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In-silico Docking Studies Of Substituted Benzotriazole As A Protease Inhibitor For SARS CoV.

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ABSTRACT

The aim of this study is to identify a potential antiviral protease inhibitor for Sars CoV using *In-silico* docking studies with the Molegro Virtual Docker (MVD). The structures of benzotriazole esters were drawn using the Chem sketch software and protein PDB ID: 1uk4 was obtained from the protein data bank. Various electron withdrawing and donating groups were substituted in the active position of benzotriazole esters and docked against Sars CoV 3Cl pro. Automatic and manual methods were selected and Flexible docking was performed and results were observed. It was found that, among the docked ligands compound 38 (best with automatic docking method) and compound 27 (best with manual docking method) was said to possess the highest ranking score. The selected lead compounds were compared with the standard drug Maraviroc with parameters such as docking score and binding capacity with the target. The lead compounds were found to possess similar binding properties as that of the standard drug Maraviroc.

Keywords: Sars-CoV, 3Cl pro, Molecular docking, Benzotriazole esters, Molegro virtual docker.

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INTRODUCTION

Severe Acute Respiratory Syndrome (SARS) is a coronavirus, identified in 2003 as an etiological agent that causes severe and acute respiratory diseases and is potentially fatal. After a few months, the World Health Organization (WHO) reported increased cases and fatality around the world. Usually, coronavirus membrane usually contains three proteins **i) SPIKE PROTEIN (S),** a type of Glycoprotein **ii) A MEMBRANE PROTEIN (M),** which spans the membrane; and, **III) AN ENVELOPE PROTEIN (E)** that is highly hydrophobic and covers the entire structure of coronavirus. Cysteine protease (papain-like) and 3Cl Protease (Chymotrypsin-like protease) are the two main proteases present in the SARS CoV [1]. The 3ClPro, accountable for replication and the infection process cleave at certain sites such as Leu, Ser, Gln, Ala, and Gly. The 3ClPro at the active site accommodates Cys145 and His41 which are accountable for natural amide synthesis and results in replication of the virus [2, 3]. For this reason, 3ClPro becomes an exemplary target for inhibition of the replication and infection process. There is no effectual antiviral drug for the treatment of SARS but combination therapy provides a symptomatic relief. Recent years have witnessed so much research for the discovery of antiviral drugs but the drugs are not effective against SARS. Certain 3ClPro inhibitors such as HIV protease inhibitor Tl-3, a thiophenecarboxylate, zinc conjugates [4] and anilides [5] were discovered but effective inhibition in the nano range against SARS was not reported. Certain stable benzotriazole esters derivatives were also discovered is late years. [6]

Maraviroc, an antiretroviral drug which is used in combinations for HIV infection also acts as an effective protease inhibitor [7]. The triazole nucleus present in the Maraviroc is chosen as a basic tool for the designing of an effective protease inhibitor. Benzotriazole, a fused aromatic benzene ring with a triazole is been focused in recent years. Certain benzotriazole and their ester derivatives possess antiviral, anti-inflammatory, antibacterial, anti-fungal, anti-tubercular properties [8]. Amongst them, benzotriazole esters show better efficacy than other derivatives. More and more benzotriazole esters have been discovered but in later years some benzotriazole esters with good bioavailability, better therapeutic effect, and minimal multiple drug resistance were identified [9] which prompted the researchers to pay a remarkable attention to this area. Docking is a preliminary step in which ability of the ligand (drug) to bind to the target can be studied computationally using modeling and docking software. The binding ability of the ligand (drug) to the active site are analyzed using certain parameters such as, docking score, Rerank score, H-bond score, electrostatic and hydrophobic interactions and other such features based upon needs and types of software used.

The aim and objective of the present study are to design a benzotriazole ester and to carry out *In-silico* docking studies against 3ClPro (PDB ID: 1uk4) by automatic and manual docking method with the manipulation of Molegro virtual docker (MVD) and compare the docking score and binding pattern of the benzotriazole ester with that of the standard drug Maraviroc. In automatic docking, cavity for binding is automatically generated by the MVD algorithm, the largest cavity is preferred in automatic docking. In manual docking, the active site of the protein is predetermined using the search space algorithm and the ligands are directed towards them for binding.

MATERIALS AND METHODS

Molecular Docking Studies:

Designing of the Benzotriazole ester:

The benzotriazole ester was designed using an evaluation version of the **CHEM SKETCH** software which was obtained from the CHEMAXON website [10]. To determine the best benzotriazole ester, various electron withdrawing and electron donating groups such as CH3, OH, CN, OCH3, SH, NO2, NH2, were substituted at the active positions R1 and R2 of the benzotriazole ring and several benzotriazole ester were designed. The structures were drawn and bond order and bond length were fixed using the auto corrector function. The structure was converted from 2D to 3D format and saved in a .mol format which is suitable for work with MVD.

Preparation of Ligands:

The designed benzotriazole esters were imported in 3D .mol format to the workspace in the Molegro virtual docker. Missing charges, bond order, and flexibility were corrected using the Ligand preparation wizard and the Bond length, atom type, molecular weight, bond angle were observed in the deck of the MVD.

Preparation of Protein (1uk4):

For this study, SARS CoV 3Clpro was chosen as the target protein. The protein 1uk4 [11] was downloaded into the workspace from the Protein Data Bank (PDB) [12] in .pdb format. Sometime the protein structure may miss some of the charges and hydrogens. Such errors are rectified using the protein preparation wizard in the MVD. The 1uk4 contains 2 chains, Chain A & B and 627 residues and 4788 atoms.

Molegro virtual docker's scoring function and algorithms:

Docking was performed in Molegro Virtual Docker [13] using the Differential evolution (MolDock optimizer) algorithm [14] merged with a cavity detection algorithm where individuals in docking are exposed to a selection process in which the poor solutions are neglected by the function of the algorithm.

Docking process:

Both manual docking and automatic docking were performed with the selected algorithm. In automatic docking, cavity detection function with expanded van der Waals force was used to determine the cavity. The cavity was defined in the X.Y and Z directions and the largest cavity was chosen for automatic docking.

In manual docking, the predetermined active site of the target protein (the Cys145 and His41) [15] was detected using the protein preparation wizard. The search space functions were used to find a cavity in the active site. The cavity closest to the Cys145 and His41 was chosen for docking.

Both the methods were performed using the MolDock Score [GRID] function with 0.30 A^o Grid resolution [16].Displaceable water molecules were added with an entropy reward of 0.50 [17]. The docking was followed by the search algorithm and parameter settings in the docking wizard.

MolDock optimizer:

In MVD, the differential evolution algorithm has a default parameter setting with Max iterations = 2000; scaling factor = 0.50; crossover rate = 0.90; with a population size of 50 which were determined using the hit and miss method. The MolDock optimizer algorithm was chosen with a max runs of 10 and energy was minimized after docking with the optimization of H-bonds [18].Offspring scheme 1 with Variance-based termination scheme was chosen along with the default parameter settings for docking.

Parameters for the Docking Scoring function:
MolDock score:

MolDock score is the measuring value of the protein-ligand interaction which is calculated from Steric (by PLP), steric (by LJ12-6), hydrogen bonds, hydrogen bonds no directionality, electrostatic (short range), electrostatic (long range), E-soft constrain penalty, E-penal, E-intra (elec), E-intra (vdw), E-intra (steric), E-intra hbond, E-intra sp2-sp2, E-intra(tors), E-intra(tors, ligand atom), E-displaced water, E-inter(water-ligand), co-factor(elec), co-factor(hbond), co-factor(vdw), E-inter (cofactor-ligand), electro and electro long. The scoring function used is a simplified Linear Potential whose parameters were fit to that of the binding data's and protein-ligand interaction [19]. Considering these factors, the ligand with high ranked MolDock score was picked out as the best one.

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Rerank score:

Predicting the experimental binding affinity of a protein-ligand complex based on the unchanged conformation of the ligand is a difficult task but the rerank score indicates ability of the ligand to bind with the protein in terms of strength. Rerank score is more significant than MolDock score, however it provides the best among same ligand of different poses which may provide a valuable information is the QSAR Studies. Considering the measure of binding affinity, we could arrive at a rough estimation about high ranked poses.

DruLiTo:

A molecule can be considered a drug if it obey the Drug Likeness rules such as the Lipinski rule of 5. DruLiTo is a public collaborated and a free software which can predict drug-likeness rules. DruLiTo uses a java library and chemistry development kit (CDK) for descriptor calculation [20].

RESULTS AND DISCUSSION

Benzotriazole ester was substituted with various electron withdrawing and electron donating groups in active positions R1 and R2 **(Figure 1)** of the benzotriazole nucleus which yielded almost 97 benzotriazole ester compounds. All the compound were docked against SARS CoV 3ClPro.

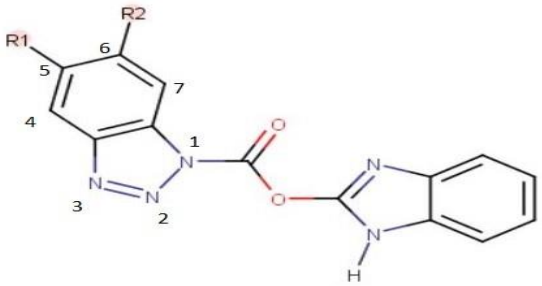

Figure 1: Designed benzotriazole ester with active position R1 and R2

Among the substituted groups the electron withdrawing groups scored the high ranked score when compared with electron donating group. Electron withdrawing groups such as NO2 (compound 27) and CN (compound 38) scored high ranks. But when the substitution of electron withdrawing group occurred at 4th and 7th positions, the docking score was greater than scores of groups substituted at the 5th and 6th positions.

MolDock score and rerank score were used to analyze the binding ability of benzotriazole with SARS CoV 3ClPro. The *in-silico* docking studies of benzotriazole against SARS CoV 3ClPro (**PDB ID: 1uk4**) based on automatic docking is represented in **Table 1(**Amongst the 97 docked compounds the best ten compounds are enumerated in the table**)** and **Table 2** represents manual docking.

Table 1: Molecular docking analysis of Benzotriazole esters on SARS CoV 3ClPro (PDB ID: 1UK4) –Automatic docking

S.No	Name	Ligand	MolDock Score	Rerank Score	H Bond
1.	[01] compound 38	R_1= CN ; R_2= CN	-169.874	-124.103	-7.52139
2.	[01] compound 92	R_1= CN ; R_2= OCH_3	-160.725	-135.052	-5.89209
3.	[00] compound 93	R_1= OCH_3 ; R_2= CN	-159.441	-125.33	-8.63945
4.	[00] compound 26	R_1= H ; R_2= NO_2	-154.436	-130.524	-16.3219
5.	[00] compound 32	R_1= OH ; R_2= NO_2	-151.87	-121.317	-8.07106
6.	[01] compound 48	R_1= CN ; R_2= NO_2	-150.332	-114.493	-8.97718
7.	[00] compound 91	R_1= OCH_3 ; R_2= NO_2	-140.671	-118.364	-12.7867
8.	[00] compound 28	R_1= SH ; R_2= NO_2	-140.519	-117.565	-8.71786
9.	[01] compound 27	R_1= NO_2 ; R_2= NO_2	-139.349	-74.0114	-13.3476
10.	[00] compound 90	R_1= NO_2 ; R_2= OCH_3	-127.349	-96.5366	-10.1589

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Table 2: Molecular docking analysis of Benzotriazole esters on SARS CoV 3ClPro (PDB ID: 1UK4) –Manual docking

S.No	Name	Ligand	MolDock Score	Rerank Score	H Bond
1.	[01] compound 27	R_1= NO_2 ; R_2= NO_2	-136.723	-101..214	-14.6235
2.	[01] compound 91	R_1= OCH_2 ;R_2= NO_2	-125.572	-92.409	-8.5144
3.	[00] compound 29	R_1= NO_2 ; R_2= SH	-122.756	-105.75	-6.83413
4.	[01] compound 33	R_1= NO_2 ; R_2= OH	-121.568	-94.3002	-10.9094
5.	[00] compound 60	R_1= NO_2 ; R_2= Br	-119.668	-88.2714	-8.29361
6.	[00] compound 74	R_1=NO_2 ; R_2= Cl	-117.619	-80.0808	-11.7153
7.	[01] compound 47	R_1= NO_2 ; R_2= CN	-116.638	-83.7241	-8.51899
8.	[00] compound 40	R_1= CN ; R_2= SH	-115.388	-84.1558	-5.8774
9.	[00] compound 48	R_1= CN ; R_2= NO_2	-112.47	-75.2986	-11.2698
10.	[00] compound 90	R_1= NO_2 ;R_2= OCH_3	-111.602	-86.7974	-8.06625

In automatic docking, compound 38 possesses high ranked score and it was found to bind with **Lys5 (A), Arg4 (A) and GLN127 (A)** residues of the target **Figure 2**.

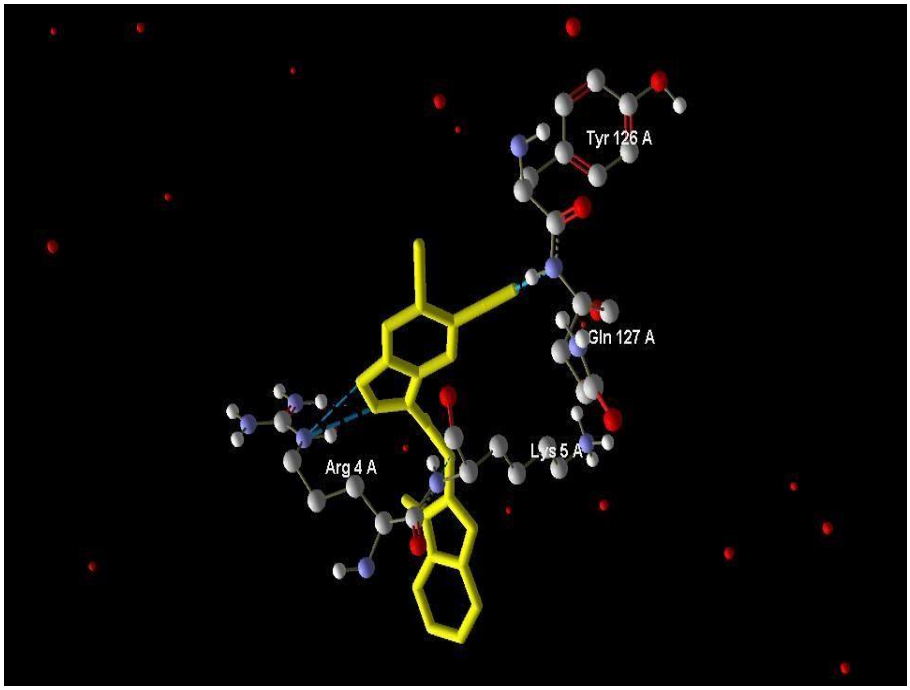

Figure 2: Automatic docking (Compound 38)

In manual docking, compound 27 possesses high ranked score and was found to bind with **Gly143 (A), Cys145 (A), Ser144 (A), Glu166 (A), HOH (51)** and **Gln189 (A) Figure 3**.

Drug-likeness of the compound was tested with the DruLiTo software and the results are reported in **Table 3.** Both compound 38 and compound 27 has passed the Lipinski rule of 5.

Table 3: Drug Likeness properties of the benzotriazole esters

S.No	Compound name	Molecular Weight	HBA	HBD	LogP
1	Compound 38	329.07	9	1	0.828
2	Compound 27	369.05	7	1	1.98

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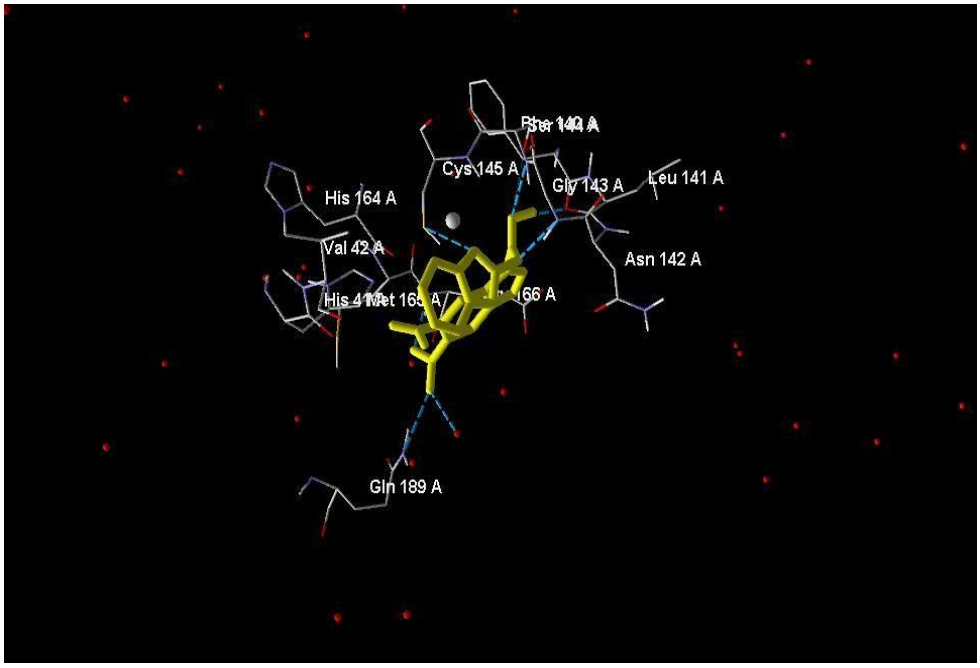
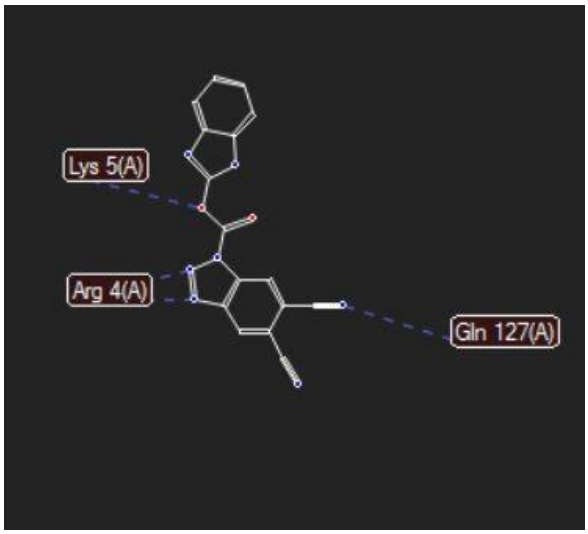


Figure 3: Manual docking (compound 27)



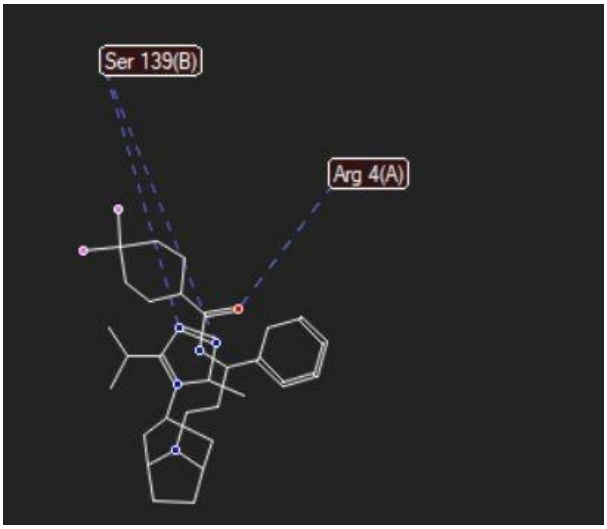


Figure 4 (a): Automatic docking (comp 38) **Figure 5 (b): Automatic docking (Maraviroc)**

Comparing the docking patterns of compound 38 and the standard drug Maraviroc, it was found that compound 38 bound to the same residue **Arg 4(A)** as Maraviroc.**(Figure 4 (a) (b))**

Similarly, docking pattern of compound 27 was compared with the standard drug Maraviroc and it was found that compound 27 bound to same residue **CYS 145(A) and Gln 189(A)** as Maraviroc. **(Figure 5 (a) (b))**

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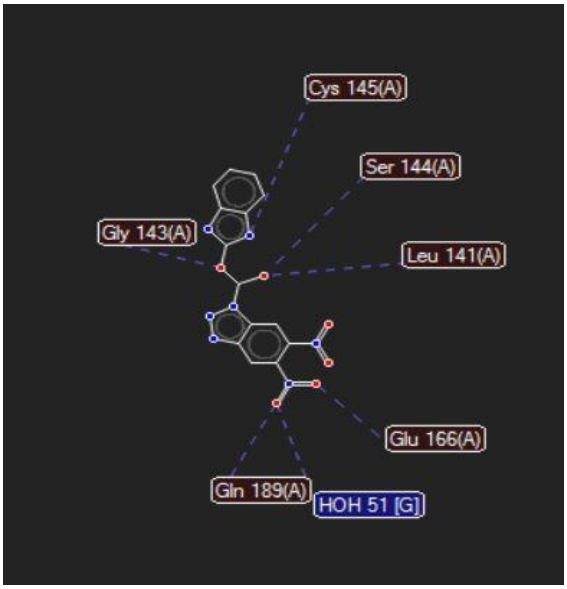

Figure 6 (a): Manual docking (comp 27)

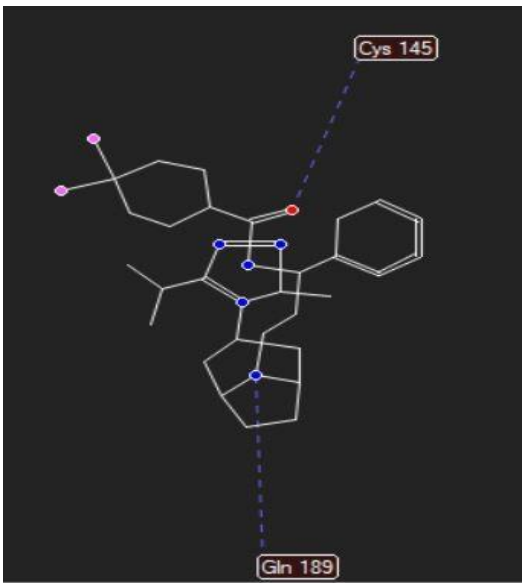

Figure 7 (b): Manual docking (Maraviroc)

CONCLUSION

From the *in-silico* docking study of the benzotriazole esters, it is evident that compound 38 and compound 27 possess nearly similar binding properties as the standard drug Maraviroc. It can be further synthesized to study the in-vitro and in-vivo antiviral activity against SARS CoV 3ClPro.

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CONFLICTS OF INTEREST: There are no conflicts of interest.

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