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Research Article

Comparative Molecular Docking Studies: Antifungal Activities of 1,4-Thiazine Derivatives.

Sindhu T.J.

Department of Pharmaceutical Chemistry, Sanjo College of Pharmaceutical Studies, Vellapara, Palakkad, India.

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***Corresponding author E-mail address:** sindhutj81@gmail.com*

ABSTRACT
The present study was designed to synthesise some novel derivatives of 1, 4- thiazines by using *o*-amino thiophenol with maleic anhydride. Further four Mannich bases were synthesised from 3-oxo-3, 4-dihydro-2H-1, 4-benzothiazin-2-yl) acetic acid with sulpha drugs, ethanol and formaldehyde. Then synthesised Mannich bases were docked against Dihydrofolate reductase complexed with NADPH and 6- methyl-5- [3-methyl-3-(3,4,5-trimethoxyphenyl) but-1-yn-1-yl] pyrimidine-2,4-diamine (UCP115A)using Argus Lab software. The results indicate the Mannich base of 1, 4-thiazines (ligand binding energy varies from -8.9046kcal/mol to -12,0457kcal/mol) shows considerable antifungal activity against *Candida albicans*. Out of the five derivatives, E4 (Sulfamethoxazole substituted 1,4- thiazine)possess best ligand pose energy (-12.0414 kcal/mol) and two hydrogen bond. The study suggests that 1, 4-Thiazine derivatives shows potent antifungal activity.

KEYWORDS
Thiazine, Argus lab, *candida albicans.*

1. INTRODUCTION

Microbial infections are becoming the most important issue for global health and economy. Among these Fungal infections are major problem in these days. The morbidity and mortality of invasive fungal infections are unacceptably high. It is an urgent need for development of new antifungal agents to treat these life-threatening invasive infections. *Candida* species are important human pathogen that is best known for causing opportunist infections in immune compromised hosts[1, 2, 3]. In continuation of synthetic work on biologically active compounds, it is interesting to note that the Cephalosporin contain a 1, 3-thiazine ring, which is active core of Cephalosporin β-lactam antibiotics. It has been observed that there is no Cephalosporin with a 1, 4- thiazine nucleus. In the light of above fact we have synthesized some new 1, 4-thiazine derivatives by changing sulphur to the fourth position from the third position by Mannich reaction[4,5,6].

The present research work was aimed at to synthesize newer, less toxic and more effective Mannich bases of 1, 4 thiazine derivatives and further compare their antifungal activities observed by molecular docking. Molecular docking plays an important role in the rational design of drugs. For molecular docking studies, the synthesised Mannich bases of thiazines were docked against Dihydrofolate reductase complexed with NADPH and 6- methyl-5- [3-methyl-3-(3,4,5-trimethoxyphenyl) but-1-yn-1-yl] pyrimidine-2,4-diamine (UCP115A) using Argus Lab software.

On this basis, we selected 3QLS as a biological target for docking study of synthesized compound[7].

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

o-aminothiophenol, maleic anhydride, formaldehyde, diethyl ether.All the solvents and chemical reagents were collected from MERCK, CHEMCO and NICE pharmaceuticals.

2.2. Methods

2.2.1. Melting point determination

The melting points of the organic compounds were determined by open capillary tube method and are uncorrected.

2.2.2. Solubility of the compounds

The solubility of the synthesized compounds was tested in various solvents like water, ethanol, methanol, chloroform, benzene etc.

2.2.3. Thin layer chromatography

TLC represents the most useful and precise technique to identify the formation of new compounds and also to determine the purity of the compound. R_f value is an important parameter and is characteristic for each compound in the same solvent system. So R_f values of the synthesized compounds were determined. The solvent system used is Acetone, methanol, chloroform (2:1:1).

$$\mathbf{R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}}$$

2.3. The Experimental Work Comprises of Proposed Scheme[8]

2.3.1. Synthesis of Mannich Bases

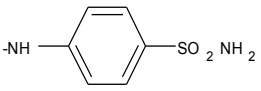
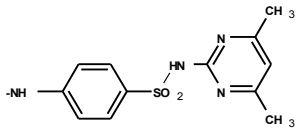
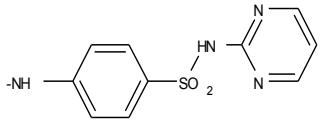
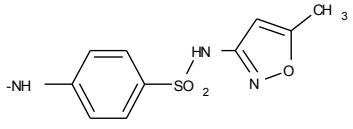
Step 1: Synthesis of (3-oxo-3, 4-dihydro-2H-1, 4-benzothiazin-2-yl) acetic acid (ST_1)

To a solution of maleic anhydride (0.05 mol) in diethyl ether (20ml) a solution of o- amino thiophenol (0.05mol) or in diethyl ether (20ml) was added. The reaction mixture was stirred at room temperature for 2 hours. The precipitate was filtered and washed with ether and recrystallized from 70% ethanol to get pure (ST_1). Yield: 95%. Melting point: 201°. Purity was assessed by TLC as a single spot (The solvent system used is Acetone, methanol, chloroform (2:1:1).

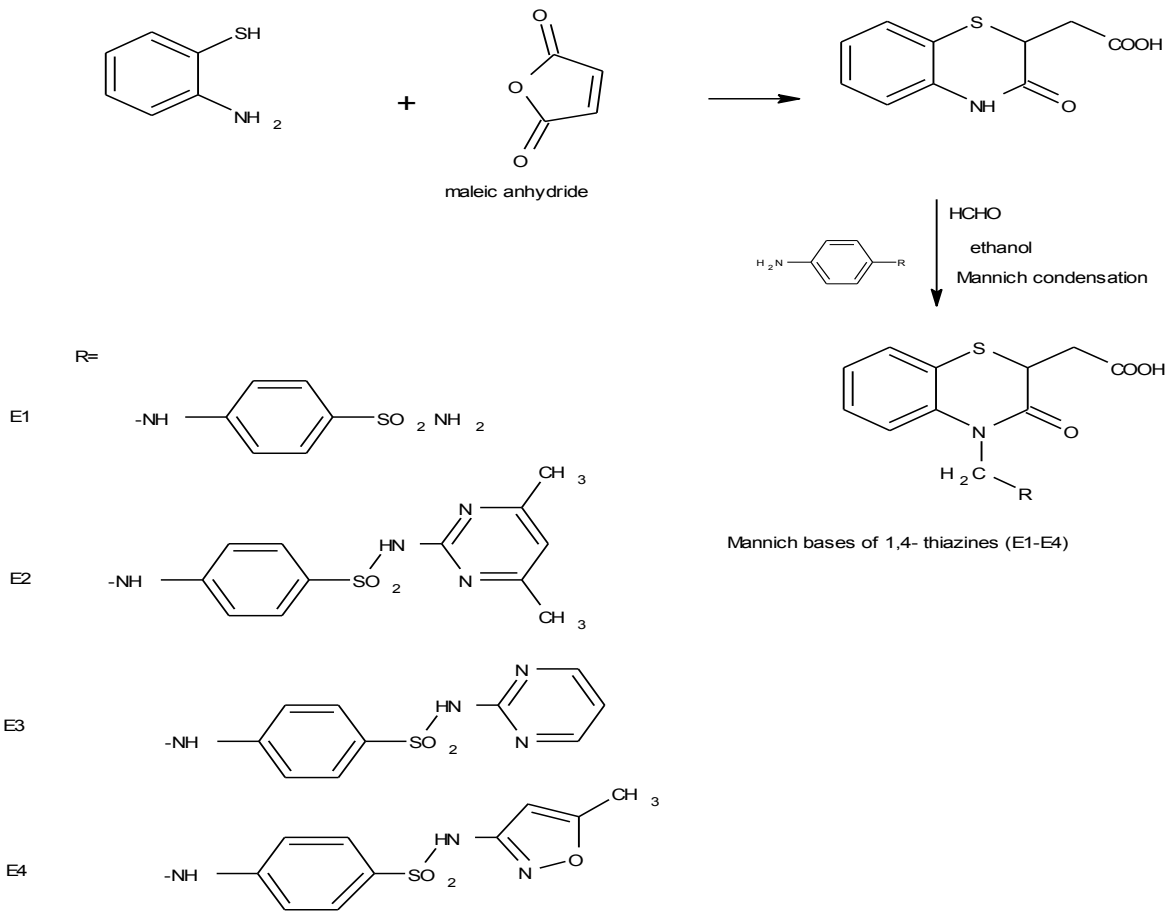
2.3.2. Step 2: Synthesis of Mannich bases of 1, 4 thiazine derivatives

A mixture of compound ($ST_{1)}$ (0.01 mol) was dissolved in ethanol (15 ml) followed by addition of sulfa drugs (0.01 mol) and formaldehyde (0.02 mol) to undergo Mannich reaction. The reactants were refluxed for 2-10 hours with continuous stirring at 70-75^{0}C.The completion of reaction was checked by TLC. After completion of reaction the mixture was poured into ice water and kept in a refrigerator overnight. The product precipitated out and was filtered, dried and recrystallised with 80% ethanol to give solid compounds. Purity was assessed by TLC as a single spot (The solvent system used is Acetone, methanol, chloroform (2:1:1).

Table 1. Physical data of newly synthesized Mannich bases of 1, 4- thiazines.

Compound	R	Molecular Formula	Mol. wt	M.P (°c)	% yield	Rf value
E1		$C_{17}H_{17}O_5S_2N_3$	407.20	233-235	75	0.73
E2		$C_{23}H_{23}O_5S_2N_5$	513.33	188-190	70	0.69
E3		$C_{21}H_{19}O_5S_2N_5$	485.0	215-217	68	0.70
E4		$C_{21}H_{20}N_4O_6S_2$	488.27	186-188	74	0.74

*solvent system: Acetone: Methanol: Chloroform (2:1:1)

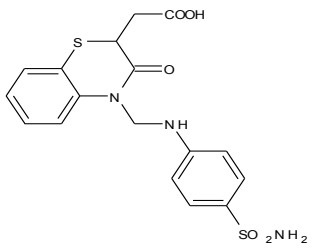


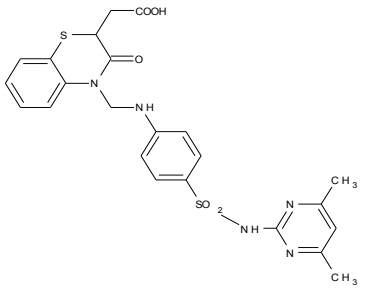
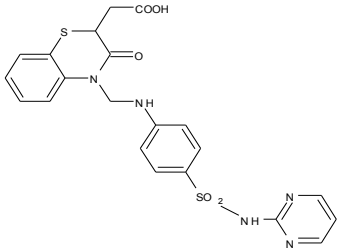
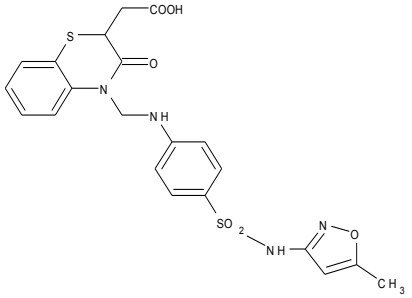
Scheme 1

2.4. Solubility of the Compounds

The solubility of the synthesized compounds was tested in water, ethanol, methanol, DMSO, chloroform, and benzene. All the compounds were soluble in ethanol, methanol and DMSO and insoluble in rest of compounds.

Table 2. IUPAC names of the synthesized compounds.

CODE NO	STRUCTURE	IUPAC NAME
E1		[4({[4(aminosulfonyl)phenyl]amino }methyl)-3-oxo-3,4-dihydro-2H-1,4benzothiazin-2-yl]acetic acid.

E2		[4({[4-(4,6-dimethyl2-pyrimidiny)aminosulfonyl)phenyl]amino}methyl)-3-oxo-3,4-dihydro-2H-1,4benzothiazin-2-yl]acetic acid
E3		([4({[4-N2-pyrimidinyl aminosulfonyl)pheny] amino}methyl)-3-oxo-3,4-dihydro-2H1,4benzothiazin-2-yl]acetic acid
E4		[4({[4-(N-(5-methyl-3-isoxazol yl)amino sulfonyl) phenyl] amino} methyl)-3-oxo-3,4-dihydro-2H1,4benzothiazin2yl]acetic acid

2.5. Molecular Docking Studies[9, 10, 11, 12.]

Molecular docking is routinely used for understanding drug information about drug receptor interactions, and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. The structures of the proteins were retrieved from Protein Data Bank. After obtaining the structure from Protein Data Bank, the possible binding sites of the protein were searched using Computed Atlas of Surface Topography of Proteins (CASTp). The synthesized compounds (inhibitor) and target protein was geometrically optimized and docked using the docking engine Argus Lab. The synthesized compounds (inhibitor) and target protein was geometrically optimized and docked using the docking engine Argus Lab.

2.5.1. Procedure

The different steps involved in the molecular docking studies include

1. **Target identification and retrieval**

Crystallographic structure of the targets of interest were obtained from Protein Data Bank and saved in standard 3D co-ordinate format. In the next step water molecules present in the protein were removed and saved the protein without water.

2. **Active site identification**
   All targets were possessing natural ligand and so active site residue identification was carried out taking advantages of the same. After obtaining the structure from Protein Data Bank, the possible binding sites of the protein were searched using Computed Atlas of Surface Topography of Proteins (CASTp).
3. **Preparation of active site**
   Hydrogen atoms missing in the PDB structure were added using Argus lab. Furthermore the atom list of the molecules were prepared, which represents the numbers of all the atom of the active site residues involved.
4. **Ligand preparation**
   The smiles formula of the drug molecules were obtained from chem. sketch. Then, using converter of the same server, PDB structure of the drug was converted into MDL MOL format which is an acceptable form for any standard docking software. Finally using Argus lab, ligand molecules were prepared by the addition of hydrogen atom.
5. **Molecular docking**
   Docking studies can be carried out using docking softwares like GOLD (genetic optimization and ligand docking), Argus lab, Autodock, GLIDE, Moligro virtual docker etc. here the software used is Argus lab. Entire process must be carried out with minimum speed and maximum accuracy.

2.6. Lipinski's Rule of Five[13, 14.]

Chemical data bases can contain thousands of molecules that could be a suitable ligand for the receptor. But no matter how good the fit is to the receptor, the candidate molecule is of no use if the absorption is poor or the drug is entered too slowly from the body.

Compounds usually must fit within the defined limits that estimate absorption, distribution metabolism and excretion. An analysis of 2,245 drugs has led a set of rules called the Lipinski's rule of five. Lipinski's rule of five is a thumb to evaluate drug likeness, if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetic in the human body including the ADME. However the rule does not predict if a compound is pharmacologically active or not. The rule is important for drug development were a pharmacologically active lead structure is optimized step wise for increased activity and selectivity. Lipinski's rule states that, in general, an orally active drug has not more than one violation of the following criteria.

- Molecular weight should not be greater than 500.
- Number of hydrogen donors (NH, OH) should be fewer than 5.
- Number of hydrogen bond acceptors should be fewer than 10.
- Calculated log P values should be less than 5.

To evaluate drug likeness, the rules are

- Partition coefficient log P is -0.4 to 5.6 ranges.
- Molar refractivity is from 40 to 130.
- Molecular weight should be in the range of 160 to 500.
- Number of atoms should be in the range of 20 to 70.(includes H bond donors (OHs and NHs) and H bond acceptors (Ns and Os)Polar surface area should not be greater than 140 A.
- This rule of five is designed as yes/no filter for the rejection of structures.

Table 3: Protein used for molecular docking studies.

ACTIVITY	ORGANISM	PROTEIN	PDB ID
Antifungal	*Candida albicans*	Dihydrofolate reductase complexed with NADPH and 6-methyl-5- [3-methyl-3-(3,4,5 trimethoxyphenyl) but-1-yn-1-yl]pyrimidine-2,4-diamine (UCP115A)	3QLS

Table 4: Summary of binding energy of all the synthesized compounds against the target protein 3QLS (Antifungal Activity against *Candida albicans).*

Compound Code	Binding Energy (Kcal / Mol)	No of Conformations	No of Hydrogen Bonds
E1	-11.5924	140	NIL
E2	-11.9342	140	NIL
E3	-12.0457	140	NIL
E4	-12.0414	140	2
Fluconazole (Standard)	-8.9046	140	3

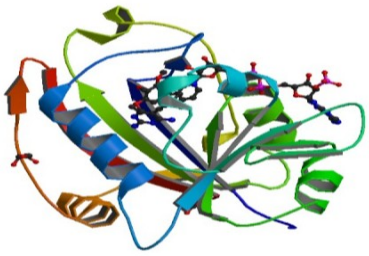


Fig. 1: Structure of 3QLS.

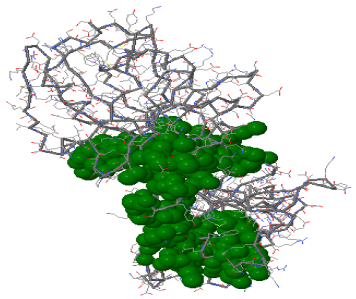


Fig. 2: Active sites of PDB ID: 3QLS.

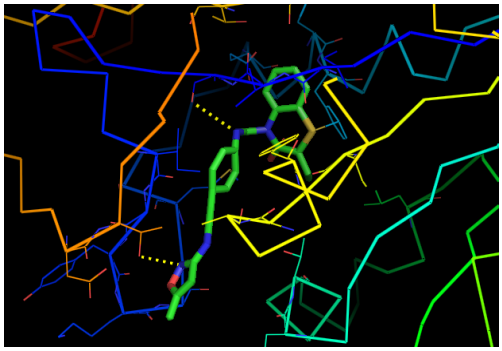


Fig. 3: Docking complex of PDB ID 3QLS with E4.

3. RESULT AND DISCUSSION

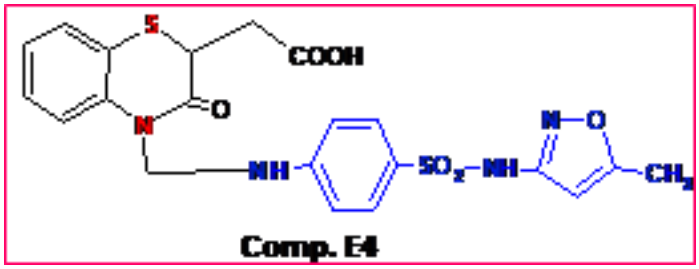
Five different 1, 4 thiazine derivatives were synthesized according to the scheme. The structures of the synthesized compounds were confirmed on the basis of physical characteristics such as M.P, TLC, solubility and % yield. Molecular docking studies of the synthesized thiazine derivatives (E1-E4) were carried out for antifungal activities using Argus lab software.

Table 5: Most active compound from docking studies.

Activity	Organism	Most Active Compound From Docking Studies
Antifungal	*Candida albicans*	Compound code: E4

Lead Molecule

Among the synthesized compounds, compounds E4 shows highest antifungal activity due to the presence of 1,4- thiazine with *sulfamethoxazole* substitution .



Comp. E4

4. CONCLUSION

Fungal infections remain one of the biggest public health problems in the 21st century. Although many active antifungal agents have been developed, the emergence of resistance to these agents is a pressing concern for human health. It is a leading cause for new challenges for the prevention, treatment and control of this deadly disease, the investigation for a new drug target is essential to continue the battle against drug resistance. The Mannich bases of 1, 4-thiazine derivatives are interesting groups of heterocyclic compounds exhibiting diverse pharmacological activities. The present work was made to find out more active new thiazine derivatives as antifungal agents by molecular docking studies. From the above results, it would be concluded that Molecular docking studies show that the Mannich bases of 1,4-thiazine derivatives possess good anti fungal activity. The life threatening nature of fungal disease as one of the current thrust area of drug research and hence development of new bioactive molecules as a challenge.

It is planned to take up experimental as well as the QSAR studies in future with suitable molecular modifications of the presently synthesized compounds with this scheme, it will be possible to find potential lead compounds for better biological activities and lesser adverse reaction in future.

Let us be optimistic that, further design of these compounds may be very useful and fruitful in the discovery of new antimicrobial agent.

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