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A RESEARCH STUDY ON EVALUATION OF ANTI-HELMINTHIC ACTIVITY IN METHANOLIC LEAF EXTRACT OF *ANANAS COMOSUS* - AN *INVITRO* AND INSILICO APROACH

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Abstract:

Helminthic infections are among the most common infection in human affecting large population of the world.As per WHO only few drugs are frequently used in the treatment of these parasite infection.in present study ethanol and methanol extract of ananas comosus leaves were investigated for their antihelminthic activity against pheretima posthuma. The four concentration (10,20,30,50 mg/ml) of each extraxt was studied. These experiment involved in the determination time of paralysis and time of death of the worms.the gradual increase in a dose exhibited, a gradual increase in the activity .Methanol extract displayed significant antihelminthic activity at the highest concentration 50mg/ml. Insilico studies have revealed Ltryptophan a natural ligand as the good binding energy score of -97.124 with target protein 1sa0 as compared with that of standard drug albendazole.

Keywords: *Antihelminthic , Ananas comosus, Methanol, pheretima posthuma ,molecular docking , ADMET profile.*

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INTRODUCTION:

Antihelminthic:

The anthelminthic is the term used to describe a drug used to treat infection of animals with parasitic worms. Currently antihelminthic drugs generally act by paralyzing the parasites (e.g by preventing muscular contraction) by damaging the worms such that the host immune system can eliminate it, or by altering parasite metabolism [1] (e.g. by affecting microtubule function).

Helminthiasis:

Soil-transmitted helminth infections are among the most common infections in the world, affecting the poorest and most disadvantaged communities. It is caused by an infestation of one or more intestinal parasitic worms. They are spread by eggs found in human faeces, which contaminate soil in areas with poor sanitation. [2] Infected individuals excrete helminth eggs in their faeces, which can contaminate the soil in areas with poor sanitation.

Helminthic:

Helminth is a generic term for worm. They are invertebrates with elongated, flat, or round bodies. Flukes and tapeworms are examples of flatworms or platyhelminths (platy from the Greek root meaning "flat"). Nematodes (from the Greek root meaning "thread") are roundworms. Helminths are large, multicellular organisms that can be seen with the naked eye once they reach the adult stage of their life cycle.

In their adult form, helminths are unable to multiply in humans and utilise numerous mechanisms of transmission to ensure reproductive success [3]. These parasites live in and feed on hosts, allowing them to obtain nutrients while interfering with the hosts' nutrient absorption.

The major groups of parasitic helminths include:

- ❖ Flukes (Trematodes)
- ❖ Tapeworms (Cestodes)
- ❖ Roundworms (Nematodes)
- ❖ Acanthocephalins (thorny-headed worms)
- ❖ platyhelminths (flatworms)

Cycle of helminths infection:

Helminths have three distinct life stages: eggs, larvae, and adults. Adult worms infect definitive hosts (those that undergo sexual development), whereas larval stages may be free-living or parasitize invertebrate vectors, intermediate, or paratenic hosts[4].

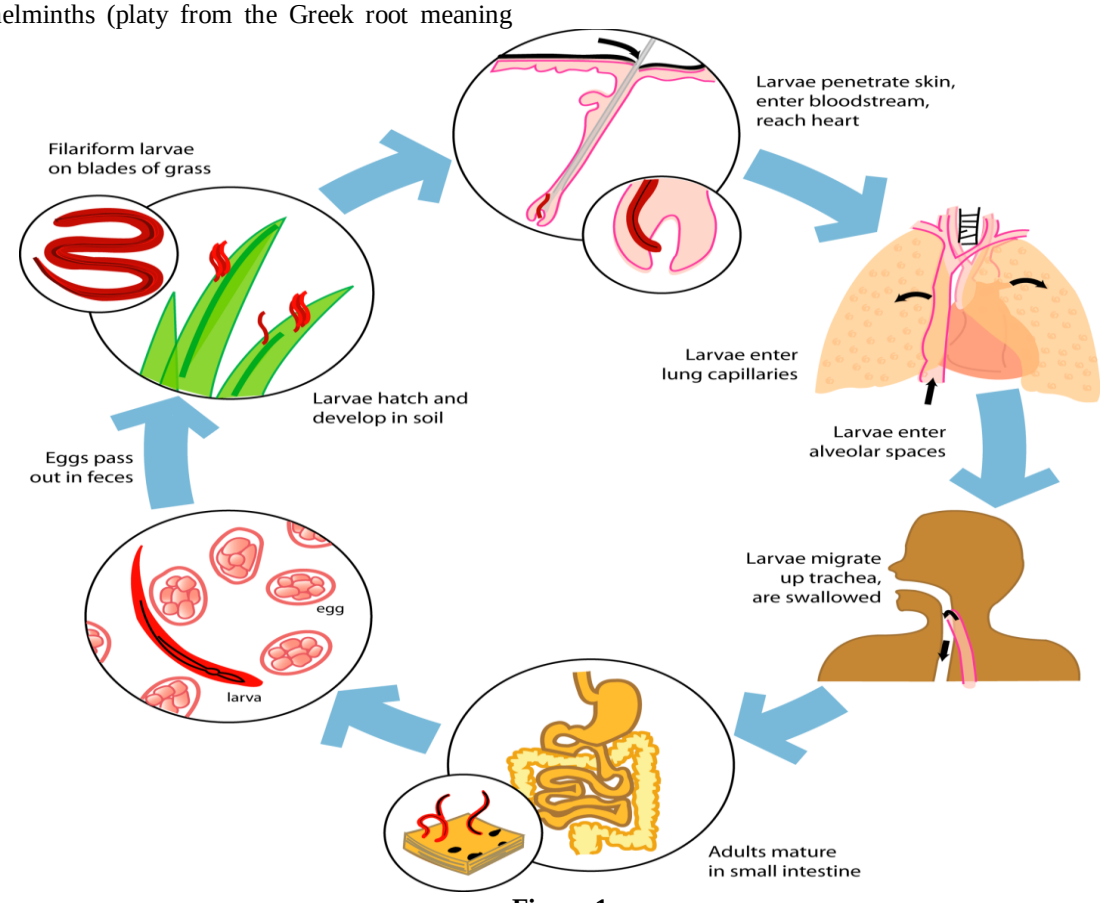


Figure 1

Signs and symptoms:

- ✓ The signs and symptoms of helminthiasis are determined by several factors like,
- ✓ Location of the infestation within the body.
- ✓ The number of worms and their volume.
- ✓ The type of damage caused by the infesting worms.
- ✓ And the body's immunological response.

Diagnosis:

I. Gross contamination:
II. Microscopy based techniques:
   a. Kato katz :
   b. Mc master technique:
   c. Flotac and mini flotac techniques:
III. Other techniques:
   a. Stool test
   b. Blood test
   c. Tape test
   d. Colonoscopy
   e. Imagine test

TREATMENT:

A. Medication
B. Mass deworming:
C. Surgery

DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex[5]. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes[6].

Molegro virtual docker

Protein- ligand docking stimulation program. escasting the binding conformation of ligands to appropriate target binding site was done using MVD.It is used in estimation of MolDock score of the ligand[7].

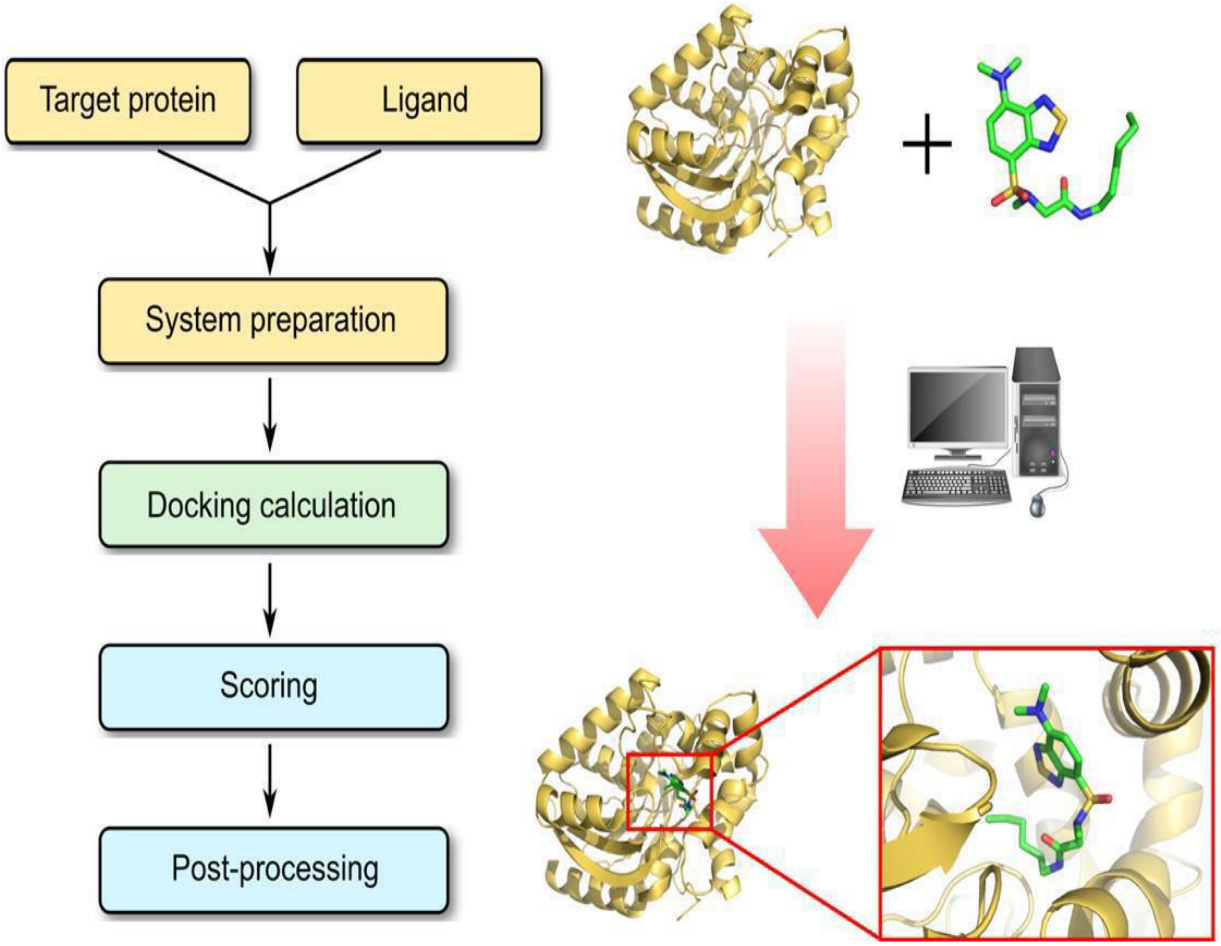


Figure 2

PLANT PROFILE

Botanical name	Ananas comosus
Synonyms	Ananas ananas
Family	Bromeliaceae
English name	Pineapple
Phylum	Vascular plant
Class	Mangaliopsida
Order	Poales
Species	Ananas comosus

Figure 3

MATERIALS AND METHODS:

Plant collection and identification:

Fresh leaves of *Ananas comosus* was collected from the local place in kanchipuram, Tamilnadu. It was authenticated by Dr.M.K.Seeni, Government analyst, State drug testing laboratory (Indian medicine) Arumbakkam, Chennai – 600 106.

Preparation of the plant extract:

- The fresh and tender leaves of Ananas comosus was collected.
- Thoroughly washed off with distilled water.
- Then the leaves were cut into small pieces.
- The leaves were thoroughly shade dried for a week.
- The dried leaves were grinded into a fine powdered.

- The powder was used for the extraction process.

Figure 4

Extraction procedure

- Extraction was carried out by two solvents 99.9% ethanol and methanol weighing approximately 500gm of powdered.
- Dissolve into 1000ml of each solvent. Maceration technique was performed.
- Then collect the filterate.A total of 10 cycles were run by continuous heating and cooling to obtain thick slurry.
- This slurry was evaporated to obtain a solid extract.

The worms

PHERETIMA POSTHUMA the worm is selected due to its anatomical and physiological resemblance with intestinal round worm parasite of human being.

Length : 4 – 5 cm

Experimental design

GROUPS	DESCRIPTION	TREATMENT	NO. OF. SPECIES
I	CONTROL	Normal saline	5
II	STANDARD	10 mg/ml albendzole (500mg)	5
III	TEST I	Methanolic l.extract (10,20,30,50mg/ml)	Each contains 5 species
IV	TEST II	Ethanolic l.extract (10,20,30,50mg/ml)	Each contain 5 species

Figure 6

Phytochemical screening

Qualitative Analysis For The Presence Of Plant Phytoconstituents Such As Carbohydrates, Flavonoids, Alkaloids, Tannins, Saponins, Phenols were carried out on the standard procedure.

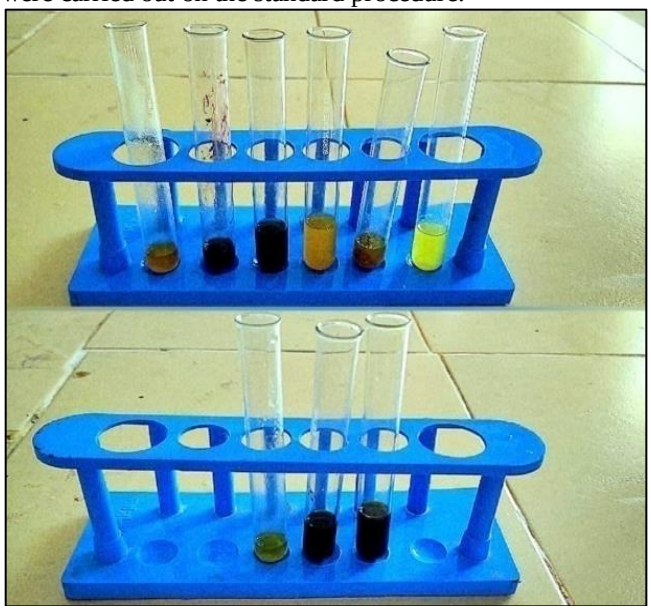


Figure 7

***INSILICO* ANALYSIS**

Swiss ADME:

Swiss ADME is a web tool used to determine the pharmacokinetic properties like absorption, distribution, metabolism and excretion. Two important chemical descriptions correlate with PK properties, the 2D polar surfaces and the lipophilicity levels in the form of atoms based LogP. Smiles notation of a ligand was imported in a workspace; the results were obtained and analyzed in the project table.

Molecular docking procedure

I. **Ligand preparation** - The selected ligand was downloaded from pubChem Database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format.
II. **Target preparation** - The target proteins which is responsible for the helminthiasis disease were selected and downloaded fr**o**m Protein Data Bank (https://www.rcsb.org/) in PDB format. The PDB id of the target molecules was 1sa0.
III. **Molecular import and preparation**
IV. **Docking**
V. **Analysis**

Target protein

Tubulin colchicine – stathmin like domain complex (Responsible for the helminthiasis disease)

Target ligand

- L- tryptophan(major phytoconstitutents present in *Ananas comosus* leaves selected as the study ligand.)

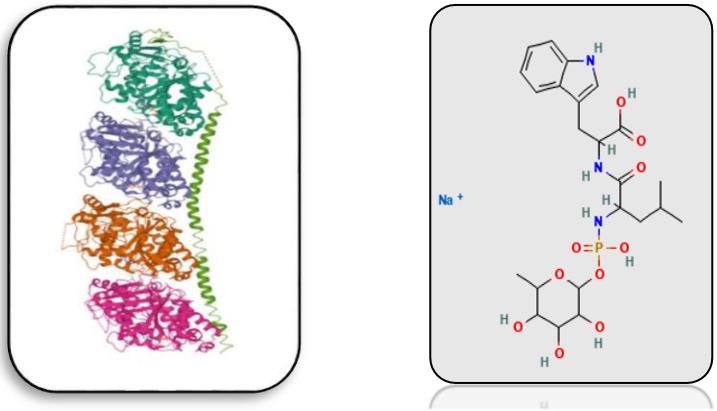


Figure 8

RESULT AND DISCUSSION:

Phytochemical screening

Phytoconstitutent	Methanol extract	Ethanol extract
Alkaloids	+	+
Flavanoids	+	+
Carbohydrates	+	+
Saponins	+	_
Tannins	+	_
Phenols	+	+

+ indicates presence

--indicates absence

TABLE 1

Anti-Helmintic Activity results of *Ananas comosus* Methanolic L.Extracts:

Extract	Concentration mg/ml	Pheretime posthum	
		Time of paralysis in min(MEAN &SEM)	Time of death in min (MEAN&SEM
Control	--	---	-
Methanol	10	27±0.509	68±0.447
	20	24±0.316	62±0.374
	30	18.4±0.4	55±0.509
	50	15±0.509	50.2±0.374
Ethanol	10	35±0.316	73±0.374
	20	30±0.374	67±0.4
	30	27±0.305	61±0.374
	50	22±0.509	56.4±0.4
Standard	10	18±0.374	55±0.312

Table-2

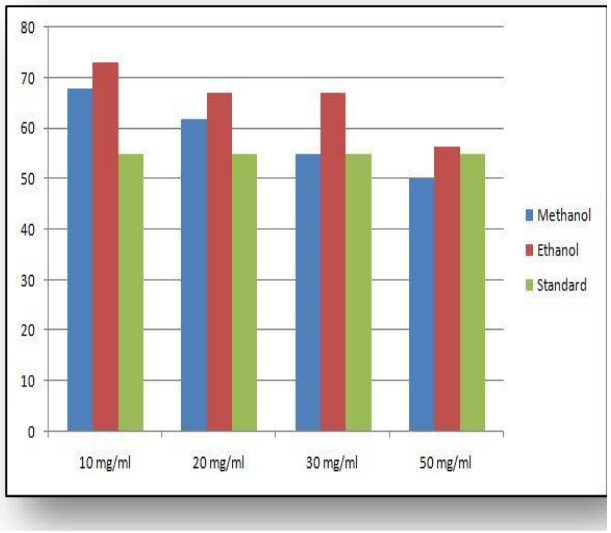


Figure 9

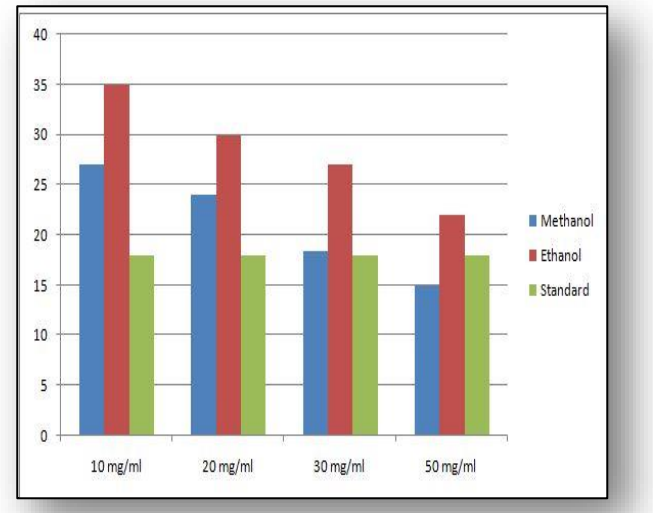


Figure 10

Physiochemical and drug likeness properties of L-tryptophan.

PROPERTY	RESULT (SWISS ADME PROFILE
Molecular formula	$C_{11}H_{12}N_{20}$
Molecular weight	204.223g/mol
Hydrogen bond donors	3
Hydrogen bond acceptors	3
Rotatable bond	3
Log p	1.12
Log s	-0.68
Bio availability score	0.55

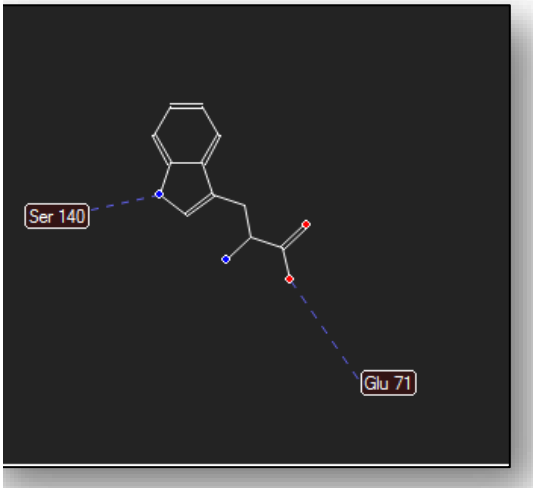
TABLE 3

Molecular Docking Score (kcal/mol) of L-tryptophan with standard drug(Albendazole) against the target.

L-TRYPTOPHAN/ STANDARD	LIGAND (PDBID)	MOL DOCKING SCORE	RERANK SCORE	H BOND
L- trypton	6305	-97.124	-77.8189	-7.82094
Standard (albendazole)	2082	-114.014	-93.0896	-7.79629

TABLE 4

Two, three-dimensional view of molecular interaction of natural L-tryptophan with the target tubulin colchicine stathmin - like domain complex



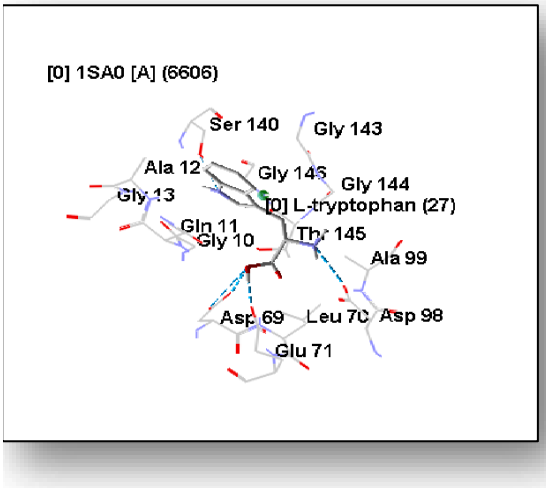
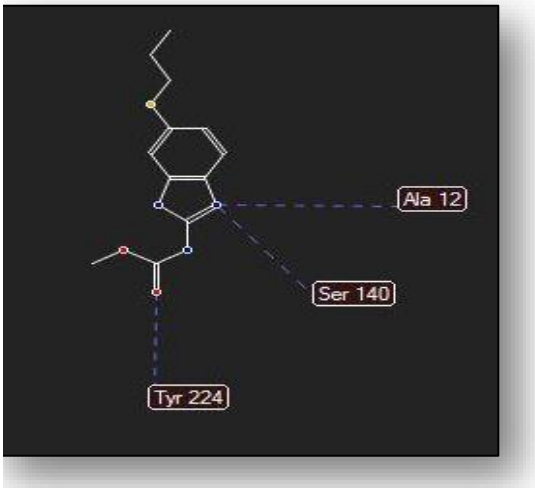


Figure 11 **figure 12**

Two ,three dimensional view of molecular interaction of standard (Albendazole) with the target tubulin colchicines stathmin-like domain complex



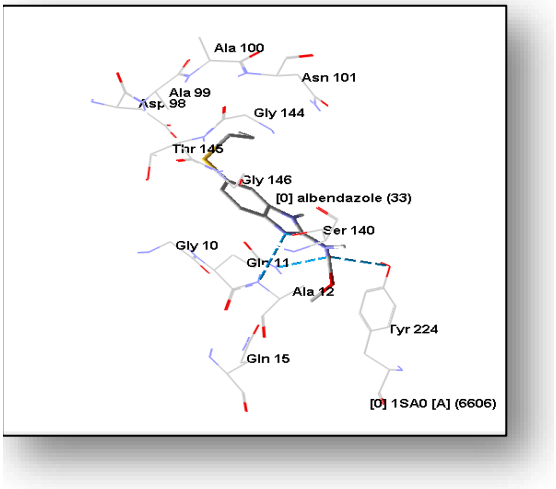


Figure 13 **figure 14**

CONCLUSION:

- All the four concentrations of *Ananas comosus L.*extracts showed significant dose dependent *A*ntiHelmintic property at 10,20,30 and 50 mg/ml concentrations.
- Results clearly indicated that 50 mg/ml concentration of the methanol extract has the highest potency as AntiHelmintic. Ethanol extract falls next to methanol in potency when compared to standard drug Albendazole.
- *Insilico* studies have revealed L-tryptophan as the GOOD binding energy score with the target protein 1sa0. They have better binding energy and also interact with active site residues.

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