



Drug Design, Docking Studies, Synthesis And Biological Evaluation Of Certain Optimized Lead Derivatives Of Triazoles As Inhibitors Of Cytochrome P450 14 - α - Demethylase

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ABSTRACT

Cytochrome P 450 14 α Demethylase or CYP51A1 is the target enzyme for antifungal drugs. These drugs inhibit the enzyme and prevent ergosterol biosynthesis in fungal cell wall resulting in antifungal action. Life threatening systemic fungal infections having become increasingly common shows the need for discovery of new antifungal drugs having more potency and broader spectrum of activity. In the view of these facts, the antifungal activity of certain triazole derivatives were designed, synthesized and evaluated. We also predicted the proposed inhibitory action of the designed drugs with the target enzyme through molecular docking studies using HEX, GLIDE and GOLD docking soft wares. The structure of CYP51A1 was studied and the active site was identified from Protein Data Bank (PDB) and literatures. Triazoles were selected as the lead moiety which was optimized by molecular docking studies. Based on the best docking scores, certain triazole derivatives were synthesized and evaluated for their antifungal action against *Candida albicans* and *Aspergillus niger*. The experimental data obtained was then compared with computational docking results. Experimentally all the synthesized compounds were found active against *Candida albicans* and inactive against *Aspergillus niger*. The halogenated derivative of triazole which showed the highest docking score was also found to exhibit greater zone of inhibition in comparison with other synthesized compounds. Thus the computational results were found to comply with the experimental results obtained. Summarizing the work, it can be concluded that the experimental data together with computational evidence can be of use in further experimental protein-ligand designs, and to provide insight into the interaction occurring in the active site.

INTRODUCTION

Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical in vitro and in vivo studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development. The process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like combinatorial chemistry high throughput screening, virtual screening, de novo design, in vitro, insilico screening and structure-based drug design.

In recent years, life-threatening systemic fungal infections have become increasingly common, especially in the immuno compromised hosts suffering from tuberculosis, cancer or AIDS and in organ transplant cases. Several clinical drugs, such as azoles have been developed to reduce the impact of fungal diseases. Among those, azoles, especially triazole antifungal agents, were used widely and efficiently.

These antifungal drugs act by inhibiting CYP51A1 or Cytochrome P450 14 α Demethylase, a necessary enzyme in the biosynthesis of ergosterol, through a mechanism in which the heterocyclic nitrogen atom (N-4 of tri-azole) binds to the heme iron atom. However, the increasing administration of antifungal agents has led to the development of fungal resistance. Genetic mutations that result in resistance to clinically used drugs, especially fluconazole, may also result in resistance to new structurally related azoles such as voriconazole and ravuconazole. The emergence of resistance shows the need of the discovery of new antifungal compounds which have broader antifungal spectra and higher therapeutic indexes than fluconazole [1].

Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist.

Structure-based drug design involve active site identification, lead discovery ,lead optimisation and molecular docking. Active site identification involves visualization of 3D structure of the target molecule using graphic tools and also analysis of the protein to find the binding pocket which derives key interaction sites within the binding pocket, and then prepares the necessary data for ligand fragment link Leads discovered using virtual screening and *de novo* design methodologies needs to be optimized to produce candidates with improved bioavailability and low toxicity Lead optimization ensures optimum binding affinity and drug likeness. Evaluation of drug-likeness involves prediction of ADMET (absorption, distribution, metabolism, excretion, toxicity) properties Lipinski's Rule of Five is a rule of thumb to evaluate druglikeness Lipinski's rule says that, in general, an orally active drug has no more than one violation of the following criteria: Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms) ,Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms) , A molecular weight under 500 daltons and an octanol-water partition coefficient $\log P$ of less than 5 .

In the field of molecular modeling, docking [2] is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking .Steps in Molecular Docking involve ligand setup ,protein setup ,start of docking calculation , evaluation of results and Preparation of figures and methods for the report.[3-6].

MATERIALS & METHODS

Computational work

Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost. The present work by computational approach used the following softwares and involves Chems sketch and Marvin sketch for the structure manipulation of drugs, Molinspiration online web service for calculation of drug likeness and bioactivity prediction and molecular docking softwares such as HEX, GLIDE and GOLD.

The target site for antifungal agents was found to be the fungal enzyme, cytochrome P 450 14 α demethylase or CYP51A1 which is involved in ergosterol biosynthetic pathway. Inhibition of the enzyme results in deficiency of ergosterol in fungal cell membrane leading to cell death. The mechanism through which antifungal action occurs is taken as the basis for design of novel antifungal agents.

The structure of the target enzyme was taken from PDB (ID: 2BZ9) The search for lead compound is focused on rational approach i.e., by using the knowledge of the receptors and their mode of interaction with the drug molecules. Among the currently used clinical antifungal drugs, triazole antifungals were found to be used widely and effectively. Hence triazole heterocyclic ring was selected as the lead compound for our present work.

More than 300 compounds were screened after incorporating different heterocyclic moieties with the triazole lead in different positions. The compounds thus obtained were subjected to the next stage i.e., lead optimization. Virtual screening of the designed compounds were done by estimating drug-likeness according to Lipinski's rule of five , using molinspiration online web service and estimating drug-receptor complex binding affinity ,using molecular docking softwares: HEX, GLIDE, GOLD.

The compounds which satisfied Lipinski's rule of five were subjected to molecular docking. Based on comparison with three docking softwares, those compounds that exhibited best docking scores were selected for synthesis and biological evaluation.

SYNTHETIC WORK

Synthesis Of methyl 4-(1H- indol- 3 -yl) butanoate:(2)

A mixture of 20.3g (0.1 mole) compound (1), methanol (6.4 ml), dichloride methane (100 ml) and con. Sulphuric acid (5 drops) was refluxed for 5 hours and cooled to 5°C .The contents were poured into ice water (100 ml). The organic layer was separated and dichloromethane was distilled off to get the crude product. The high vacuum distillation of this crude product afforded the pure compound 2 which is recrystallised from methanol. Purity of this ester was established by single spot on the TLC plate .Solvent system used: Methanol: Chloroform (3:1).

Synthesis Of methyl 4-(1H- indo l- 3 -yl) butanehydrazide: (3)

To the compound 2 (21.7 g, 0.1 mol) in ethanol (20 ml), hydrazine hydrate (99%, 2 ml) was added in drops with stirring and refluxed for a period of 2 hours. After cooling the solution was poured into crushed ice. The solid separated was filtered, dried and recrystallised from methanol. The purity of the compound was established by single spot on TLC plate. Solvent system: Methanol: Chloroform (3:1).

Synthesis Of potassium {[methyl 4-(1H- indol- 3 -yl) butanehydrazido] methanethioyl} sulfanide: (4)

Potassium hydroxide (0.15 mol, 8.4 g) was dissolved in 200 ml absolute ethanol. 0.1 mol acid hydrazide (13.7 g) and 0.15 mol carbon disulphide (11.4 ml) was added. The mixture diluted with 150 ml ethanol, stirred for 16 hours. The resulting solution was then diluted with 250 ml dry ether and the precipitated solids which was obtained was filtered ,washed with dry ether ,dried at 65°C. The products obtained were employed in the next step without further purification.

Synthesis Of N-(3-[3-(1H indol 3-yl) propyl]-5-sulfanyl-4H-1,2,4-triazole-4-yl]-4-substituted benzamide: (5a-5d)

A suspension of compound 4 (0.1 mol) was treated with various phenyl hydrazides (to get the corresponding triazole derivative) and water (5ml) by heating under reflux for 6 hours and reflux was continued for another 2 hours when profused

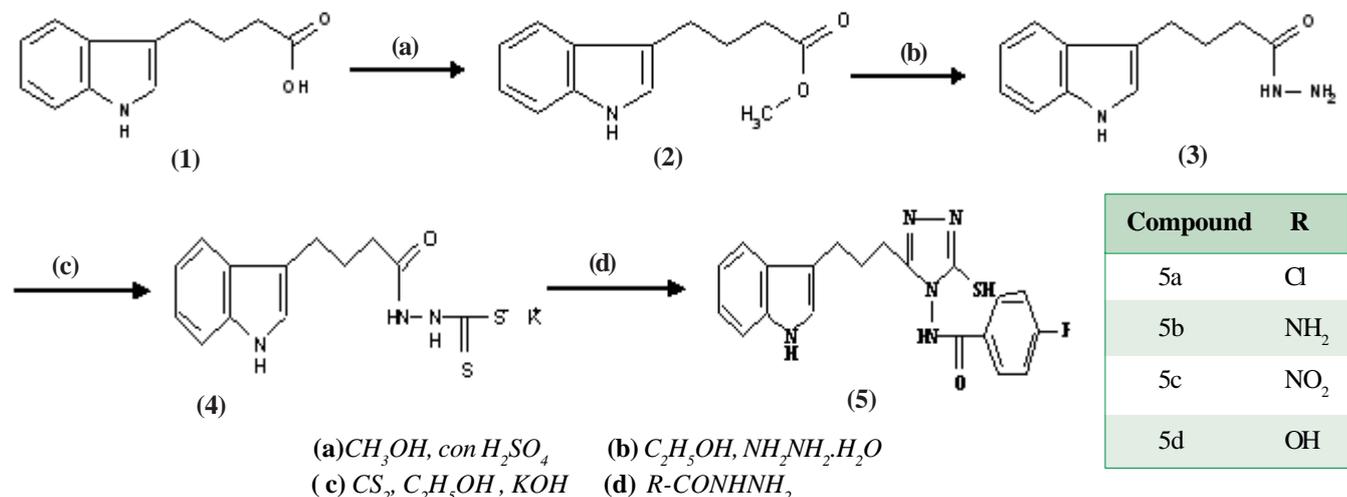


Figure 1: Scheme for synthesis of triazole derivatives leading to compounds 5a-5d

Antifungal activity

Sabouraud Dextrose agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 25°C just before inoculation.

The organism (*Candida albicans* NCIM 3471, *Aspergillus niger* NCIM 596) were inoculated in the plates prepared earlier by dipping sterile swab in the inoculum removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking a swab all over the surface of the medium three times rotating the plates through the angle of 60° after each application. Finally the swab was pressed round the edges of the agar surface. The inoculum was left to dry at room temperature with lid closed. Sterile discs containing the test standard and blank were placed in the petridish aseptically. 10 ml/disc of saturated solutions were used and DMSO reagent used as the vehicle.

The petridishes were incubated at 25°C for 24-48 hours after placing them in refrigerator for one hour to facilitate uniform diffusion. Observations were made for zone of inhibition round the discs and compared with that for Fluconazole disc (25mcg/disc). All the compounds were tested for antifungal activity [9-10].

Anti oxidant activity

The DPPH assay measures hydrogen atom or electron donating activity and hence provides a measure of free radical scavenging antioxidant activity. DPPH is a purple coloured stable free radical which becomes reduced to yellow colored diphenyl picryl hydrazine. DPPH is a stable free radical that can accept an electron or hydrogen radical and thus can be converted into a stable diamagnetic molecule. DPPH has an odd electron and so

evolution of hydrogen sulphide was observed and clear solution resulted. Dilution of the reaction mixture with cold water (50 ml) and subsequent acidification with HCl (1:1) gave a white precipitate. It was filtered, washed with water and recrystallised from aqueous ethanol (80%) [7-8]. The scheme for synthesis is shown in (Fig 1)

has a strong absorption band at 517 nm. When this electron becomes paired off the absorption decreases stoichiometrically with respect to the number of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to the capacity of numerous molecules to act as free radical scavengers.

1.5ml of 0.2mM of DPPH solution was added to 1.5ml of different concentrations of the drug solutions. Another series of solutions were prepared by taking 1.5ml of different concentrations of drug solutions and 1.5 ml of methanol. The above solutions were allowed to react at room temperature for 30min. After 30min the absorbance values were measured at 517 nm and converted to percentage of scavenging activity [11-12] which was calculated by using the following formula

$$\% \text{ of Scavenging activity} = \frac{(A_b + A_s) - A_m}{A_m} \times 100$$

A_b = Absorbance of 1.5ml DPPH + 1.5ml methanol

A_m = Absorbance of 1.5ml DPPH + 1.5ml drug solution

A_s = Absorbance of 1.5ml drug solution + 1.5ml methanol

RESULTS & DISCUSSION

Of the various compounds screened, triazole derivatives (5a-5d) showed good docking scores by comparing with three docking softwares (Fig 2-4). The compounds also satisfied Lipinski's rule of Five and were selected for synthesis. Among synthesized compounds, 5a showed best docking value i.e., lowest energy value or GLIDE score in GLIDE commercial software and the highest value or fitness function in GOLD commercial software. HEX freeware showed best score (lowest E_{value} or energy value) for the compound 5d which scored second highest in the commercial softwares i.e., GLIDE and GOLD softwares.

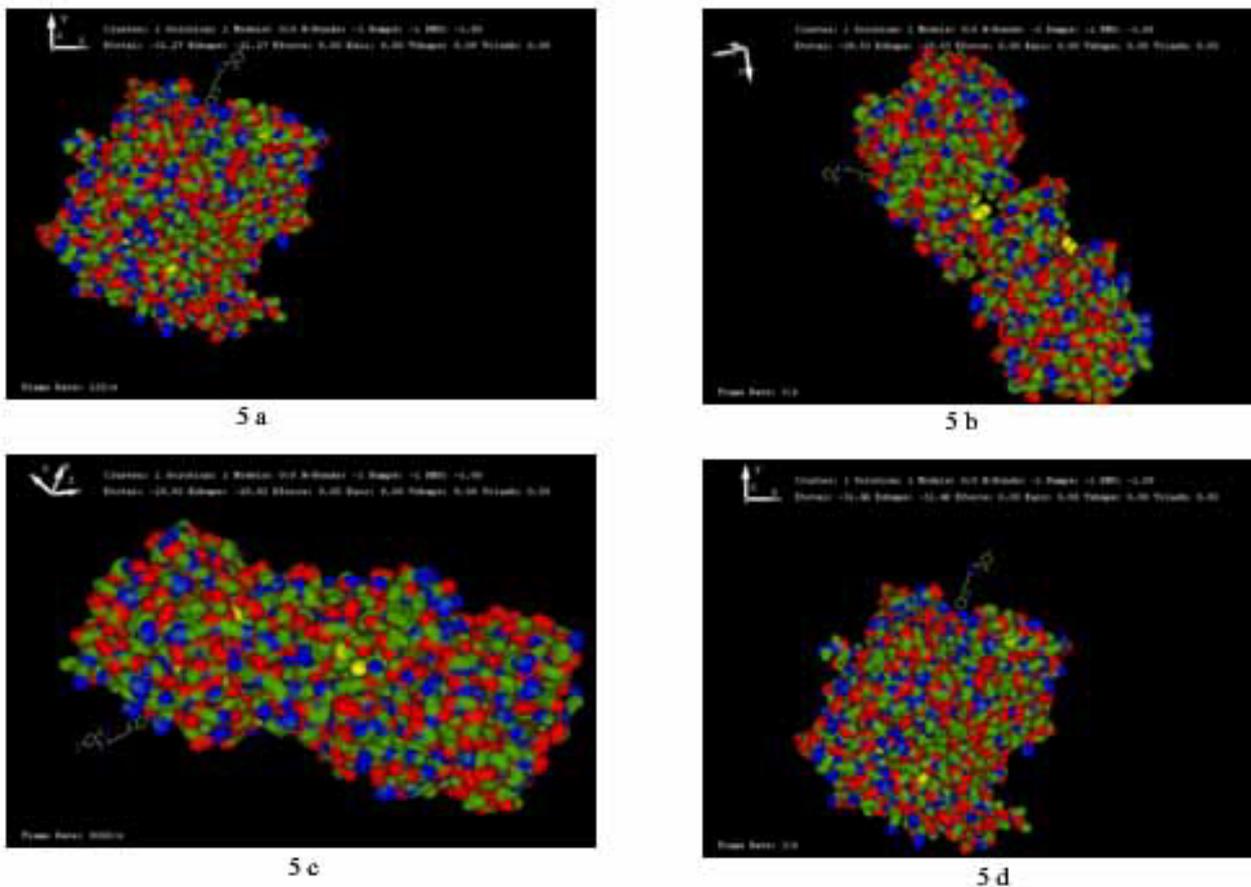


Figure 2: Molecular Docking of compounds 5a-5d with CYP51A1 in HEX software

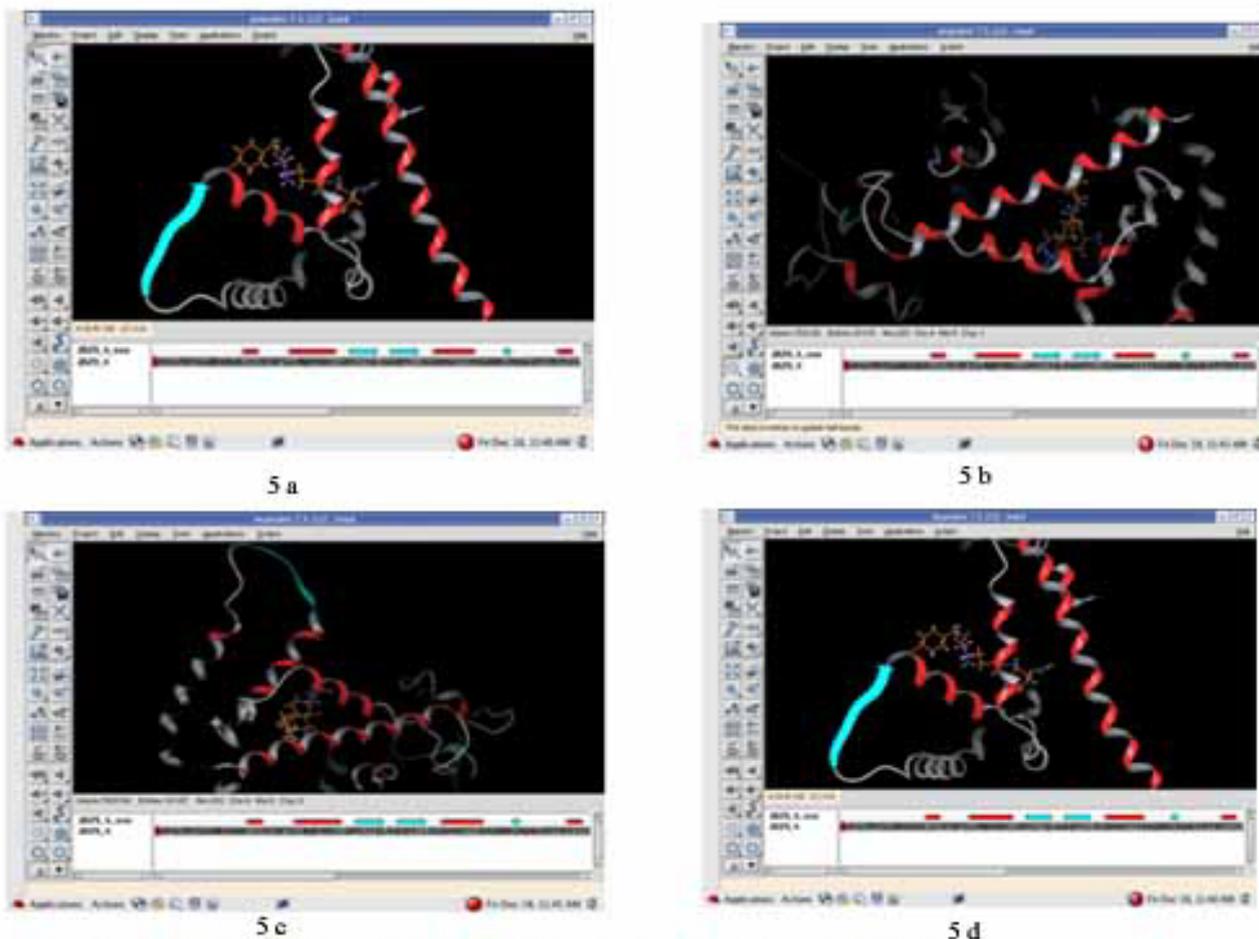
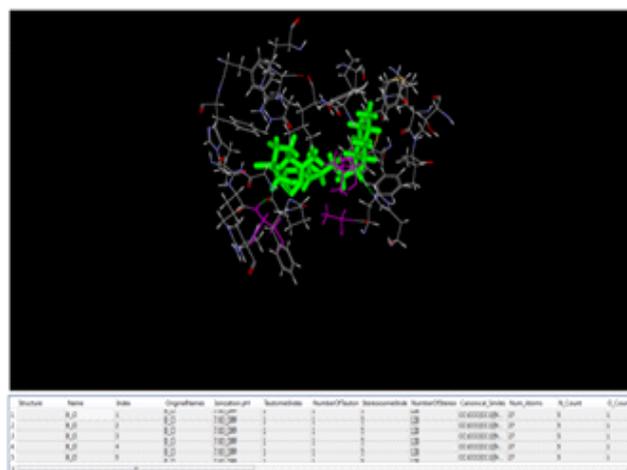
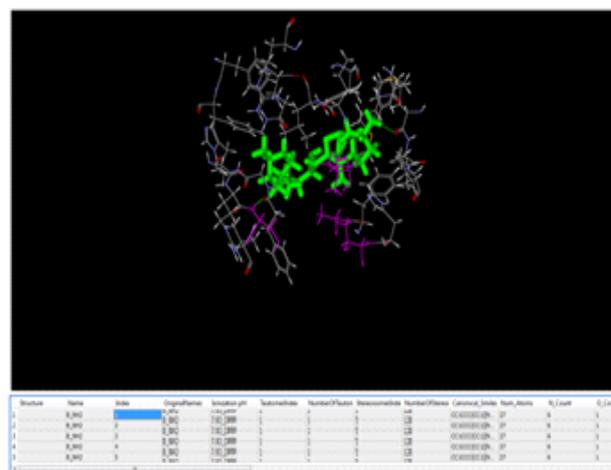


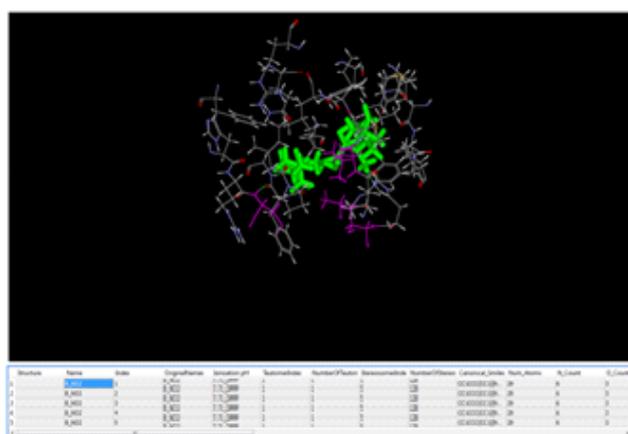
Figure 3 :Molecular Docking of compounds 5a-5d with CYP51A1 in GLIDE software



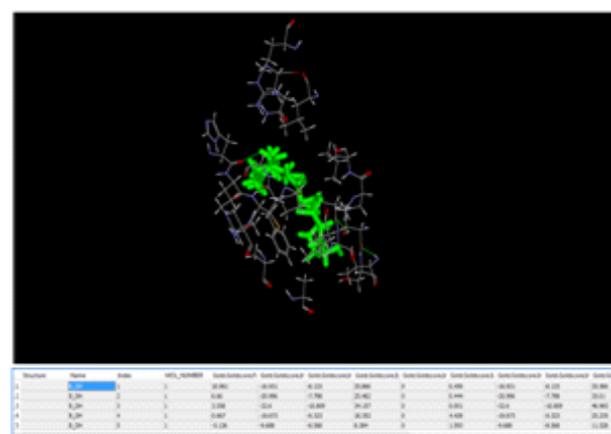
5 a



5 b



5 c



5 d

Figure 4: Molecular Docking of compounds 5a-5d with CYP51A1 in GOLD software

The synthesized compounds were physically characterized (Table 1). All the compounds synthesized were found active against *Candida albicans* and were inactive against *Aspergillus niger* at a concentration of 500 μg . The compound 5a showed highest zone of inhibition followed by the compound 5d at 500 μg concentration. The experimental antifungal activity results are in compliance with computational results that had predicted the bioactivity.

The compound 5a showed the best docking value and the best antifungal activity. In comparison with the standard antifungal agent fluconazole, the synthesized compounds showed lesser docking score in HEX freeware but comparatively better docking score in GLIDE and GOLD commercial softwares. However anti fungal studies proved the compounds to exhibit slightly lesser activity than the standard drug Fluconazole (Table 2).

Table 1: Physical Characterisation Data Of The Synthesised Compounds 5a-5d

S.No	Compound	R	Molecular formula	Molecular weight	Melting point	Percentage yield	R _i value
1.	5a	Cl	C ₂₀ H ₁₈ ClN ₅ OS	411.908	246.4	86%	0.74
2.	5b	NH ₂	C ₂₀ H ₂₀ N ₆ OS	392.477	125.7	85%	0.58
3.	5c	NO ₂	C ₂₀ H ₁₈ N ₆ O ₃ S	422.46	231.7	77%	0.84
4.	5d	OH	C ₂₀ H ₁₉ N ₅ O ₂ S	393.462	128.1	70%	0.85

All the synthesized compounds showed good anti oxidant activity except compound 5a which showed weak to moderate activity (Table 3). All compounds showed decrease in absorbance with increase in concentration which ensured

increased percentage of free radical scavenging or antioxidant activity. The compound 5c showed promising activity when compared with that of standard ascorbic acid.

Table 2: Comparison of computational results of molecular docking with experimental antifungal activity of compounds 5a-5d

Compound	Docking scores			Anti fungal activity (Zone of inhibition in mm)	
	HEX	GLIDE	GOLD	<i>C.albicans</i> (500 µg)	<i>Asp. niger</i> (500 µg)
5a	- 31.27	- 7.764885	57.08	18	-
5b	-28.53	-5.788779	55.07	14	-
5c	-20.92	-6.095630	55.40	16	-
5d	-31.46	-6.204759	55.67	17	-
Fluconazole	-39.52	-7.2364	25.68	24	21

Table 3: Results of anti oxidant activity of compounds 5a-5d by DPPH assay method

Compound	% Anti Oxidant Activity		
	.1mM	.2mM	.5mM
5a	13.37%	28.48%	55.83%
5b	53.48%	66.86%	87.80%
5c	95.34%	97.09%	98.81%
5d	20.93%	34.88%	64.53%
Ascorbic acid	96.96%	97.82%	99.61%

Physical Characterization data of best of the synthesized compounds are as follows:

(5a): Mp : 246.4°C ; λ_{\max} : 283 nm ; IR(KBr): 3063 (aromatic CH),2557.15 (SH),1685(CO-NH),1492.63(CN),678.82(C-S),925.664(C-C),1087.66(CCl), 3417.24 (NH), 848.525(1,4-disubstitutedbenzene);LC-MSm/z:412(M⁺1),151,187,271,394.

(5d): Mp : 393.462°C ; λ_{\max} : 264.4 nm ; IR(KBr): 3023.84 (aromatic CH),2580.29 (SH), 1681.62(CO-NH), 1511.92(CN), 694.248(C-S), 952.663(C-C), 1234.22(C-O of phenol), 3428.81(NH), 848.525(1,4-disubstituted benzene); LC MS m/z:394 (M⁺1).

CONCLUSION

All the synthesized compounds were found active against *Candida albicans* and inactive against *Aspergillus niger*. The experimental data thus obtained was found to comply with computational results as the same compound 5a showed the highest docking score and exhibited greater zone of inhibition in comparison with other synthesized compounds. The compounds also exhibited antioxidant activity. The compound 5d showed promising activity.

Summarizing the work, it can be concluded that the experimental data together with computational evidence can be of use in further experimental protein-ligand designs, and to provide insight into the interaction occurring in the active site and the origins of variations in the corresponding binding free energy thus yielding invaluable information for the design

and prediction of activity of a second generation of inhibitors within a reasonable length of time.

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