INSILICO DESIGNING OF 6-ARYL-3-(3-HYDROXYPROPYL)-7H-1,2,4-TRIAZOLO[3,4-B][1,3,4]THIADIAZINES DERIVATIVES AND ITS ANTIFUNGAL ACTIVITY ON ASPERGILLUS FLAVUS

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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 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 6-Aryl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines by correlating electronic, steric and lipophobic properties of the substituents against the biological activity of *Aspergillus flavus.*

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 derivative with phenyl and alkyl substituents are effective antifungal agents against *A.flavus.* It shows that heterocycles having lipophobic property are pharmacologically potential against *A.flavus.*

Key words:- Virtual screening, 5-(3 Chloro-1-benzothien-2 yl)-4-phenyl-4H—1,2,4 triazole-3-thiol, insilico

INTRODUCTION

Virtual screening (VS) is a computational technique used in drug discovery research. 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 , antimycobacterial, antimycotic ,antifungal and antidepressant agents. Meanwhile, N-acylated aminoacids are known for their hepatoprotective and antimicrobial effects [Pintilie *et al.*, 2007].

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]

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]. These insilico techniques are used in pharmaceutical companies in the process of drug discovery.

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 to locate the novel drug target in *Aspergillus flavus*.

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 14-α sterol demethylase in *Aspergillus flavus* is in FASTA format which was as follows:

WFPFIGSTISYGMDPYRFFFNCREKYGDIFTFYLLGKKTTVYLGTKGNDFILNGKLROVCAEEVY SPLTTPVFGRHVVYDCNAK

2 HOMOLOGY MODELING

Structure of protein from its sequence with an accuracy is provided by Homology modeling .

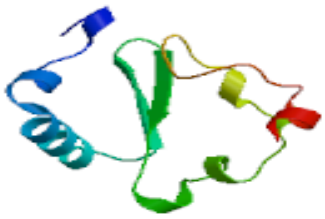


Fig1. Secondary structure of enzyme 14-α sterol demethylase (Cyt P450) in *Aspergillus flavus*

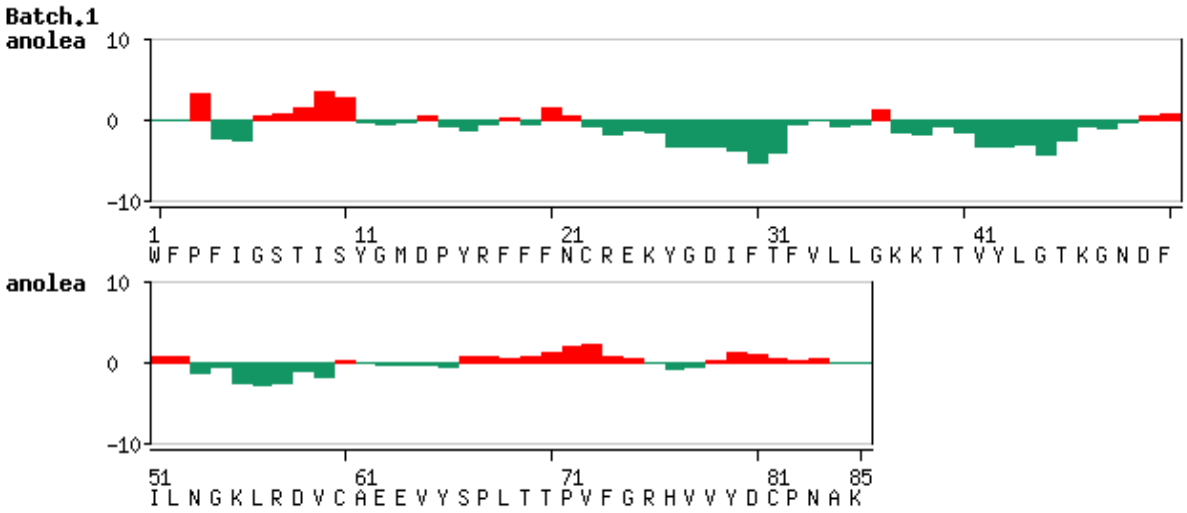
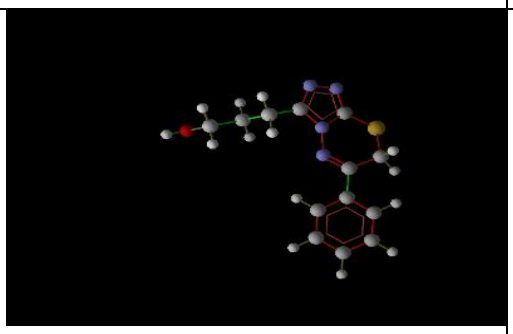
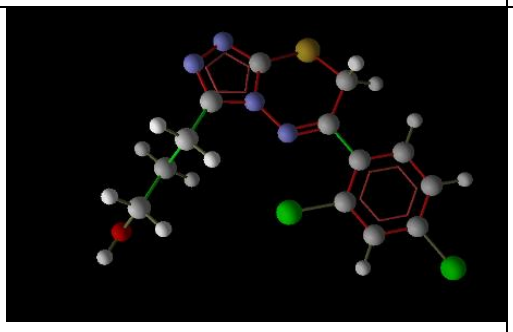


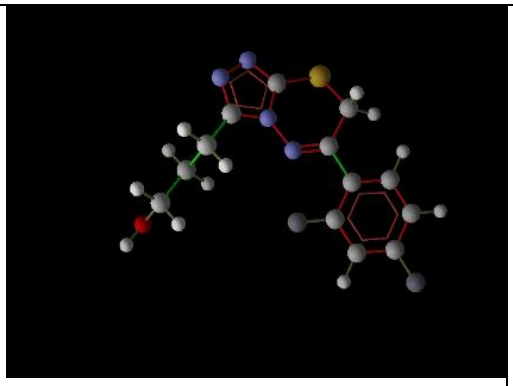
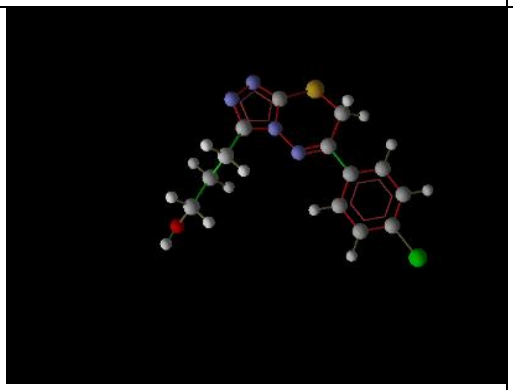
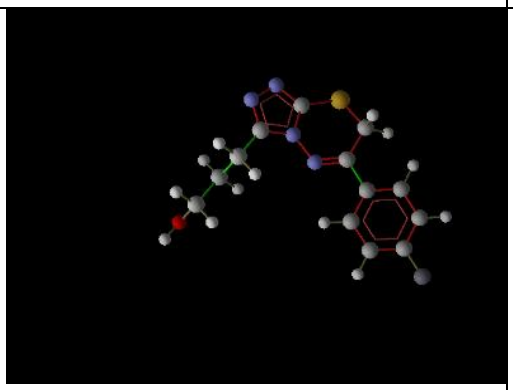
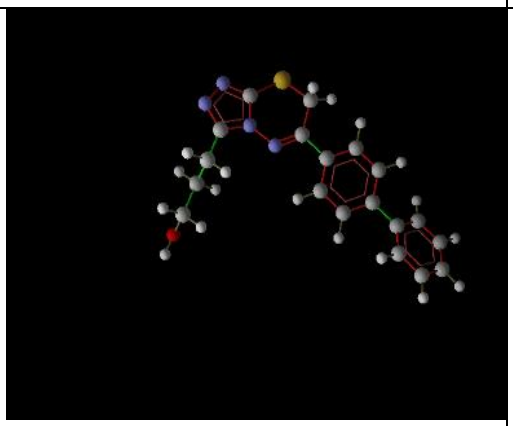
Fig 2. Amino acid sequence (residue) in 14-α sterol demethylase (Cyt P450) in *Aspergillus flavus* .

3. GENERATION OF LIBRARY OF TRIAZOLES

Library is generated in mol 2 format. 3D structure and properties of the derivatives were tabulated as follows:

Nitrogen ***Carbon*** ***Sulphur*** ***Oxygen*** ***Chlorine***

S.No	*3 D Structure*	*Properties*
4.3.1(a)		*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***
4.3.1(b)		*Molecular Weight* ***:343.232*** *Molecular Formula*: ***$C_{13}H_{12}Cl_2N_4OS$*** *Smile*:***OCCCc2nnc3SCC (c1ccc(Cl)cc1Cl)=Nn23*** *IUPAC Name*:***6-(2,4-dichlorophenyl)-3-(3-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines*** *Heavy atoms*: ***21*** *Torsion* ***4***

4.3.1(c)		*Molecular Weight* ***:310.322*** *Molecular Formula*: $C_{13}H_{12:}F_2N_4OS$ **Smile**: OCCCc2nnc3SCC (c1ccc(F)cc1F)=Nn23 *IUPAC Name*:***6-(2,4-difluorophenyl)-3-(3-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines*** *Heavy atoms*: ***21*** *Torsion* ***:4***
4.3.1(d)		*Molecular Weight* ***:308.787*** *Molecular Formula*: $C_{13}H_{13}ClN_4OS$ **Smile**: OCCCc2nnc3SCC (c1ccc(Cl)cc1)=Nn23 *IUPAC Name*: ***6-(4-Chloro phenyl)-3-(3-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines*** *Heavy atoms*: ***20*** *Torsion* ***:4***
4.3.1(e)		*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-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines*** *Heavy atoms*: ***20*** *Torsion* ***:5***
4.3.1(f)		*Molecular Weight* ***: 350.437*** *Molecular Formula*: $C_{19}H_{18}N_4OS$ **Smile**: OCCCc3nnc4SCC (c2ccc(c1ccccc1)cc2)=Nn34 *IUPACName*: ***6-(di phenyl)-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b][1,3,4] thiadiazines*** *Heavy atoms*: ***25*** *Torsion* ***:4***

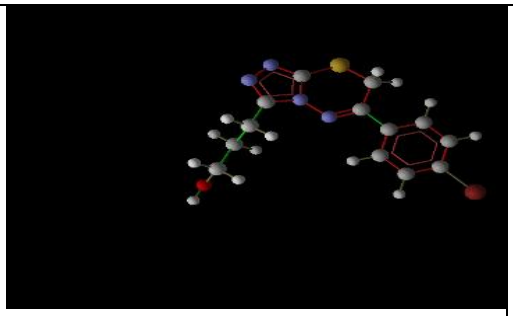
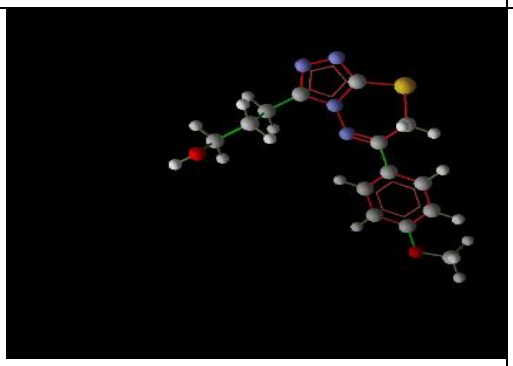
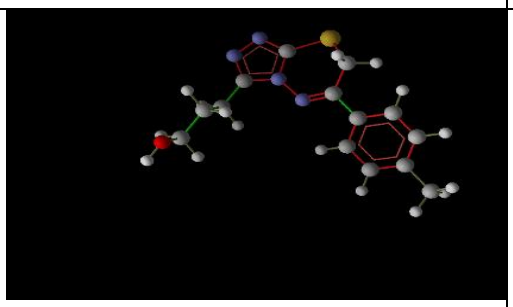
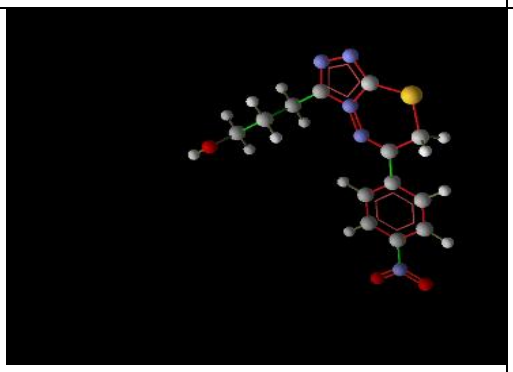
4.3.1(g)		*Molecular Weight* ***: 353.238*** *Molecular Formula*: ***$C_{13}H_{13}BrN_4OS$*** *Smile*: ***OCCCc2nnc3SCC*** ***(c1ccc(Br)cc1)=Nn23*** *IUPACName*: ***6-(4-bromophenyl)-3-(3-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b][1,3,4] thiadiazines*** *Heavy atoms*: ***20*** *Torsion* ***:5***
4.3.1(h)		*Molecular Weight* ***: 304.367*** *Molecular Formula*: ***$C_{14}H_{16}N_4O_2S$*** **Smile**: COc3ccc(C2=Nn1c(CCCO)nnc1SC2)cc3 *IUPAC Name*:***6-(4-methoxyphenyl)-3-(3-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b][1,3,4] thiadiazines*** *Heavy atoms*: ***21*** *Torsion* ***:4***
4.3.1(i)		*Molecular Weight* ***: 288.368*** *Molecular Formula*: ***$C_{14}H_{16:}N_4OS$*** **Smile**: Cc3ccc(C2=Nn1c(CCCO)nnc1SC2)cc3 *IUPAC Name*: ***6-(4-methylphenyl)-3-(3-hydroxy propyl)-7H-1,2,4-triazolo[3,4-b][1,3,4] thiadiazines*** *Heavy atoms*:***20*** *Torsion* ***:4***
4.3.1(j)		*Molecular Weight* ***: 318.331*** *Molecular Formula*: ***$C_{13}H_{13}N_5O_3S$*** **Smile:**OCCCc2nnc3SCC(c1ccc(N(=O)=O)cc1)=Nn23 *IUPAC Name*: ***6-(4-nitro phenyl)-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b][1,3,4] thiadiazines*** *Heavy atoms*:***22*** *Torsion* ***:5***

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

Attachment of an aliphatic side chain containing a hydroxyl (-OH) to the 3rd position of the fused heterocycle could bring about

ch changes in its solubility. [Jin *et al*., 2007]

Table1:- 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 *A.flavus.*

Molecular Formula	Mol Dock Score	Re-rank Score	H-Bond
$C_{13}H_{14}N_4OS$	-78.8099	-63.9395	-2.5
$C_{13}H_{12}Cl_2N_4OS$	-86.3357	-66.8831	-2.5

$C_{13}H_{12}F_2N_4OS$	-82.3121	-62.4552	0
$C_{13}H_{13}ClN_4OS$	-82.5149	-64.0806	0
$C_{13}H_{13}FN_4OS$	-79.5912	-62.7963	-1.54135
$C_{19}H_{18}N_4OS$	-97.372	-73.8211	-4.85841
$C_{13}H_{13}BrN_4OS$	-83.1625	-66.4556	-2.5
$C_{14}H_{16}N_4O_2S$	-81.6519	-55.4034	-2.5
$C_{14}H_{16}N_4OS$	-83.7258	-66.5611	0
$C_{13}H_{13}N_5O_3S$	-86.6892	-56.5321	0

The Mol Dock Score shows that (6-(di phenyl)-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines) has the least energy calculation (-97.372) and 6-phenyl-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b] [1,3,4] thiadiazines has the maximum energy calculation (-78.8099).

The interaction between drug molecules and bio-molecules that is amino acids has been represented with the help of pictures capture by MVD.

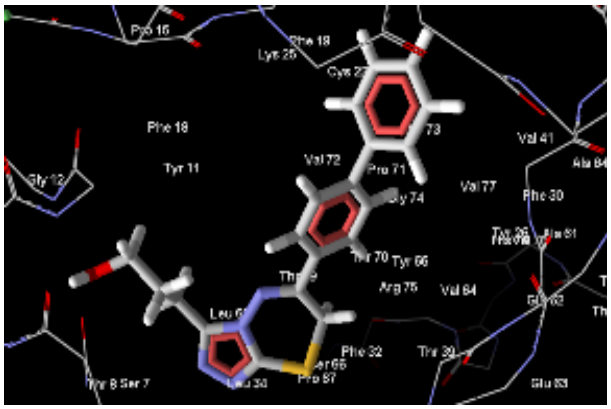


Fig 4.(a) Residue around the cavity with which receptor binds.

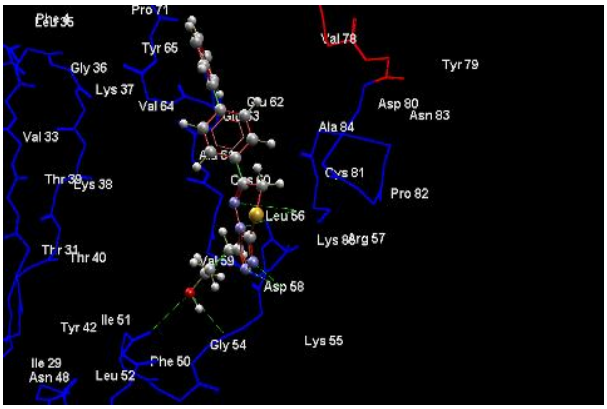


Fig 4.(b) Hydrogen bond interaction between lead compound and Gly 54, Lie 51, Asp68, Leu 56 residues

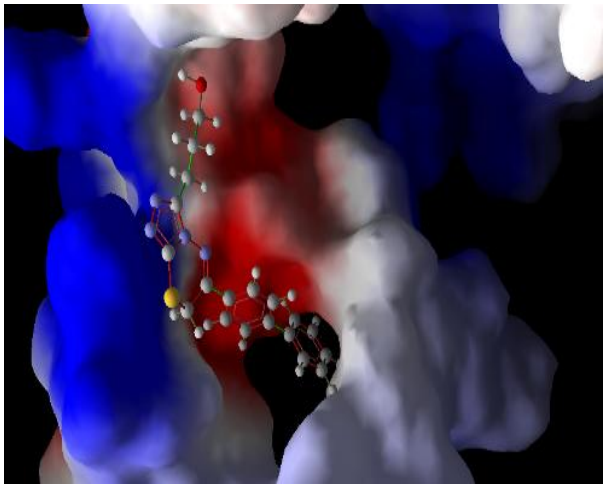


Fig 4.(c) Binding position of ligand at the surface of receptor

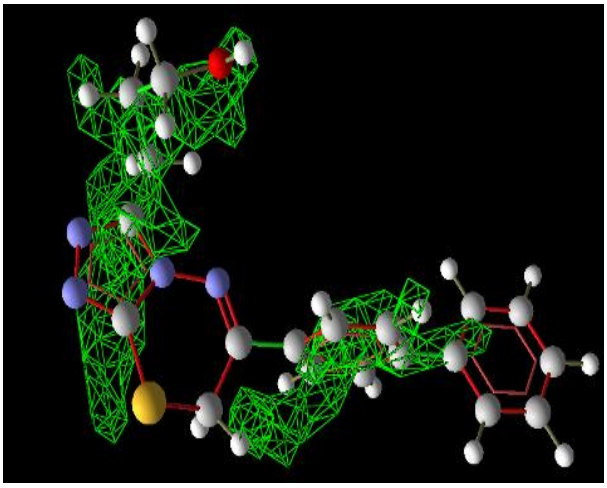


Fig 4.(d) Binding of ligand at the cavity of enzyme

Fig 4 (a-d): Interaction between top scorer derivative "6-(di phenyl)-3-(3-hydroxypropyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines" with 14- α sterol demethylase present in *Aspergillus flavus*

The mechanism of the foresaid reaction suggesting the pharmacological properties of the Triazole can be explained by the study of **Guerra *et al.*, 2003**. According to which Triazole block ergosterol synthesis through the inhibition of 14- α demethylase.

The results have been corroborated by the studies of **Bretner *et al.*** in 2005, according to which elongation of aliphatic chain results in enhancement of their inhibitory potential. The result is also supported by **Seydel *et al.*** in 1976 who reported that substitution of electron donating groups to the ring nitrogen yields compounds with decreased antimicrobial action.

SUMMARY AND CONCLUSION

The frequency of serious infection of *Aspergillus flavus* is rising continuously, this increase in fungal infections has been accompanied by the development of new and less toxic antifungal agents. Nitrogen heterocycles are of a special interest because they constitute an important class of natural and non-natural products. 1,2,4 triazole and its derivatives are found to be associated with various biological activities. They are well known to posses an array of physiological activities and are widely used in pharmaceuticals.

The study was concerned with virtual screening of 1,2,4 triazole derivatives and their potential application on *A.flavus*. The present work has been performed to get the effective drug like molecule from the derivatives of 6-aryl-3-(3-hydroxy propyl) -7H-1,2,4 triazolo [3,4-b][1,3,4] thiadiazines through the chemoinformartic approach.

The structure of drug like molecule was drawn and submitted by a server ie; CADD groups- chemoinformatic tools and users services option to derive the pdb library of 1,2,4 triazole in mol 2 format. The sequence of amino acid in cytochrome P450 in *Aspergillus flavus* was retrieved from NCBI in fasta format. This sequence was then used to get the 3D structutre of cypP450 with the help of SWISS model. Drug target identification and docking of the ligand molecule with the protein was done with the help of MVD software. The triazole derivative with least negative docking score is supposed to be the most effective drug. The bioavailability, absorption, and toxicity of the drug like molecules were studied by pharma-algorithm. Absorption rate should be high, so that these molecules must be available for biological system. Thus on the basic information obtained from the ADMET properties both study time and cost can be saved along with the life. The results obtained demonstrate that derivatives of 6-aryl-3-(3-hydroxy propyl)-7H-1,2,4 triazolo [3,4-b] [1,3,4]thiadiazines derivatives containing aryl and alkyl hydrophobic group have potential pharmacological properties.

Therefore, homology based rational drug designing can be a successful approach for designing of potent antifungal drug.

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