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Research Article

INSILICO EVALUATION OF 6-ARYL-3-(3-HYDROXYPROPYL)-7*H*-1,2,4-TRIAZOLO[3,4-*B*][1,3,4]THIADIAZINES DERIVATIVES AND 4-BENZYLIDENE-AMINO-4,5 DIHYDRO- 2H 1,2,4 TRIAZOLE-5 ONE AS ANTIFUNGAL ACTIVITY ON *FUSARIUM SOLANI*

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ABSTRACT

In silico methods help in identifying drug targets via Chemoinformatic tools. Chemoinformatics is the mixing of those information resources to transform data into information and information into knowledge for the intended purpose of making better decisions faster in the area of drug lead identification and optimization. In the present work Virtual Screening of 6-Aryl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo [3,4-b] [1,3,4] thiadiazines and 4-benzylidene-amino-4,5 dihydro- 2h 1,2,4 triazole-5 one derivatives has been performed in order to identify those structures that most likely to bind to a drug target, typically a protein receptor or enzyme. Studies have been performed for a series of substituted derivatives by correlating electronic, steric and lipophobic properties of the substituents against the biological activity of *Fusarium solani.* The work has been performed in silico using NCBI {Database}, Cactus server for protein format conversion {Database}, Swiss model {Server}, Molegro Virtual Docker {Software} to predict the activity of compounds from their structures. The results obtained demonstrate that derivatives with halo group at ortho position and alkyl substituents are effective antifungal agents against *Fusarium solani* and also para substituted halo group decreases the potency of derivatives. It shows that heterocycles having lipophobic property are pharmacologically potential against *Fusarium solani*.

Keywords: Virtual screening, 5-(3 Chloro-1-benzothien-2 yl)-4-phenyl-4H—1,2,4 triazole-3-thiol, 4-benzylidene-amino-4,5 dihydro- 2h 1,2,4 triazole-5 one, *insilico.*

INTRODUCTION

Virtual screening (VS) is a computational technique used in pharmaceutical companies in the process of drug discovery [Baldi, 2005]. The purpose of virtual screening is to come up with hits of novel chemical structure that bind to the macromolecular target of interest. Several five membered heterocyclic compound for instance pyrroles, imidazole, oxazole, itraconazol have been shown to be promising antimicrobial agents [Gallardo *et al.,* 2007]. Moreover, the triazoles have attracted widespread attention due to their diverse applications as antibacterial, antimycotic, antifungal and antidepressant agents.

Triazole is advantageous due to its broad range of application in the treatment of both superficial and systemic fungal infections and it also shows greater affinity for fungal rather than mammalian Cytochrome P-450 enzymes for ex. Fluconazole for treatment of Histoplasmosis. [Sheehan *et al.,* 1999]. The commonly used triazole antifungal drugs work by inhibition of the fungal Cytochrome P450

14- α demethylase. This interrupts the conversion of lanosterol to ergosterol, a component of the fungal cell membrane. The fungal infections still remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents. Immunocompromised patients are particularly at risk of developing these infections, with *Aspergillus* sp. that are resistant to antifungal agents, making treatment options a concern. [Nickie and Pharm , 2003]. Nitrogen containing heterocycles are frequently found in privileged structures (pharmacophores) but their incorporation sometimes possess special problems (multistep sequences, lack of generality, preparation from acyclic precursors, etc). Consequently, the design and development of procedures for the generation of new heterocycles receives growing interest.[Azizian *et al*., 2006]

The cell wall of pathogens containing mannoproteins, chitins, and α and β-glucans play an important role in protection, cell morphology, cell rigidity, metabolism, ion exchange, primary interaction with the host and resistance to host cell-mediated immune function.[Munoz *et al.*, 2006]. Thus, novel targets have been explored in an attempt to overcome the problems derived from the exploitation of traditional targets. The antimicrobial identification using experimental techniques is invariably very expensive, requires extensive pains and labour. Therefore, *in silico* techniques, which have the power to cut down these unavoidable steps, would be valuable [Schneider and Fechner, 2005].

In the current study attempts have been made to do the *in silico* analysis of different derivatives of 6-aryl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo [3,4-b] [1,3,4] thiadiazines and 4-benzylidene-amino-4,5 dihydro- 2h 1,2,4 triazole-51,2,4-triazole to locate the novel drug target in *Fusarium solani*.

MATERIALS AND METHODS

Materials

Databases, softwares & online servers used during the study are as follows:

- PDB {http://www.pdb.org}
- NCBI {Database: www.ncbi.nlm.nih.gov}
- Cactus server for protein format conversion {Database}
- Swiss model {Server :- http:/swissmodel.expasy.org}
- Molegro Virtual Docker {Software}
- Pharma algorithm {Database}

Methodology

- Sequence retrieval
- Homology modeling
- Generation of ligand library
- Virtual screening of the ligand library for minimum energy calculation
- In silico adme/tox analysis of drug like molecules

RESULTS AND DISCUSSION

1. Sequence Retrieval

Retrieval of amino acid sequence of Taxane 13-α hydroxylase in *Fusarium solani* is in FASTA format which was as follows:

- **Taxane 13-α hydroxylase *[Fusarium solani]***

EESIGIVRAALSRFLGPQALQNHFAKMSSGI
QRHINEKWKGKDEVTVLPLVKDLVFSVASRL
FFGITEEHLQEQLHNLLEVILVGSFSVPLNIPG
FSYHKAMQARATLADIMTSLIEKRRNELRAG
TASENQDLLSVLLTFTDERGNSLADKEILDNF
SMLLHGSYDSTNSPLPMLIKVRASNPETI

Homology Modeling

Homology based modeling of enzymes was done by Swiss Model server [Arnold et al., 2006] and the structural homologue, which was used as a template for this model, is Taxane 13-α hydroxylase from *Fusarium solani*. Ribbon model and active sites are represented in fig 1 and fig 2.

Generation of library of triazoles

Library is generated in mol 2 format. 3D structure and properties of the derivatives were tabulated in figure 3 and fig 4:

Attachment of an aliphatic side chain containing a hydroxyl (-OH) to the 3rd position of the fused heterocycle could bring about changes in its solubility. [Jin *et al*., 2007].

4-(Benzylidenamino)4,5-dihydro-1H-1,2,4-triazole-5-one derivative has weak acidic properties. There is one weak acidic group N-CH_3 and one ether group attached to benzene ring. [Yuksek *et al.*, 2005]

Docking between 6-aryl-3-(3-hydroxy propyl)-7H-1,2,4 triazolo [3,4-b][1,3,4] thiadiazines derivatives with enzyme taxane13-α hydroxylase in *Fusarium solani* has been performed by Molegro virtual docker. The molecule having minimum energy calculation has been selected as the lead compound. Docking score is shown in table1.

Derivative 1c has minimum re-rank score (-79.2564) and 1b has the maximum energy calculation (-77.2243).

The results have been corroborated by the studies of Sherin M. *et al*., according to which the phenyl ring of is freely rotated around the torsional angle of the carbon-nitrogen double

bond providing proper hydrophobic interaction with the surrounding pocket residues.

These ligands performed better lipophylic recognition within the binding pocket due to the embedding of halo-substituted phenyl ring within hydrophobic aromatic rings of the surrounding residues.

The result is supported by Khan *et al*. (2009), Sayed (2006), Hussain *et al. (*2008) according to which, compound having *ortho*-phenyl residue found less resistance and greater activities to pass the cell wall in comparison to its *para* and *ortho* analogs, respectively. QSAR studies governed by " Craig plot " with regard to the various substituents in a new drug molecule. The chloro group essentially enhance both electron withdrawing characteristics and hydrophobicity in the 'drug like molecule' by virtue of their σ +ve and π +ve effects.

Docking score of derivatives of "4-benzylidene-amino-4,5 dihydro- 2H 1,2,4 triazole-5 one" with *F.solani* is given in table 2. Compound 2c has minimum (-85.7839) and 2e has maximum (-63.3927) energy calculation. This result is supported by Pintilie *et al.*, according to which a triazole compound with a methyl-phenyl moiety on the heterocyclic ring shows inhibitory effect against fungus. This could be explained by electropositive effect of methyl group attached to the phenyl moiety because of the known favorable influence of electron donating groups on the potency of the heterocyclic nuclei.

Thus the interaction between drug molecules and bio-molecules that is amino acid residue in enzyme have been represented with the help of pictures capture by MVD in fig 5 and fig 6.

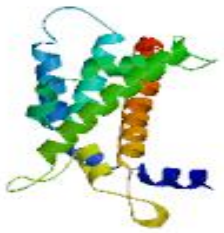


Fig. 1: Secondary structure of enzyme taxane13-α hydroxylase in *Fusarium solani*

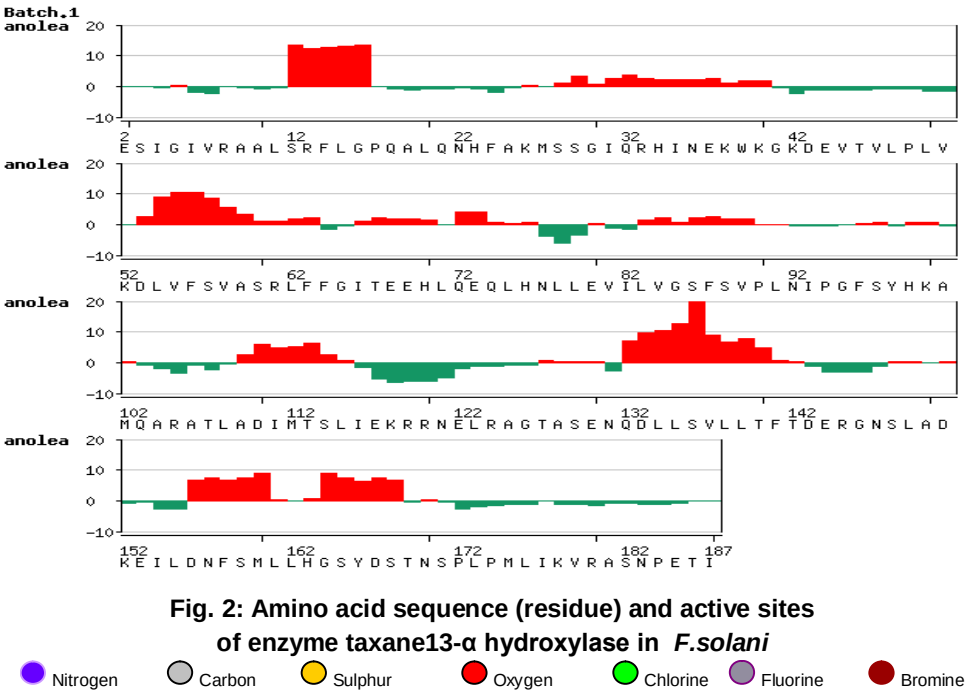
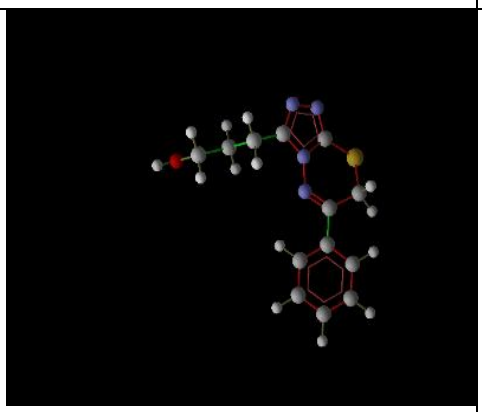
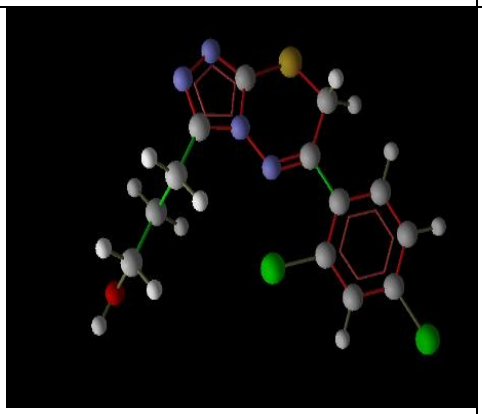
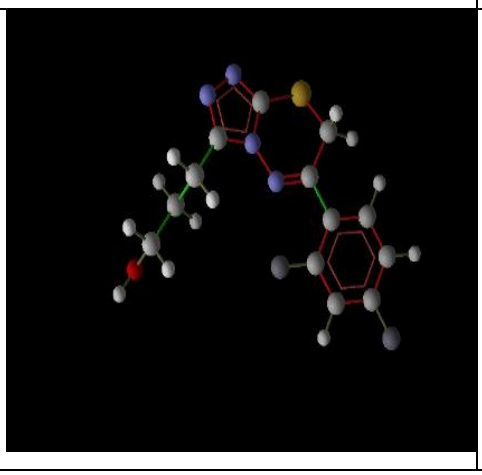


Fig. 2: Amino acid sequence (residue) and active sites of enzyme taxane13-α hydroxylase in *F.solani*

S. No.	3 D Structure	Properties
1a.		Molecular Weight : 274.341 Molecular Formula: $C_{13}H_{14}N_4OS$ Smile: OCCCc2nnc3SCC (c1ccccc1)=Nn23 IUPAC Name: 6-phenyl-3-(3- hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines Heavy atoms: 19 Torsion : 4
1b.		Molecular Weight :343.232 Molecular Formula: $C_{13}H_{12}Cl_2N_4OS$ Smile:OCCCc2nnc3SCC (c1ccc(Cl)cc1Cl)=Nn23 IUPACName:6-(2,4-dichloro phenyl) -3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines Heavy atoms: 21 Torsion 4
1c.		Molecular Weight :310.322 Molecular Formula: $C_{13}H_{12}F_2N_4OS$ Smile: OCCCc2nnc3SCC (c1ccc(F)cc1F)=Nn23 IUPAC Name:6-(2,4-difluoro phenyl)- 3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines Heavy atoms: 21 Torsion :4

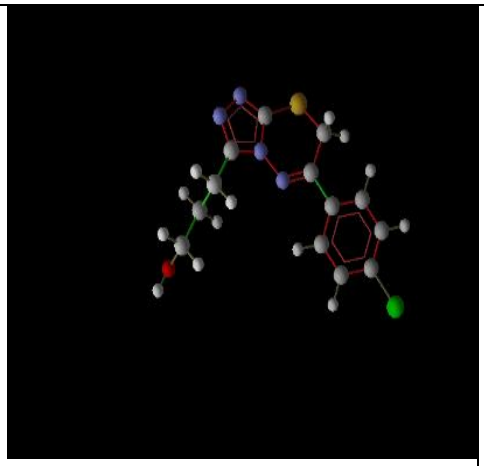
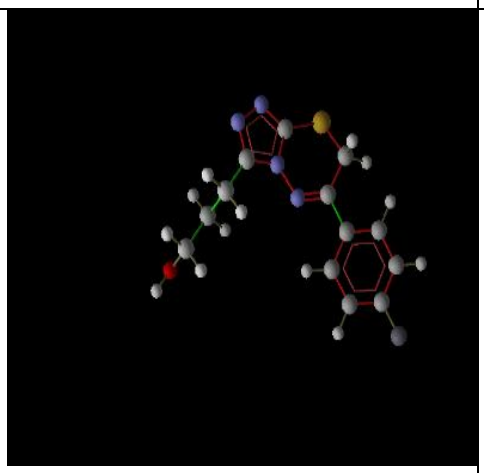
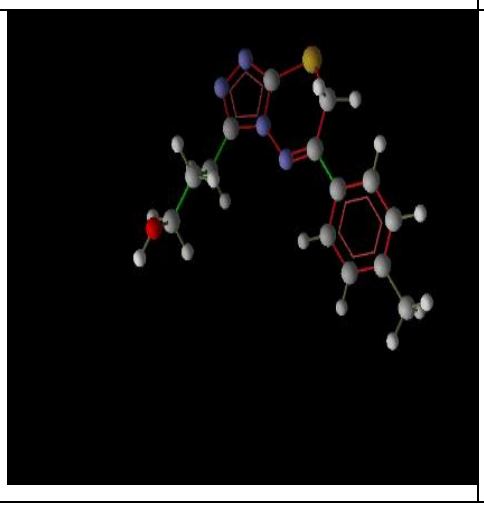
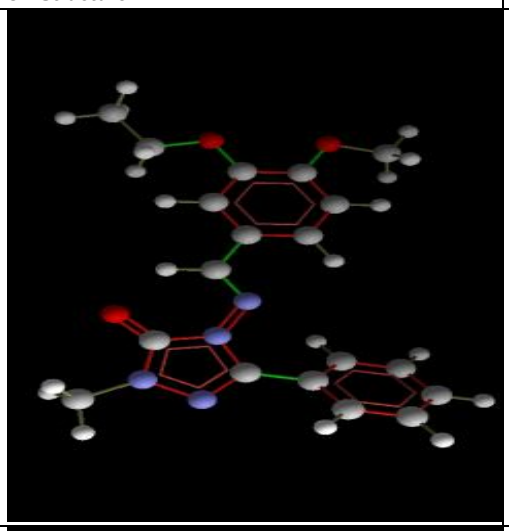
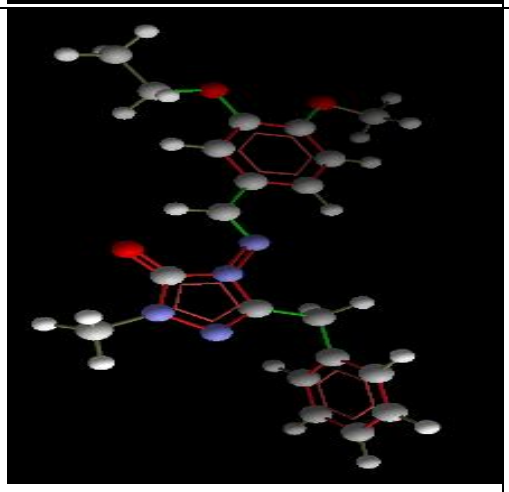
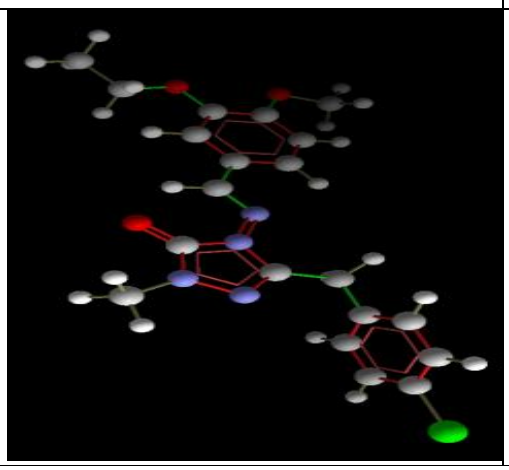
1d.		Molecular Weight :308.787 Molecular Formula: $C_{13}H_{13}ClN_4OS$ Smile: OCCCc2nnc3SCC (c1ccc(Cl)cc1)=Nn23 IUPACName: 6-(4-Chloro phenyl)-3- (3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines Heavy atoms: 20 Torsion :4
1e.		Molecular Weight : 292.332 Molecular Formula: $C_{13}H_{13}FN_4OS$ Smile: OCCCc2nnc3SCC (c1ccc(F)cc1)=Nn23 IUPACName: 6-(4-fluoro phenyl)-3- (3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines Heavy atoms: 20 Torsion :5
1f.		Molecular Weight : 288.368 Molecular Formula: $C_{14}H_{16}N_4OS$ Smile: Cc3ccc(C2=Nn1c(CCCO) nnc1SC2)cc3 IUPACName: 6-(4-methyl phenyl)- 3-(3-hydroxypropyl)-7H-1,2,4- triazolo[3,4-b][1,3,4] thiadiazines Heavy atoms:20 Torsion :4

Fig. 3(a-f): Derivatives of 6-aryl-3-(3-hydroxy propyl)-7H-1,2,4 triazolo[3,4-b][1,3,4] Thiadiazines

S. No.	3D Structure	Properties
2a.		Molecular Weight : 352.387 Molecular Formula: $C_{19}H_{20}N_4O_3$ Smile: CCOc3cc(C=Nn2c(c1ccccc1)nn (C)c2=O)ccc3OC IUPAC Name: 1-methyl-3-phenyl-4-(3-ethoxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazole-5-one Heavy atoms: 26 Torsion 6
2b.		Molecular Weight : 366.414 Molecular Formula: $C_{20}H_{22}N_4O_3$ Smile: CCOc3cc(C=Nn2c(Cc1ccccc1) nn(C)c2=O)ccc3OC IUPAC Name: 1-methyl-3-benzyl-4-(3-ethoxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazole-5-one Heavy atoms: 27 Torsion 7
2c.		Molecular Weight : 400.859 Molecular Formula: $C_{20}H_{21}N_4O_3$ Smile: CCOc3cc(C=Nn2c(Cc1ccc(Cl)cc1) nn(C)c2=O)ccc3OC IUPAC Name:1-methyl-3-chloro benzyl-4-(3-ethoxy-4-methoxybenzyliden-amino)-4,5-dihydro-1H-1,2,4-triazole-5-one Heavy atoms: 28 Torsion 7

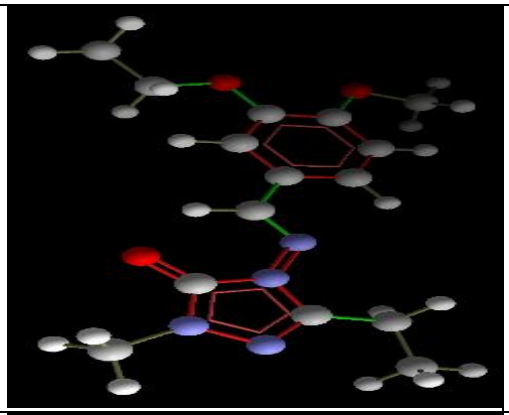
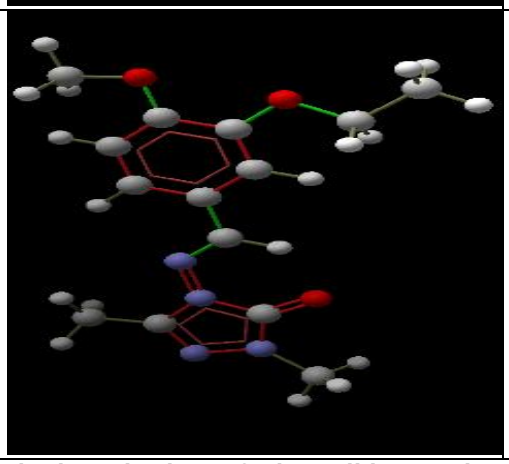
2d.		Molecular Weight : 304.344 Molecular Formula: $C_{15}H_{20}N_4O_3$ Smile: CCOc2cc(C=Nn1c(C)nn(C)c1=O)ccc2OC IUPAC Name: 1-methyl-3-ethyl-4 -(3-ethoxy-4-methoxybenzylidenamino) -4,5-dihydro-1H-1,2,4-triazole-5-one Heavy atoms: 22 Torsion 6
2e.		Molecular Weight : 290.318 Molecular Formula: $C_{14}H_{18}N_4O_3$ Smile: CCOc2cc(C=Nn1c(C)nn (C)c1=O)ccc2OC IUPAC Name: 1-methyl-3-methyl-4- (3-ethoxy-4-methoxybenzylidenamino) -4,5-dihydro-1H-1,2,4-triazole-5-one Heavy atoms: 21 Torsion 5

Fig. 4(a-e): Derivatives of 4-benzylidene-amino-4,5 dihydro- 2H 1,2,4 triazole-5 one

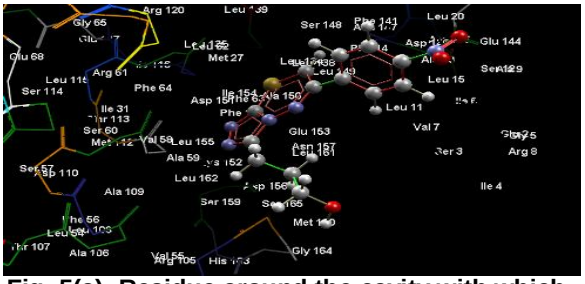


Fig. 5(a): Residue around the cavity with which receptor binds.

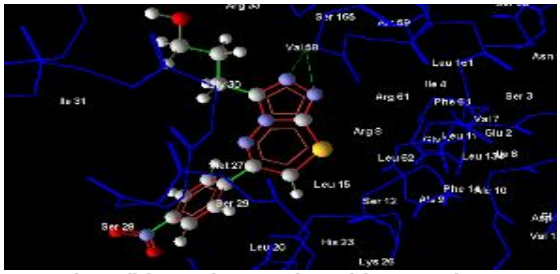


Fig. 5(b): Hydrogen bond interaction between lead compound and Val 68 residue

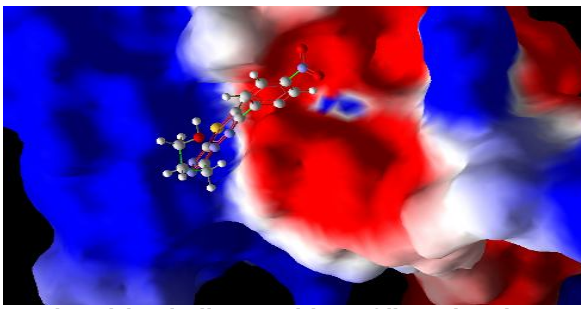


Fig. 5(c): Binding position of ligand at the surface of receptor

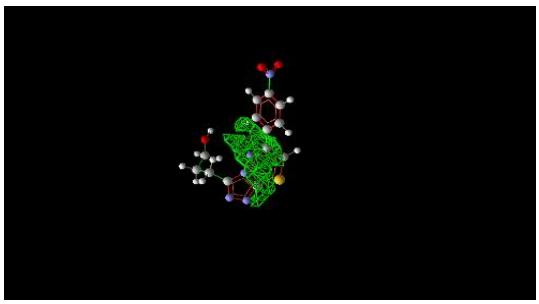


Fig. 5(d) Binding of ligand at the cavity of enzyme

Fig. 5(a-d): Interaction between top scorer derivative "6-(4-nitro phenyl)-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines" with *Fusarium solani*

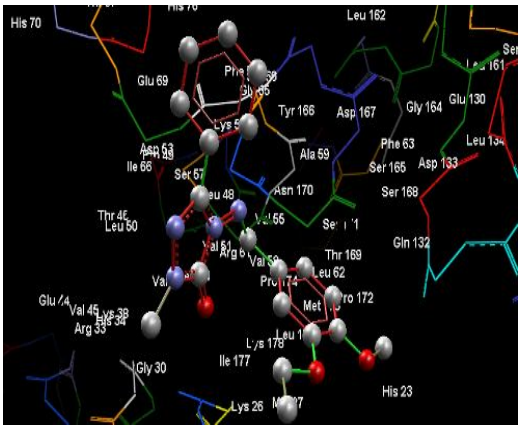


Fig. 6(a): Residue around the cavity with which receptor binds

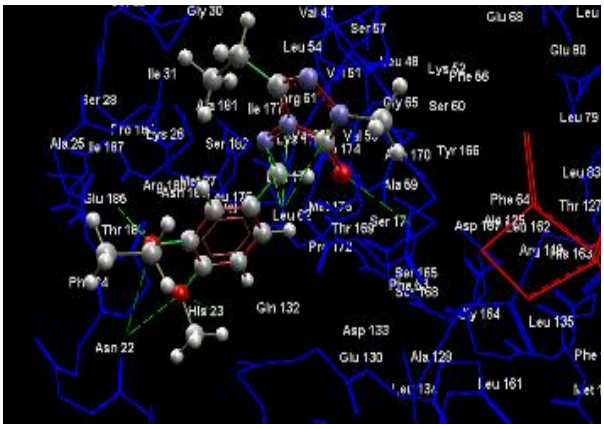


Fig. 6(b): Hydrogen bond interaction between lead compound and Asn22, Leu 62 and Ser17 residues

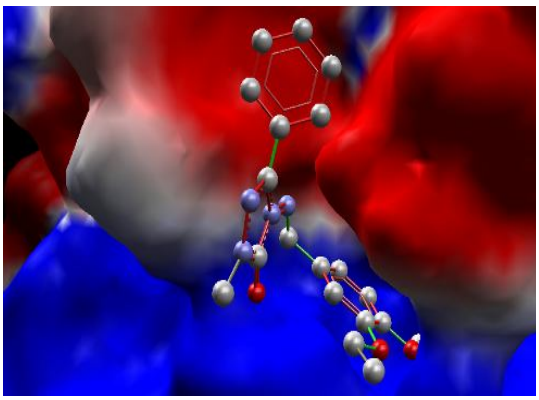


Fig. 6(c): Binding position of ligand at the surface of receptor

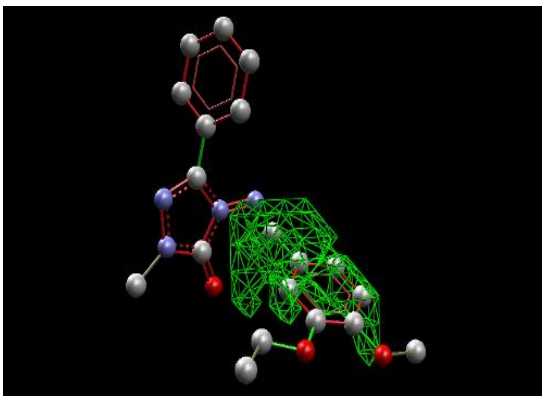


Fig. 6(d): Binding of ligand at the cavity of enzyme

Fig. 6(a-d): Interaction between top scorer derivative "1-methyl-3-phenyl-4-(3-ethoxy-4-methoxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazole-5-one" with *Fusarium solani.*

Table 1: Docking Score of "6-aryl-3-(3-hydroxy propyl)-7H-1,2,4 triazolo [3,4-b][1,3,4] thiadiazines" with enzyme present in *F.solani*

S. No. of derivatives	Molecular Formula	Mol Dock Score	Re-rank Score	H-Bond
3a.	$C_{13}H_{14}N_4OS$	-92.7651	-77.3397	-5.4329
3b.	$C_{13}H_{12}Cl_2N_4OS$	-100.252	-77.2243	-2.28459
3c.	$C_{13}H_{12}F_2N_4OS$	-99.0889	-79.2564	-2.5
3d.	$C_{13}H_{13}ClN_4OS$	-99.8125	-78.7441	-2.5
3e.	$C_{13}H_{13}FN_4OS$	100.031	-78.3679	-2.5
3f.	$C_{14}H_{16}N_4OS$	-99.7080	-78.6896	-2.5

Table 2: Docking score of derivatives of "4-benzylidene-amino-4,5 dihydro- 2H 1,2,4 triazole-5 one" with *F.solani*

S. No. of derivatives	Molecular Formula	Mol Dock Score	Re-rank Score	H-Bond
4a.	$C_{19}H_{20}N_4O_3$	-110.299	-63.6151	-1.84691
4b.	$C_{20}H_{22}N_4O_3$	-110.299	-74.9228	0
4c.	$C_{20}H_{21}N_4O_3$	-103.788	-85.7839	0
4d.	$C_{15}H_{20}N_4O_3$	-107.24	-69.3272	-5.2556
4e.	$C_{14}H_{18}N_4O_3$	-102.032	-63.3927	-5.2503

CONCLUSION

The results obtained demonstrate that halo substitution at ortho position in 6-aryl-3-(3-hydroxypropyl)-7*h*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines derivative and alkyl substituted 4-benzylidene-amino-4,5 dihydro-2h 1,2,4 triazole-5 one derivative is the most effective against *Fusarium solani.*

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