100.05
Centratherin	-91.04	-91.49	-105.31	-100.36	-95.62	-110.98
Goyazenolide	-102.56	-101.32	-100.50	-106.82	-90.69	-103.10
Lynchopholide	-91.00	-93.64	-101.21	-101.44	-85.64	-102.69
8β-Hydroxyzaluzanin D	-34.17	-76.59	-88.96	-84.58	-70.26	-96.21
Oleanolic acid	2.24	-69.14	-75.19	-65.72	-62.19	35.14
Ursolic acid	66.37	-80.75	-44.82	-70.61	-66.63	90.20
Betulinic acid	53.29	-72.08	-83.05	-69.64	-72.83	45.28

Table A.8: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with pteridine reductase 2 and sterol 14α-demethylase.

Ligand	Pteridine Reductase 2		Sterol 14-Alpha Demethylase			
	1MXF	1MXH	2WX2	3K1O	3KHM	3KSW
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-88.61	-85.88	-72.15	-81.76	-69.76	-64.97
Arborinine	-88.30	-91.65	-64.12	-74.19	-73.12	-59.55
Kokusagine	-94.51	-84.18	-73.95	-74.46	-74.44	-68.54
N-Methyl-1-hydroxy-3-methoxyacridone	-88.53	-84.53	-65.74	-57.05	-64.56	-56.67
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-95.49	-94.42	-90.02	-94.57	-88.61	-81.54
Skimmianine	-93.52	-81.44	-71.05	-79.84	-71.62	-66.01
Dicenitrinone	-101.45	-93.66	-85.20	-73.00	-84.62	-78.73
Dugetine	-99.67	-94.18	-72.25	-82.46	-84.99	-70.58
Dugetine-β-*N*-oxide	-96.32	-103.37	-79.91	-79.06	-82.77	-81.24
N-Methylglaucine	-83.54	-84.53	-84.30	-64.92	-85.49	-74.40
N-Methyltetrahydropalmitine	-60.70	-110.38	-87.36	-84.79	-74.93	-69.54
Caaverine	-97.62	-95.75	-68.91	-67.54	-69.74	-60.35
Coclaurine	-99.04	-99.70	-83.41	-87.56	-80.50	-71.65
Corytuberine	-81.51	-84.41	-81.20	-80.93	-81.95	-62.04
Domesticine	-86.44	-94.74	-83.07	-83.40	-73.13	-81.03
Glazovine	-79.09	-90.79	-73.18	-72.17	-75.85	-66.69
Isoboldine	-86.85	-90.96	-75.18	-70.70	-77.77	-70.47
Laurotetanine	-99.45	-82.50	-85.10	-81.78	-79.24	-81.06
N-Methylcoclaurine	-85.48	-100.20	-89.76	-87.63	-86.75	-75.66
Nordomesticine	-97.04	-108.24	-85.88	-77.76	-78.07	-79.55
Norisoboldine	-93.54	-101.72	-83.58	-82.08	-70.36	-77.99
Pallidine	-73.09	-85.35	-86.59	-80.47	-77.39	-71.21
Sarachine	-74.16	-114.12	-97.61	-95.57	-88.27	-84.04
5-Methoxycanthin-6-one	-95.32	-85.40	-64.77	-72.60	-71.92	-67.65
Canthin-6-one	-85.73	-80.32	-58.78	-59.59	-39.23	-60.14

Ligand	Pteridine Reductase 2		Sterol 14-Alpha Demethylase			
	1MXF	1MXH	2WX2	3K1O	3KHM	3KSW
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-85.93	-104.07	-90.84	-85.92	-89.99	-79.97
18-Acetoxy-13-15-diene-cassanoic acid	-108.15	-85.85	-85.96	-91.85	-60.94	-76.03
18-Hydroxycassan-13,15-diene	-86.83	-91.46	-68.45	-57.02	-62.27	-64.98
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-95.32	-102.13	-81.80	-87.26	-84.15	-66.37
6β-18-Dihydroxycasssan-13,15-diene	-85.50	-95.59	-71.49	-77.20	-65.78	-71.57
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-90.28	-95.53	-73.22	-82.04	-83.65	-58.66
Geranygeraniol	-96.57	-106.24	-95.22	-84.65	-86.12	-81.37
5-epi-Icetexone	-92.75	-94.03	-80.78	-88.98	-84.40	-67.98
Alpinitine	-93.40	-87.74	-76.52	-79.30	-69.69	-64.38
5,6,7-Trihydroxy-4′-methoxyflavone	-101.42	-101.71	-86.73	-84.48	-81.97	-76.48
Ganglin	-94.58	-92.55	-76.66	-75.48	-74.76	-69.17
Ganglin-3-methyl ether	-95.21	-95.75	-76.99	-78.82	-76.47	-70.66
Luteolin	-99.79	-101.12	-85.47	-82.34	-81.84	-77.36
Pinobanksin 3-acetate	-94.79	-93.59	-82.06	-76.00	-69.94	-68.08
Pinobanksin	-92.33	-87.63	-77.72	-73.74	-72.27	-69.34
Pinocembrin	-89.14	-91.82	-78.91	-73.69	-70.66	-64.24
Quercetin-3-methyl ether	-106.91	-102.76	-87.45	-87.80	-89.33	-80.71
Tectochrysin	-95.29	-97.40	-2.45	-78.90	-76.76	-74.90
Aristolignan	-122.47	-108.90	-97.87	-92.54	-97.57	-79.58
Calopiptin	-120.26	-115.33	-99.07	-84.16	-92.93	-86.13
Galgravin	-120.31	-122.77	-93.55	-101.73	-85.64	-87.21
Ganschisandrine	-110.97	-105.11	-92.92	-100.95	-82.18	-79.11
Machilin G	-124.14	-115.86	-100.35	-96.75	-87.73	-97.39
Nectandrin A	-114.21	-106.10	-100.35	-99.70	-81.79	-78.87
Nectandrin B	-108.13	-108.03	-101.67	-95.41	-89.69	-76.20
Conocarpan	-88.94	-88.76	-82.94	-82.52	-73.79	-75.24
Eupomatenoid-3	-108.81	-93.15	-92.58	-95.55	-84.82	-90.46
Eupomatenoid-5	-105.26	-103.79	-96.96	-93.86	-83.82	-91.62
Eupomatenoid-6	-97.67	-95.81	-88.44	-85.96	-83.43	-85.86
Grandisin	-98.00	-118.66	-101.83	-90.33	-93.80	-88.40

Ligand	Pteridine Reductase 2		Sterol 14-Alpha Demethylase			
	1MXF	1MXH	2WX2	3K1O	3KHM	3KSW
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-127.31	-117.04	-101.85	-104.76	-100.87	-101.44
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-110.29	-111.55	-106.42	-111.66	-105.10	-102.42
4,5-di-(*E*)-O-Caffeyoquinic acid	-133.07	-136.06	-148.58	-114.54	-105.27	-102.01
Caffeic Acid	-76.53	-73.57	-65.30	-69.17	-61.54	-60.85
Isonoholaenic acid	-103.36	-83.11	-80.86	-89.05	-80.57	-83.92
3-Heptadecyl-5-methoyphenol	-65.45	-78.39	-76.64	-76.69	-56.59	-59.44
Embelin	-73.75	-93.99	-67.57	-73.17	-63.20	-48.62
1,2,4-Trihrooxyheptadecane	-97.83	-93.53	-101.11	-96.31	-75.52	-77.32
1,2,4-Trihydrooxyheptadec-16-ene	-86.18	-98.42	-86.05	-94.84	-80.50	-72.58
1,2,4-Trihydrooxyheptadec-16-yne	-90.33	-100.38	-104.68	-84.40	-79.73	-85.69
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-96.86	-113.71	-105.25	-94.35	-80.84	-83.45
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-85.35	-103.03	-109.68	-98.88	-83.65	-80.23
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-94.97	-101.93	-96.62	-91.25	-79.91	-76.08
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-84.80	-103.01	-101.71	-92.99	-93.43	-76.42
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-84.43	-104.55	-94.41	-91.98	-78.62	-81.29
Helenalin	-80.26	-79.83	-75.03	-78.30	-75.04	-64.07
Mexicanin	-76.51	-86.03	-75.36	-63.25	-78.11	-63.94
15-Deoxygoyazenolide	-102.33	-93.45	-92.32	-92.55	-91.56	-80.12
Centratherin	-42.89	-100.29	-91.41	-98.05	-94.70	-95.62
Goyazenolide	-108.46	-97.68	-100.96	-92.47	-96.19	-86.60
Lynchopholide	-101.69	-94.80	-96.45	-92.22	-83.61	-90.36
8β-Hydroxyzaluzanin D	-74.65	-89.09	-79.11	-86.62	-83.39	-77.39
Oleanolic acid	-64.39	-97.46	-5.34	-78.02	-47.90	-73.46
Ursolic acid	-52.21	-85.16	-93.15	-54.58	-54.05	-55.83
Betulinic acid	-70.63	-97.80	-45.54	-82.15	-69.83	-76.53

Table A.9a: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with miscellaneous enzyme targets.

Ligand	Fe-Superoxide Dismutase	Histidyl-tRNA Synthetase		Old Yellow Enzyme	Glucokinase	
	2GPC	3HRK	3LC0	3ATZ	2Q2Ra	2Q2Rb
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-73.42	-84.52	-79.23	-87.37	-83.88	-79.30
Arborinine	-70.91	-74.73	-70.56	-61.83	-85.08	-85.80
Kokusagine	-78.60	-80.18	-71.12	-78.42	-84.22	-83.73
N-Methyl-1-hydroxy-3-methoxyacridone	-69.36	-79.82	-71.16	-70.82	-82.01	-82.53
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-76.47	-80.25	-83.41	-84.35	-100.85	-106.08
Skimmianine	-82.53	-80.43	-74.49	-71.50	-88.21	-91.26
Dicenitrinone	-73.55	-80.56	-80.08	-87.73	-108.00	-106.19
Dugetine	-76.32	-90.59	-74.94	-84.99	-101.16	-101.00
Dugetine-β-*N*-oxide	-74.71	-91.29	-77.08	-87.38	-81.53	-86.15
N-Methylglaucine	-69.04	-79.94	-80.53	-60.72	-99.84	-112.39
N-Methyltetrahydropalmitine	-75.01	-77.41	-84.63	-73.94	-83.61	-81.15
Caaverine	-72.40	-85.64	-71.92	-79.84	-94.60	-89.29
Coclaurine	-73.76	-90.45	-78.64	-78.45	-102.72	-100.84
Corytuberine	-66.64	-69.07	-83.01	-81.64	-94.73	-91.92
Domesticine	-63.96	-86.13	-82.03	-82.56	-98.43	-98.21
Glazovine	-70.02	-72.33	-74.00	-81.48	-98.03	-83.35
Isoboldine	-65.50	-81.66	-68.39	-83.28	-90.61	-90.05
Laurotetanine	-70.49	-71.96	-75.94	-78.82	-100.76	-101.35
N-Methylcoclaurine	-79.96	-86.20	-81.97	-81.96	-93.15	-88.99
Nordomesticine	-69.94	-87.96	-76.88	-80.36	-100.61	-100.37
Norisoboldine	-69.20	-87.39	-72.17	-81.91	-91.26	-91.98
Pallidine	-65.42	-87.30	-81.22	-71.60	-102.08	-87.15
Sarachine	-79.18	-91.78	-82.82	-75.54	-89.95	-90.03
5-Methoxycanthin-6-one	-71.29	-82.19	-74.08	-74.46	-83.96	-83.36
Canthin-6-one	-66.78	-73.33	-69.72	-69.88	-77.82	-77.85
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-66.38	-99.96	-93.40	-109.58	-98.67	-109.93

Ligand	Fe-Superoxide Dismutase	Histidyl-tRNA Synthetase		Old Yellow Enzyme	Glucokinase	
	2GPC	3HRK	3LC0	3ATZ	2Q2Ra	2Q2Rb
18-Acetoxy-13-15-diene-cassanoic acid	-70.45	-95.26	-92.48	-83.74	-108.07	-99.72
18-Hydroxycassan-13,15-diene	-62.28	-84.72	-69.34	-73.26	-84.58	-86.93
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-71.91	-85.08	-87.22	-84.49	-85.61	-86.78
6β-18-Dihydroxycasssan-13,15-diene	-68.37	-87.73	-72.11	-72.80	-88.67	-89.69
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-63.56	-95.18	-66.55	-76.22	-96.27	-87.99
Geranygeraniol	-94.58	-102.75	-89.05	-96.90	-105.98	-111.82
5-epi-Icetexone	-67.76	-85.71	-69.36	-79.91	-89.36	-88.53
Alpinitine	-73.41	-81.71	-73.14	-83.00	-91.35	-91.82
5,6,7-Trihydroxy-4′-methoxyflavone	-75.21	-89.25	-78.95	-87.98	-93.16	-94.65
Ganglin	-72.75	-93.19	-79.98	-80.64	-90.72	-84.32
Ganglin-3-methyl ether	-73.32	-86.88	-74.81	-79.82	-90.63	-92.93
Luteolin	-81.16	-97.17	-76.41	-88.19	-97.02	-97.80
Pinobanksin 3-acetate	-70.61	-80.83	-83.16	-79.71	-89.08	-82.18
Pinobanksin	-73.31	-91.20	-72.17	-78.32	-87.38	-88.51
Pinocembrin	-71.53	-88.00	-76.14	-78.21	-83.52	-84.63
Quercetin-3-methyl ether	-82.43	-101.48	-89.10	-83.32	-106.81	-104.71
Tectochrysin	-75.42	-85.88	-71.34	-78.55	-91.46	-90.94
Aristolignan	-91.21	-104.33	-77.40	-90.25	-101.69	-97.19
Calopiptin	-77.30	-108.38	-84.13	-93.28	-105.17	-111.27
Galgravin	-96.42	-92.86	-86.29	-94.38	-106.84	-107.03
Ganschisandrine	-85.64	-80.39	-91.70	-87.85	-103.83	-106.79
Machilin G	-81.33	-93.43	-92.27	-95.09	-108.67	-111.26
Nectandrin A	-93.45	-91.03	-87.92	-99.04	-107.95	-110.13
Nectandrin B	-88.17	-101.75	-82.26	-102.09	-99.12	-116.29
Conocarpan	-90.91	-94.24	-83.15	-86.22	-98.22	-101.57
Eupomatenoid-3	-97.51	-90.70	-88.12	-92.38	-109.87	-111.01
Eupomatenoid-5	-85.74	-96.95	-87.16	-93.16	-109.85	-109.37
Eupomatenoid-6	-86.61	-85.88	-76.83	-83.95	-100.81	-101.05
Grandisin	-83.60	-95.60	-103.41	-103.85	-108.74	-109.82
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-91.63	-110.55	-111.91	-112.53	-112.76	-113.97

Ligand	Fe-Superoxide Dismutase	Histidyl-tRNA Synthetase		Old Yellow Enzyme	Glucokinase	
	2GPC	3HRK	3LC0	3ATZ	2Q2Ra	2Q2Rb
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-93.41	-117.20	-100.40	-116.80	-118.75	-123.65
4,5-di-(*E*)-O-Caffeyoquinic acid	-99.57	-129.93	-108.66	-127.95	-124.50	-144.95
Caffeic Acid	-67.59	-78.60	-70.64	-75.49	-75.62	-75.45
Isonoholaenic acid	-87.30	-96.52	-85.10	-91.52	-99.79	-95.20
3-Heptadecyl-5-methoyphenol	-61.01	-77.46	-55.90	-64.60	-76.30	-73.71
Embelin	-75.72	-97.77	-73.05	-73.15	-70.81	-78.10
1,2,4-Trihrooxyheptadecane	-93.13	-111.05	-81.84	-84.25	-95.66	-97.61
1,2,4-Trihydrooxyheptadec-16-ene	-92.47	-91.84	-84.78	-91.17	-92.47	-98.12
1,2,4-Trihydrooxyheptadec-16-yne	-84.72	-89.32	-76.18	-90.09	-103.35	-101.99
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-90.13	-88.43	-81.61	-85.41	-98.36	-100.84
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-94.48	-89.19	-72.21	-85.03	-88.57	-112.02
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-90.75	-100.09	-69.12	-77.18	-86.66	-100.49
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-94.06	-120.81	-87.42	-93.68	-101.38	-97.77
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-89.22	-101.97	-83.50	-95.12	-101.77	-94.28
Helenalin	-69.10	-81.87	-79.34	-69.75	-79.65	-79.51
Mexicanin	-69.19	-89.37	-76.38	-71.66	-83.50	-81.88
15-Deoxygoyazenolide	-72.32	-95.20	-93.27	-91.55	-94.50	-99.05
Centratherin	-81.65	-83.46	-93.42	-93.72	-96.04	-97.26
Goyazenolide	-76.19	-92.52	-89.58	-103.18	-93.95	-89.66
Lynchopholide	-77.61	-93.90	-88.91	-92.52	-96.98	-101.98
8β-Hydroxyzaluzanin D	-67.15	-88.67	-76.49	-77.26	-86.43	-86.55
Oleanolic acid	-16.37	-23.63	-74.27	-67.10	-58.95	-68.53
Ursolic acid	-33.15	-51.49	-70.51	-66.98	-38.77	-63.35
Betulinic acid	-44.01	-94.31	-78.08	-79.30	-94.31	-91.23

Table A.9b: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with miscellaneous enzyme targets.

Ligand	Glyceraldehyde-3-Phosphate Dehydrogenase				Lipoamide Dehydro-genase	Cyclophilin
	1K3T	1ML3	1QXS	3IDS	2QAE	1XQ7
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-69.93	-77.60	-63.22	-72.23	-98.38	-75.47
Arborinine	-65.24	-80.30	-64.52	-76.40	-84.90	-65.67
Kokusagine	-59.42	-86.71	-67.64	-81.89	-107.74	-76.21
N-Methyl-1-hydroxy-3-methoxyacridone	-71.13	-81.22	-59.74	-76.29	-84.58	-65.61
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-80.27	-88.96	-80.00	-69.77	-88.12	-83.52
Skimmianine	-68.67	-68.54	-68.99	-73.08	-104.17	-77.25
Dicenitrinone	-83.61	-87.36	-70.75	-84.55	-87.01	-82.35
Dugetine	-75.49	-72.48	-69.15	-68.55	-97.95	-85.22
Dugetine-β-*N*-oxide	-74.29	-70.50	-72.14	-73.58	-93.16	-81.63
N-Methylglaucine	-72.87	-70.24	-68.23	-68.72	-93.14	-74.49
N-Methyltetrahydropalmitine	-70.07	-73.42	-73.85	-67.34	-86.26	-74.06
Caaverine	-68.35	-62.48	-58.97	-59.09	-74.84	-71.69
Coclaurine	-75.01	-79.96	-70.24	-71.64	-93.29	-81.70
Corytuberine	-64.58	-68.47	-65.99	-69.57	-82.27	-73.83
Domesticine	-70.62	-85.96	-67.64	-85.43	-92.29	-73.56
Glazovine	-71.16	-72.88	-62.05	-75.82	-88.88	-86.56
Isoboldine	-71.56	-73.31	-65.68	-78.49	-91.40	-65.01
Laurotetanine	-80.27	-85.91	-67.28	-84.69	-89.35	-74.70
N-Methylcoclaurine	-85.29	-78.26	-66.18	-74.65	-93.91	-81.03
Nordomesticine	-69.82	-85.98	-67.47	-85.50	-95.84	-78.89
Norisoboldine	-84.40	-85.35	-65.65	-83.25	-92.25	-78.02
Pallidine	-68.15	-66.49	-71.76	-72.28	-84.26	-66.65
Sarachine	-78.27	-63.56	-71.31	-78.79	-100.68	-64.09
5-Methoxycanthin-6-one	-61.88	-74.27	-54.10	-73.03	-75.04	-73.18
Canthin-6-one	-66.39	-72.49	-52.89	-66.13	-69.20	-69.17
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-81.16	-72.85	-77.83	-78.45	-88.18	-86.50
18-Acetoxy-13-15-diene-cassanoic acid	-81.95	-65.54	-70.83	-74.11	-95.95	-78.50

Ligand	Glyceraldehyde-3-Phosphate Dehydrogenase				Lipoamide Dehydro-genase	Cyclophilin
	1K3T	1ML3	1QXS	3IDS	2QAE	1XQ7
18-Hydroxycassan-13,15-diene	-66.46	-64.64	-66.85	-69.79	-78.18	-74.11
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-77.61	-59.34	-70.62	-64.70	-101.34	-79.47
6β-18-Dihydroxycasssan-13,15-diene	-65.27	-75.00	-64.10	-73.25	-74.77	-68.67
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-74.72	-80.00	-61.59	-67.86	-77.97	-67.34
Geranygeraniol	-80.62	-87.51	-83.11	-86.65	-117.95	-93.81
5-epi-Icetexone	-68.65	-47.28	-67.27	-63.41	-118.29	-73.25
Alpinitine	-70.09	-79.20	-66.59	-74.26	-97.88	-80.88
5,6,7-Trihydroxy-4′-methoxyflavone	-82.46	-84.10	-61.65	-81.02	-97.67	-84.64
Ganglin	-73.49	-78.86	-66.64	-79.03	-104.69	-80.43
Ganglin-3-methyl ether	-72.55	-78.20	-71.16	-73.34	-90.26	-81.22
Luteolin	-80.73	-86.61	-56.52	-88.12	-101.32	-80.00
Pinobanksin 3-acetate	-74.29	-79.00	-59.96	-74.97	-118.19	-71.23
Pinobanksin	-80.90	-81.01	-63.74	-76.67	-101.70	-76.05
Pinocembrin	-68.27	-79.03	-58.34	-76.26	-96.20	-71.08
Quercetin-3-methyl ether	-77.44	-79.06	-83.23	-84.44	-102.70	-83.11
Tectochrysin	-84.09	-79.69	-62.84	-80.52	-106.64	-67.98
Aristolignan	-86.61	-84.85	-81.54	-84.07	-103.18	-95.17
Calopiptin	-82.77	-92.54	-79.81	-88.68	-113.85	-100.41
Galgravin	-78.47	-77.62	-82.89	-84.23	-117.80	-87.44
Ganschisandrine	-85.83	-84.62	-76.82	-76.90	-112.07	-83.80
Machilin G	-87.34	-91.58	-80.19	-90.86	-111.95	-94.05
Nectandrin A	-82.98	-81.23	-91.46	-86.11	-110.97	-89.50
Nectandrin B	-79.80	-89.51	-83.05	-79.37	-102.65	-91.76
Conocarpan	-80.51	-78.26	-70.59	-76.27	-88.13	-75.81
Eupomatenoid-3	-97.09	-99.09	-83.73	-99.80	-104.74	-73.20
Eupomatenoid-5	-90.03	-93.61	-85.49	-94.55	-113.65	-79.80
Eupomatenoid-6	-88.25	-88.65	-78.92	-93.20	-102.76	-79.50
Grandisin	-71.99	-90.64	-88.50	-81.97	-73.80	-96.54
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-85.76	-85.35	-96.04	-72.92	-79.22	-105.10

Ligand	Glyceraldehyde-3-Phosphate Dehydrogenase				Lipoamide Dehydro-genase	Cyclophilin
	1K3T	1ML3	1QXS	3IDS	2QAE	1XQ7
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-98.68	-87.86	-95.83	-83.01	-105.57	-108.18
4,5-di-(*E*)-O-Caffeyoquinic acid	-117.86	-99.40	-97.20	-115.36	-130.66	-103.03
Caffeic Acid	-72.66	-70.22	-59.72	-76.46	-76.17	-75.95
Isonoholaenic acid	-81.06	-82.62	-77.01	-88.89	-110.42	-82.27
3-Heptadecyl-5-methoyphenol	-56.54	-59.05	-40.98	-55.37	-93.62	-63.41
Embelin	-50.23	-63.51	-55.06	-48.61	-91.63	-68.56
1,2,4-Trihrooxyheptadecane	-89.76	-90.69	-78.14	-59.76	-102.67	-75.37
1,2,4-Trihydrooxyheptadec-16-ene	-85.71	-78.21	-78.56	-80.46	-92.16	-73.44
1,2,4-Trihydrooxyheptadec-16-yne	-78.94	-73.17	-72.38	-67.62	-109.70	-85.63
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-83.78	-73.09	-86.42	-59.34	-99.91	-85.50
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-85.70	-76.87	-79.41	-61.79	-99.35	-83.75
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-75.60	-62.95	-75.35	-71.54	-100.92	-82.76
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-76.59	-73.37	-72.49	-70.19	-98.35	-92.50
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-72.96	-87.95	-86.85	-77.59	-93.83	-90.28
Helenalin	-69.15	-76.54	-57.43	-76.79	-79.12	-83.72
Mexicanin	-66.53	-74.17	-61.97	-69.23	-87.90	-83.43
15-Deoxygoyazenolide	-86.48	-86.81	-83.35	-82.36	-88.28	-81.12
Centratherin	-93.30	-88.99	-87.18	-86.53	-114.48	-88.62
Goyazenolide	-91.25	-92.12	-82.52	-86.03	-91.26	-90.23
Lynchopholide	-87.79	-87.15	-86.01	-83.94	-99.35	-74.23
8β-Hydroxyzaluzanin D	-78.66	-61.53	-68.62	-71.77	-87.45	-70.55
Oleanolic acid	-77.88	-54.10	-55.64	-68.91	-20.46	-54.49
Ursolic acid	-70.24	-53.92	-58.21	-21.52	-52.21	-15.35
Betulinic acid	-84.59	-74.67	-68.42	-61.89	-52.71	-51.17

Table A.9c: Lowest-energy docked poses (re-rank scores) for anti-trypanosomal agents with miscellaneous enzyme targets.

Ligand	Triosephos-phate Isomerase	Ribose 5-Phosphate Isomerase Type B			DUTPase	Pyruvate Kinase
	2OMA	3K7S	3K8C(A)	3K8C(B)	1OGK	3QV9
4-Methoxy-6-[2-(methylamino)phenyl]-2*H*-pyran-2-one	-71.72	-81.52	-78.47	-62.33	-75.71	-67.05
Arborinine	-65.28	-86.06	-77.26	-39.93	-70.47	-62.74
Kokusagine	-70.64	-83.38	-78.47	-41.86	-76.90	-64.59
N-Methyl-1-hydroxy-3-methoxyacridone	-62.54	-81.19	-76.28	-39.39	-68.47	-61.42
rel-(7*R*,8*R*)-8-[(*E*)-3-Hydroxy-3-methyl-1-butenyl]-4,8-dimethoxy-5,6,7,8-tetrahydrofuro[2,3,-b]quinoline-7-yl acetate	-70.40	-86.84	-69.82	-36.72	-91.29	-68.98
Skimmianine	-67.16	-85.56	-81.02	-66.55	-73.42	-66.75
Dicenitrinone	-68.72	-91.07	-78.75	-55.48	-80.42	-71.42
Dugetine	-69.19	-91.53	-66.45	-49.73	-75.99	-62.76
Dugetine-β-*N*-oxide	-66.85	-95.75	-60.34	-54.50	-73.28	-58.64
N-Methylglaucine	-39.83	-74.34	-45.91	-29.86	-74.18	-60.94
N-Methyltetrahydropalmitine	-64.48	-85.92	-67.60	-41.51	-80.31	-69.89
Caaverine	-66.65	-77.06	-74.73	-0.96	-61.22	-64.19
Coclaurine	-76.98	-90.23	-83.87	-60.02	-85.69	-67.89
Corytuberine	-75.91	-78.72	-46.69	-37.85	-70.32	-42.98
Domesticine	-67.35	-78.44	-78.04	-44.65	-71.67	-59.90
Glazovine	-73.65	-75.87	-73.12	-51.87	-71.40	-65.98
Isoboldine	-78.28	-76.51	-43.66	-43.03	-67.79	-56.29
Laurotetanine	-79.63	-76.63	-77.42	-37.07	-71.06	-56.43
N-Methylcoclaurine	-81.40	-81.64	-77.04	-46.71	-84.42	-71.10
Nordomesticine	-74.64	-85.57	-82.28	-54.58	-69.94	-58.54
Norisoboldine	-75.31	-76.06	-64.33	-38.61	-76.95	-47.89
Pallidine	-69.22	-49.76	-53.82	-52.88	-69.14	-76.90
Sarachine	-56.73	-62.72	-69.99	-48.67	-73.80	-71.06
5-Methoxycanthin-6-one	-66.43	-79.95	-80.13	-34.87	-66.29	-62.03
Canthin-6-one	-63.70	-76.97	-76.79	-34.61	-66.95	-55.74
ent-9α-Hydroxy-15β-*E*-cinnamoyloxy-16-kauren-19-oic acid	-77.58	-48.01	-49.23	-69.18	-81.41	-80.86
18-Acetoxy-13-15-diene-cassanoic acid	-62.95	-63.83	-44.86	-41.27	-82.72	-67.99

Ligand	Triosephos-phate Isomerase	Ribose 5-Phosphate Isomerase Type B			DUTPase	Pyruvate Kinase
	2OMA	3K7S	3K8C(A)	3K8C(B)	1OGK	3QV9
18-Hydroxycassan-13,15-diene	-50.59	-68.92	-71.08	-36.00	-74.05	-64.15
6β,13β-Dihydroxy-18-acetoxy-cassan-14(17),15-diene	-66.42	-88.54	-81.95	-52.53	-78.32	-77.78
6β-18-Dihydroxycasssan-13,15-diene	-35.53	-71.10	-70.62	-45.61	-75.69	-63.14
6β-Hydroxy-18-acetoxy-cassan-13,15-diene	-62.22	-72.08	-73.20	-50.31	-68.21	-62.65
Geranygeraniol	-71.19	-98.67	-94.29	-71.31	-93.22	-83.95
5-epi-Icetexone	-64.89	-87.65	-71.26	-42.78	-53.91	-44.32
Alpinitine	-61.82	-84.71	-79.83	-74.31	-79.10	-73.15
5,6,7-Trihydroxy-4′-methoxyflavone	-71.04	-86.81	-85.13	-58.01	-89.71	-64.58
Ganglin	-71.08	-82.64	-79.75	-61.65	-78.30	-69.22
Ganglin-3-methyl ether	-72.82	-84.10	-84.17	-52.27	-78.08	-71.14
Luteolin	-74.59	-91.58	-91.54	-66.79	-81.60	-72.37
Pinobanksin 3-acetate	-71.53	-87.71	-85.65	-54.13	-71.86	-72.67
Pinobanksin	-68.90	-82.17	-80.20	-52.88	-79.32	-68.52
Pinocembrin	-68.95	-79.06	-78.26	-42.10	-79.18	-67.38
Quercetin-3-methyl ether	-75.97	-98.63	-96.31	-63.30	-83.59	-75.44
Tectochrysin	-69.91	-85.77	-79.44	-59.14	-74.55	-55.75
Aristolignan	-74.26	-95.09	-82.46	-47.08	-92.93	-71.88
Calopiptin	-72.56	-100.06	-74.44	-43.84	-91.29	-63.10
Galgravin	-51.37	-98.28	-77.35	-54.03	-82.44	-79.52
Ganschisandrine	-69.11	-87.33	-90.43	-55.77	-89.28	-80.82
Machilin G	-78.13	-98.34	-84.28	-78.33	-93.15	-79.33
Nectandrin A	-70.87	-91.90	-80.60	-68.50	-87.51	-80.70
Nectandrin B	-53.20	-105.27	-94.05	-52.05	-34.06	-75.54
Conocarpan	-71.44	-87.95	-78.33	-65.10	-80.24	-74.63
Eupomatenoid-3	-74.45	-87.57	-83.88	-59.56	-98.90	-84.28
Eupomatenoid-5	-74.59	-95.79	-91.41	-53.81	-83.24	-82.47
Eupomatenoid-6	-61.03	-85.31	-83.61	-55.65	-89.37	-78.10
Grandisin	-75.61	-93.42	-94.19	-39.32	-91.77	-76.06
rel-(7*R*,8*R*,7′*R*, 8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	-84.89	-89.18	-100.74	-53.74	-87.23	-84.75

Ligand	Triosephos-phate Isomerase	Ribose 5-Phosphate Isomerase Type B			DUTPase	Pyruvate Kinase
	2OMA	3K7S	3K8C(A)	3K8C(B)	1OGK	3QV9
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan	-87.76	-94.02	-81.57	-68.06	-93.70	-85.20
4,5-di-(*E*)-O-Caffeyoquinic acid	-100.80	-110.38	-116.75	-111.33	-133.04	-107.65
Caffeic Acid	-58.95	-76.65	-75.28	-80.14	-69.39	-61.30
Isonoholaenic acid	-70.40	-102.39	-81.84	-85.11	-92.53	-79.08
3-Heptadecyl-5-methoyphenol	-51.68	-76.57	-59.37	-72.88	-52.72	-46.49
Embelin	-58.60	-86.77	-70.17	-76.83	-64.59	-59.34
1,2,4-Trihrooxyheptadecane	-70.96	-86.50	-85.64	-55.09	-89.79	-78.51
1,2,4-Trihydrooxyheptadec-16-ene	-65.49	-87.90	-82.46	-61.40	-84.17	-73.46
1,2,4-Trihydrooxyheptadec-16-yne	-72.51	-88.27	-81.28	-63.19	-90.10	-76.89
1-Acetoxy-2,4-dihydroxyheptadec-16-ene	-62.85	-86.97	-87.35	-67.78	-101.48	-74.76
1-Acetoxy-2,4-dihydroxyheptadec-16-yne	-66.53	-82.83	-87.10	-52.52	-93.61	-76.97
4-Acetoxy-1,2-dihydroxyheptadec-16-ene	-68.91	-87.60	-86.20	-48.57	-97.12	-70.21
4-Acetoxy-1,2-dihydroxyheptadec-16-yne	-69.88	-96.95	-64.90	-35.84	-90.15	-77.68
(*E*)-1,2,4-Trihydroxynonadec-6-ene	-67.52	-95.83	-85.67	-56.92	-95.19	-81.62
Helenalin	-68.09	-90.56	-86.82	-39.47	-70.54	-67.87
Mexicanin	-69.40	-86.99	-80.43	-46.49	-71.97	-71.48
15-Deoxygoyazenolide	-87.58	-99.27	-90.00	-56.05	-77.60	-77.36
Centratherin	-92.59	-105.14	-101.08	-47.98	-88.05	-82.48
Goyazenolide	-93.01	-79.04	-90.26	-53.55	-87.66	-58.93
Lynchopholide	-89.19	-102.52	-94.18	-60.04	-61.26	-82.03
8β-Hydroxyzaluzanin D	-60.30	-98.61	-93.57	-46.60	-74.10	-67.68
Oleanolic acid	-55.82	-46.96	25.39	-33.37	-52.09	-12.88
Ursolic acid	-16.83	-7.59	16.05	-40.49	135.65	-27.94
Betulinic acid	-61.24	-67.45	-74.41	-46.77	-40.88	-54.25

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