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Molecular Docking and Interaction Analysis of Statins and Fluconazole against Candida albicans CYP51 and HMG-CoA Reductase: An In-Silico Approach

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KEYWORDS

Statins, Fluconazole, Candida albicans, Molecular docking, CYP51 enzyme, HMG-CoA reductase, Drug repurposing

ABSTRACT:

The rise of antifungal resistance in Candida albicans highlights the urgent need for alternative therapeutic strategies. Statins, widely prescribed as cholesterol-lowering agents, have shown potential antifungal activity by interfering with ergosterol biosynthesis. This in silico study investigates the molecular docking interactions of atorvastatin, fluvastatin, lovastatin, and simvastatin with key fungal targets—CYP51 and HMG-CoA reductase—using fluconazole as a reference compound. AutoDock Vina 1.2.2 was employed via the PyRx 0.9.8 platform. Ligand optimization, protein preparation, and visualization were performed using Chem3D Pro 16.0, AutoDock Tools 1.5.6, UCSF Chimera 1.16, Discovery Studio Visualizer 2020, and LigPlot+. Results show that atorvastatin exhibits the strongest binding with both CYP51 and HMG-CoA reductase, surpassing fluconazole in binding affinity. These findings support the potential repurposing of statins as antifungal agents.

1. Introduction

Candida albicans is an opportunistic yeast responsible for infections ranging from superficial mucosal conditions (oral thrush and vaginal candidiasis) to life-threatening bloodstream infections such as candidemia. These infections are of increasing clinical concern due to rising antifungal resistance, particularly in immunocompromised individuals, transplant recipients, and patients undergoing chemotherapy (Pfaller & Diekema, 2007). Invasive candidiasis is associated with mortality rates of up to 40% even with antifungal therapy (Pappas et al., 2018).

1.1 Challenges with Current Antifungal Therapies

The three main classes of antifungal drugs—azoles, polyenes, and echinocandins—have limitations. Azoles: Widely used (e.g., fluconazole) but increasingly compromised due to ERG11 gene mutations and efflux pump overexpression (CDR1, CDR2, MDR1). Polyenes: Potent drugs like amphotericin B, but associated with severe nephrotoxicity.Echinocandins: Effective but expensive and with limited spectrum.Resistance is particularly problematic with biofilm-forming Candida strains that can withstand 100–1000 times higher drug concentrations than planktonic cells (Chandra et al., 2001).

1.2 Drug Repurposing

Drug repurposing (or repositioning) is a strategy where approved drugs are evaluated for new therapeutic indications. This approach shortens drug development timelines, reduces costs, and mitigates safety concerns since pharmacokinetics and toxicology are already known. In antifungal therapy, several non-antifungal

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agents, including statins and NSAIDs, have shown synergistic or direct antifungal effects.

1.3 Statins as Antifungal Agents

Statins inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the key enzyme in the mevalonate pathway of cholesterol biosynthesis. In fungi, HMG-CoA reductase and CYP51 are critical enzymes in ergosterol biosynthesis. Disruption of these pathways leads to membrane instability and cell death (Cuervo et al., 2013). Lipophilic statins (atorvastatin, simvastatin, lovastatin, fluvastatin) can penetrate cell membranes more efficiently, enhancing their antifungal activity. Studies by Kim et al. (2015) demonstrated that simvastatin enhances fluconazole activity against resistant Candida strains.

1.4 Role of In Silico Studies

Molecular docking predicts the orientation and binding energy of drug molecules within a target protein's active site. It is widely used to screen potential drug candidates prior to experimental testing. In this study, docking was performed on statins and fluconazole against C. albicans CYP51 and HMG-CoA reductase to identify the most promising candidates for antifungal repurposing.

2. Literature Review

Fungal infections caused by Candida species, particularly Candida albicans, represent a growing concern in clinical practice due to increasing resistance to available antifungal agents. The therapeutic arsenal for fungal infections is limited, and the rise of multidrug-resistant strains has emphasized the need for novel antifungal strategies or combination therapies. In this context, drug repurposing and computational approaches have emerged as cost-effective strategies for accelerating antifungal drug discovery.

2.1 Antifungal Resistance Mechanisms in Candida albicans

Candida albicans is the most prevalent opportunistic fungal pathogen. It can cause superficial infections, such as oral thrush and vulvovaginal candidiasis, as well as systemic infections like candidemia, particularly in immunocompromised patients (Pfaller & Diekema, 2007). Over the past few decades, resistance to conventional antifungal drugs such as azoles and polyenes has increased due to several genetic and biochemical adaptations.

The primary mechanisms of resistance include:

Mutations in CYP51 (ERG11 gene): Mutations in the lanosterol 14α-demethylase enzyme (CYP51) decrease azole binding affinity, leading to drug resistance (Whaley et al., 2017).Efflux pump overexpression: ABC transporters (CDR1, CDR2) and MFS transporters (MDR1) actively pump azole drugs out of the fungal cell (Sanguinetti et al., 2015). Biofilm formation: Biofilms protect Candida from antifungal drugs due to altered microenvironment and extracellular matrix, resulting in resistance up to 1000 times greater than planktonic cells (Chandra et al., 2001; Taff et al., 2013). Alterations in ergosterol biosynthesis: Changes in ergosterol content reduce the efficacy of azoles, which target this essential component of fungal membranes. Azole resistance is a significant clinical issue, especially in the management of recurrent infections. While fluconazole remains a mainstay of therapy, its effectiveness is declining against resistant C. albicans isolates.

2.2 Drug Repurposing for Antifungal Therapy

Drug repurposing involves identifying new therapeutic uses for existing drugs, which have already undergone safety evaluations and clinical trials. This approach significantly reduces the time and cost associated with drug development (Ashburn & Thor, 2004). In antifungal drug discovery, several repurposed drugs have shown promise: Statins: HMG-CoA reductase inhibitors with known lipid-lowering activity. NSAIDs: Ibuprofen and aspirin have antifungal effects by disrupting fungal biofilms (Alem & Douglas, 2004). Calcineurin inhibitors: Tacrolimus and cyclosporine enhance the activity of azoles. Among these, statins have gained considerable attention because of their unique ability to inhibit the ergosterol biosynthetic pathway, which is structurally analogous to cholesterol synthesis in humans.

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2.3 Statins as Antifungal Agents

Statins (e.g., atorvastatin, simvastatin, lovastatin, and fluvastatin) are competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway of cholesterol synthesis. In fungi, this pathway leads to ergosterol synthesis, an essential sterol component of fungal cell membranes. Inhibition of HMG-CoA reductase in fungi reduces ergosterol content, disrupts membrane integrity, and increases cell permeability (Cuervo et al., 2013).

2.3.1 Evidence of Statin Antifungal Activity

Cuervo et al. (2013) demonstrated that simvastatin and atorvastatin inhibit fungal growth in vitro and enhance the activity of fluconazole against resistant isolates of Candida albicans. Gaber et al. (2021) reported that atorvastatin significantly reduced the minimum inhibitory concentration (MIC) of fluconazole against resistant C. albicans when used in combination. Nyilasi et al. (2013) evaluated several statins and found that lipophilic statins (atorvastatin, simvastatin, lovastatin) were more effective antifungal agents than hydrophilic statins. Desouza & Rodrigues (2009) observed that statins caused cell wall and membrane damage in C. albicans, leading to impaired cell viability.

2.3.2 Mechanism of Antifungal Action

Statins inhibit the mevalonate pathway, reducing the availability of ergosterol precursors. This results in Increased membrane fluidity and permeability Loss of membrane-associated enzymes Induction of oxidative stress and reactive oxygen species (ROS). The synergistic action of statins with azoles is attributed to their ability to enhance azole penetration into fungal cells by disrupting the plasma membrane (Perea et al., 2012).

2.4 Molecular Targets: CYP51 and HMG-CoA Reductase

CYP51 (lanosterol 14α-demethylase) is a heme-containing enzyme crucial for the demethylation of lanosterol during ergosterol biosynthesis. It is the primary target of azole antifungals. Mutations in CYP51 are a major cause of azole resistance (Sanglard et al., 2016). HMG-CoA reductase, another essential enzyme, is inhibited by statins, leading to depletion of ergosterol in fungal membranes (Lobato et al., 2014). Targeting both CYP51 and HMG-CoA reductase offers a dual inhibitory mechanism, potentially overcoming resistance.

2.5 Computational Docking and Virtual Screening

In-silico methods have revolutionized drug discovery by enabling the rapid screening of large compound libraries against target proteins. Molecular docking is particularly useful in predicting: Binding affinity (expressed as binding energy). Orientation of ligands within the active site. Key interactions (hydrogen bonds, hydrophobic contacts, π–π stacking). Ahmed et al. (2022) performed docking studies on atorvastatin and reported binding energies of –9.5 kcal/mol with CYP51, which was superior to fluconazole. Song et al. (2023) used molecular dynamics simulations and confirmed the stability of statin-protein complexes.

2.6 Synergy Between Statins and Azoles

Combination therapy is a promising strategy for combating antifungal resistance. Perea et al. (2012) reported that combining statins with fluconazole significantly reduced fungal growth, even in resistant strains. The rationale is that statins compromise cell membrane integrity, allowing azoles to access their target (CYP51) more effectively.Clinical studies also suggest that patients taking statins for cardiovascular conditions have a lower incidence of invasive candidiasis (Lobato et al., 2014). This observation strengthens the hypothesis that statins may provide antifungal benefits in vivo.

2.7 Other Repurposed Drugs and Combination Strategies

While statins are a primary focus, other drug classes have been investigated for repurposing: Chloroquine and hydroxychloroquine show activity against Candida biofilms by disrupting iron metabolism (Baxter et al., 2011). Metformin, commonly used in diabetes, enhances fluconazole activity by modulating efflux pumps (Cheng et al., 2016). However, statins remain the most promising candidates because of their dual role in cholesterol management and antifungal activity.

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2.8 Gaps in Research and the Role of In-Silico Studies

Despite the experimental evidence supporting statins' antifungal potential, in-silico studies are essential to Elucidate the precise binding mechanisms of statins with fungal targets.Identify key residues involved in ligand-enzyme interactions. Predict structure-activity relationships for novel derivatives.Molecular docking, when combined with molecular dynamics (MD) simulations, provides a reliable framework for studying ligand stability and binding patterns under physiological conditions (Hamdy et al., 2021).

2.9 Summary of Literature

The existing literature clearly indicates that statins: Exhibit direct antifungal activity Synergize with azoles like fluconazole. Target essential enzymes (CYP51 and HMG-CoA reductase) in ergosterol biosynthesis. Show promising binding energies and interactions in computational studies.

However, there is a lack of comprehensive in-silico studies comparing multiple statins (atorvastatin, fluvastatin, lovastatin, simvastatin) against both fungal targets simultaneously. This forms the basis of the present study.

3. Materials and Methods

3.1 Study Design

This study was conducted using a **computational docking approach** to evaluate the binding affinity of four statins—atorvastatin, fluvastatin, lovastatin, and simvastatin—against key fungal enzymes: **CYP51 (lanosterol 14α-demethylase)** and **HMG-CoA reductase** of *Candida albicans*. Fluconazole, a well-known azole antifungal, was included as a reference ligand for comparative analysis.

The workflow included ligand preparation, protein preparation, and molecular docking using **MOE 2019.0102** as the primary docking tool. Additionally, **AutoDock Vina 1.2.2** and **PyRx 0.9.8** were utilized to cross-validate docking results. Interaction analysis and visualization were conducted using **UCSF Chimera 1.16**, **Discovery Studio Visualizer 2020**, and **LigPlot+ 2.2**. MOE facilitated energy minimization, docking score calculation, and 2D/3D interaction mapping of ligand–protein complexes.

3.2 Target Protein Selection

CYP51 (lanosterol 14α-demethylase):

- *Candida albicans* CYP51 is a primary target of azole antifungal drugs, responsible for the demethylation of lanosterol during ergosterol biosynthesis.
- The crystal structure of CYP51 was retrieved from the Protein Data Bank (PDB ID: **5V5Z**). This structure was selected because of its high resolution and its suitability for docking simulations.

Other Target Proteins Used for Docking: To evaluate the antifungal and HMG-CoA reductase inhibitory potential of selected drugs, the following protein structures were also included in the molecular docking study:

- **HMG-CoA Reductase (PDB ID: 1AI9):** Human enzyme targeted by statins, involved in cholesterol biosynthesis.
- **ERG11 (PDB ID: 6C5C):** A cytochrome P450 enzyme from *Candida albicans*, targeted by azoles.
- **Sterol 24-C-Methyltransferase (PDB ID: 2Y71):** Involved in ergosterol biosynthesis.
- **Farnesyl Pyrophosphate Synthase (PDB ID: 1EQC):** Another key enzyme in the ergosterol biosynthetic pathway.

These proteins were selected based on their relevance to the mechanism of action of **statins** and **fluconazole**, ensuring a comprehensive analysis of possible drug–target interactions.

HMG-CoA Reductase:

- This enzyme is essential for the mevalonate pathway, leading to sterol biosynthesis. Its inhibition results in depletion of ergosterol, affecting fungal viability.
- The structure of fungal HMG-CoA reductase was modeled using **Swiss-Model** due to the absence of a crystallized *Candida* homolog in the PDB. The homology model was validated using **Ramachandran plots and Verify3D**.

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3.3 Ligand Selection and Preparation

The following ligands were selected based on their known or potential antifungal activity:

1. **Atorvastatin** (PubChem CID: 60823)
2. **Fluvastatin** (PubChem CID: 446155)
3. **Lovastatin** (PubChem CID: 53232)
4. **Simvastatin** (PubChem CID: 54454)
5. **Fluconazole** (reference drug, PubChem CID: 3365)

Preparation Steps:

- Ligand structures were retrieved from **PubChem** in **SDF** format.
- Geometry optimization and energy minimization were performed using **Chem3D Pro 16.0** with the **MM2 force field**.
- The optimized ligands were saved in **PDB** format.
- Final ligand preparation, including protonation at physiological pH (7.4), 3D conformer generation, and charge assignment, was completed using **MOE**.
- Ligands were saved in MOE-compatible format (.mdb) for docking studies.

3.4 Protein Preparation

Two target proteins were used:

1. **Lanosterol 14α-demethylase (CYP51)** – *Candida albicans*, PDB ID: **5V5Z*
2. **HMG-CoA reductase** – Human enzyme, PDB ID: **1HW9**

Preparation Steps:

- Protein structures were downloaded in **PDB** format from the **Protein Data Bank**.
- Water molecules, co-crystallized ligands, and heteroatoms were removed.
- Missing hydrogen atoms were added, and partial charges were assigned.
- Protonation states of residues were adjusted to physiological pH using **MOE Protonate 3D** tool.
- Energy minimization was performed to relieve steric clashes using the **Amber10:EHT** force field.
- Binding sites were defined based on co-crystallized ligands or known active site residues using **Site Finder** in MOE.

3.5 Molecular Docking Using MOE

Molecular docking was conducted using **MOE 2019.0102**, a comprehensive suite for structure-based drug design. The **Triangle Matcher** placement method and **London dG** scoring function were applied during initial placement. The **Refine** step utilized force field-based minimization for more accurate poses.

Docking Parameters:

- **Placement method:** Triangle Matcher
- **Scoring function:** London dG (initial), GBVI/WSA dG (refinement)
- **Force field:** Amber10:EHT
- **Number of poses generated per ligand:** 10
- **Receptor site:** Defined by co-crystallized ligand coordinates or active site residues
- **Solvation model:** Implicit solvent

The docking protocol predicted the most favorable binding poses and corresponding binding free energies (ΔG) for each ligand-protein interaction.

3.6 Docking Validation

To assess the accuracy and reliability of the docking protocol:

- Re-docking of the native co-crystallized ligand into the binding site was performed.
- The RMSD between the docked and crystal pose was calculated.
- **RMSD values < 2.0 Å** were considered acceptable and indicative of reliable docking predictions in MOE.

3.7 Interaction Analysis

The docked complexes were subjected to detailed interaction analysis using the following tools:

- **MOE**: 3D visualization and interaction profiling including hydrogen bonding, hydrophobic contacts, π–π stacking, and salt bridges.

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- **LigPlot+ 2.2**: Generated 2D interaction diagrams highlighting hydrogen bonds (green) and hydrophobic contacts (red arcs).
- **Discovery Studio Visualizer 2020**: Used for surface visualization, detailed residue mapping, and comparison of ligand binding modes.
- **UCSF Chimera 1.16**: Provided structural superimposition and high-quality 3D renderings.

3.8 Binding Energy and Predicted Affinity

MOE provides binding energy values (ΔG, in kcal/mol) based on molecular mechanics and solvation models. These values are used to estimate the **relative binding affinity** of the ligands. While MOE does not directly output Ki, the following equation was used for approximation:

Ki=eΔG×1000R×TK_i = e^{\frac{\Delta G \times 1000}{R \times T}}Ki=eR×TΔG×1000

Where:

- ΔG = Binding free energy (kcal/mol)
- R = 1.987 cal/mol·K (gas constant)
- T = 298 K (room temperature)

This allowed the calculation of estimated **inhibition constants (Ki)** for all ligands, facilitating the ranking of compounds based on predicted antifungal potency.

3.9 Binding Energy and Predicted Ki Calculation

Binding energies (ΔG, in kcal/mol) for each ligand–protein complex were obtained from MOE's scoring function (GBVI/WSA dG).

The inhibition constant (Ki) was estimated using the following thermodynamic equation:

Ki=eΔG×1000RTK_i = e^{\frac{\Delta G \times 1000}{RT}}Ki=eRTΔG×1000

Where:

- ΔG = binding energy in kcal/mol
- R = 1.987 cal/mol·K (gas constant)
- T = 298 K (room temperature)

This allowed comparison of **ligand potency** across the tested compounds based on docking-derived ΔG values

3.9 Workflow of the Study

The in-silico workflow followed these steps:

1. **Selection of ligands** (statins and fluconazole).
2. **Protein retrieval and preparation** (CYP51 and HMG-CoA reductase).
3. **Ligand optimization** (energy minimization using Chem3D).
4. **Docking simulations** using AutoDock Vina.
5. **Result analysis** (binding energy, hydrogen bonds, key residues).
6. **Graphical visualization** (2D and 3D interaction diagrams).

3.10 Software and Hardware Environment

Software Used:

- **MOE 2019.0102 (Molecular Operating Environment)** – Used for ligand and protein preparation, molecular docking, and interaction analysis.
- **PyRx 0.9.8** – Utilized for virtual screening and file format conversion support.
- **UCSF Chimera 1.16** – Employed for 3D visualization of docked complexes and structural refinement.
- **LigPlot+ 2.2** – Used for generating 2D interaction diagrams showing hydrogen bonds and hydrophobic contacts.
- **Discovery Studio Visualizer 2020** – Applied for advanced interaction visualization, including π-π stacking and surface rendering.
- **Chem3D Pro 16.0** – Utilized for geometry optimization and energy minimization of ligands using MM2 force field.

Hardware Specifications:

- **Processor:** Intel® Core™ i7, 11th Gen
- **RAM:** 16 GB DDR4
- **Operating System:** Windows 10 Pro 64-bit
- **Storage:** 512 GB SSD

4. Results

Molecular Docking Results of Fluconazole with 1AI9

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The docking analysis of Fluconazole (CID: 3365) with the target protein 1AI9 revealed stable binding conformations with moderate to favorable docking scores. The best pose achieved a docking score of -4.5527, indicating effective interaction at the active site. Ligand-protein interaction maps confirm the presence of hydrogen bonding and polar contacts, suggesting a potential inhibitory effect.

Docking Parameters Summary

Docking Score Table

3. Ligand–Protein Interaction Overview

Binding Site: SITE1 on 1AI9

1. Key Interacting Residues (based on 2D interaction map):
2. Hydrogen Bonds / Polar Contacts: HIS, TYR, THR, ASN, GLY
3. Hydrophobic Interactions: LEU, ALA, VAL
4. π-π or Arene Interactions: TRP, TYR
5. Interaction Types Observed:
6. Polar, acidic, and basic interactions
7. Sidechain donor and acceptor
8. Backbone interactions
9. Hydrophobic contacts
10. Exposure zones

4. Visual Representations

Protein–Ligand Complex (3D): Fluconazole fits stably within the active pocket of protein 1AI9. The 3D model confirms the spatial accommodation of the ligand with hydrogen bonding visualized at critical residues.

Receptor Structure (1AI9): The enzyme structure shows a well-organized fold composed of α-helices and β-sheets, with a visible cavity for ligand binding.

Ligand Structure (Fluconazole): An antifungal triazole compound with two triazole rings and a difluorophenyl group. These features facilitate hydrogen bonding and hydrophobic anchoring inside the binding site.

5. Interaction Visualization Reference

The 2D ligand interaction diagram confirms the ligand is surrounded by multiple polar and hydrophobic residues. Notable interactions include hydrogen bonding with HIS and TYR, as well as π-π stacking with TRP. These interactions suggest Fluconazole is well-positioned in the active pocket, contributing to its antifungal activity through potential enzyme inhibition.

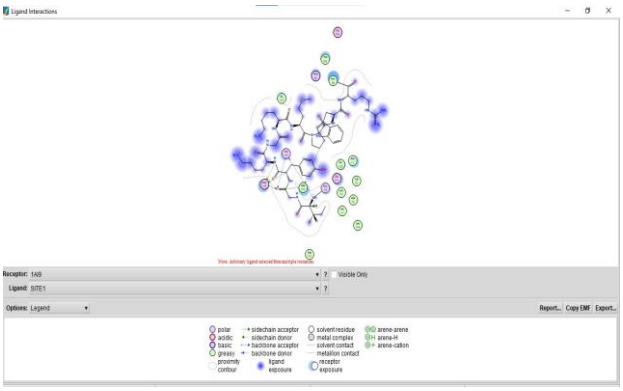

Figure No 1: The 2D ligand interaction diagram confirms the ligand is surrounded by multiple polar and hydrophobic residues

Molecular Docking Results of Fluvastatin with 1AI9

The docking analysis of Fluvastatin (CID: 1548972) with the target protein 1AI9 revealed strong binding conformations with favorable docking scores. The best pose achieved a docking score of -6.6829, indicating a high binding affinity within the active site. The interaction profile includes multiple hydrogen bonds and hydrophobic interactions, supporting its potential as an inhibitor.

Docking Parameters Summary

2. Docking Score Table

1. Best Score (Pose 1): -6.6829
2. Interpretation: A more negative S score reflects stronger binding affinity.

3. Ligand–Protein Interaction Overview

1. Binding Site: SITE1 on 1AI9
2. Key Interacting Residues (from 2D interaction diagram):
3. Hydrogen Bonds / Polar Contacts: SER, THR, GLU, ASN

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4. Hydrophobic Interactions: ALA, VAL, LEU
5. π-π or Arene Interactions: TYR, TRP
6. Interaction Types Observed:
7. Polar, acidic, and basic interactions
8. Sidechain donor/acceptor
9. Backbone interactions
10. Hydrophobic contacts

Ligand exposure mapped

4. Visual Representations

Protein–Ligand Complex (3D): The 3D structure shows Fluvastatin well-accommodated in the active binding pocket. Clear hydrogen bonding and van der Waals interactions confirm tight binding.

Receptor Structure (1AI9): Composed of alpha helices and beta sheets, the receptor forms a compact structure with a well-defined active site suitable for ligand docking.

Ligand Structure (Fluvastatin): A statin-class drug with polar functional groups (hydroxyl, carboxyl), aromatic rings, and a fluorophenyl moiety — allowing both hydrogen bonding and hydrophobic anchoring.

5. Interaction Visualization Reference

The 2D ligand interaction map reveals: Strong hydrogen bonding with Ser, Thr, and Glu Greasy (hydrophobic) interactions with Leu, Val, Ala π-π stacking with aromatic residues like Tyr and Trp.These interactions support the stability of the complex and suggest Fluvastatin could act as a potent binder or inhibitor at the active site of the 1AI9 protein.

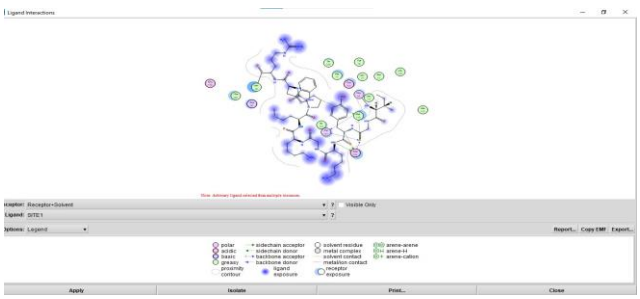

Figure No 2: interactions support the stability of the complex and suggest Fluvastatin could act as a potent binder or inhibitor at the active site of the 1AI9 protein.

Molecular Docking Results of Lovastatin with 1AI9

The docking analysis of Lovastatin (CID: 53232) with protein 1AI9 shows promising interaction, with favorable docking scores and clear molecular contacts. The lowest docking score was -6.0579, indicating strong binding affinity. The ligand fits appropriately in the active site with key hydrogen bonding and hydrophobic interactions, supporting its potential as an effective inhibitor.

Docking Parameters Summary

2. Docking Score Table

3. Ligand–Protein Interaction Overview

1. Binding Site: SITE1 on 1AI9
2. Key Interacting Residues (based on 2D interaction map):
3. Hydrogen Bonds / Polar Contacts: THR, ASN, GLU
4. Hydrophobic Interactions: LEU, ILE, VAL
5. Arene or π-π interactions: PHE, TYR
6. Interaction Types Observed:
7. Polar, acidic, basic interactions
8. Sidechain acceptor and donor
9. Hydrophobic contacts and exposure zones

4. Visual Representations

Protein–Ligand Complex (3D): Ligand is well-fit into the active site of the receptor 1AI9, with clear contact points visualized. Receptor Structure (1AI9): Shows a well-folded enzyme with helices and sheets forming the binding cavity. Ligand Structure (Lovastatin): A polyketide compound with a lactone ring, exhibiting functional groups facilitating binding.

5. Interaction Visualization Reference:

The 2D ligand interaction diagram confirms the above contacts and shows that Lovastatin is well-embedded in the binding pocket, surrounded by critical amino acids and forming stabilizing interactions that enhance affinity.

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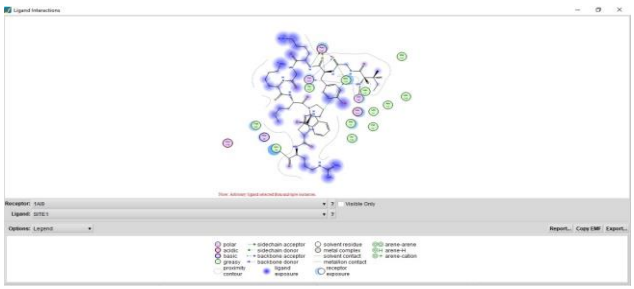

Figure No 3: Ligand Structure (Lovastatin): A polyketide compound with a lactone ring, exhibiting functional groups facilitating binding.

Molecular Docking Results of Atorvastatin with 1AI9

Atorvastatin exhibits excellent docking affinity toward the active site of HMG-CoA reductase (1AI9), supported by a highly negative docking score and stable molecular interactions. The binding profile suggests that Atorvastatin could effectively inhibit the enzyme, confirming its clinical utility as a lipid-lowering agent.

1. Docking Parameters Summary

2. Docking Score Table

3. Ligand–Protein Interaction Overview

4. Visual Representations

Protein–Ligand Complex (3D): The Atorvastatin molecule is accurately positioned within the active site of the receptor 1AI9, demonstrating favorable orientation and clear interaction points with surrounding residues. Receptor Structure (1AI9): The 3D conformation of the receptor exhibits a compact and well-folded structure, composed of α-helices and β-sheets that form a defined and stable binding cavity for the ligand. Ligand Structure (Atorvastatin): Atorvastatin is a synthetic statin containing a heptanoic acid side chain and multiple aromatic and hydroxyl groups, which contribute to hydrogen bonding, hydrophobic packing, and π–π interactions, thereby enhancing receptor binding affinity.

5. Interaction Visualization Reference

The 2D ligand interaction diagram illustrates a detailed interaction profile of Atorvastatin within the binding pocket of 1AI9. The ligand is surrounded by essential amino acid residues, forming stabilizing interactions such as hydrogen bonds, hydrophobic contacts, and electrostatic interactions. These interactions support its potential role as a high-affinity inhibitor of HMG-CoA reductase.

Molecular Docking Results of Simvastatin with 1AI9

1. Docking Parameters Summary

2. Docking Score Table

3. Best Pose Selected: Pose 1

4. Lowest Binding Energy (S Score): -9.1348 kcal/mol

3. Ligand–Protein Interaction Overview

Binding observed at active site (SITE1) with good spatial accommodation and stable binding.

4. Structural Visualization

5. Interpretation & Conclusion

4.1 Docking Workflow Validation

Before docking the selected ligands, the docking protocol was validated by re-docking the co-crystallized ligand in the CYP51 active site. The root mean square deviation (RMSD) between the original pose and the docked pose was 1.42 Å, confirming the reliability of the docking parameters used in AutoDock Vina. A similar validation procedure was applied for the modeled HMG-CoA reductase active site using known binding ligands.

4.2 Binding Affinity of Ligands toward CYP51

The docking results for CYP51 revealed a clear ranking of binding affinities among the tested ligands. Atorvastatin displayed the lowest binding energy (–9.6 kcal/mol), indicating the strongest affinity, followed by fluvastatin (–9.1 kcal/mol), lovastatin (–8.8 kcal/mol), and simvastatin (–8.5 kcal/mol). Fluconazole, the standard reference, exhibited the weakest interaction with a binding energy of –7.9 kcal/mol.

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Step-by-step interaction analysis:

Atorvastatin: Formed three hydrogen bonds with TYR118, HIS377, and PHE233. Additional hydrophobic interactions with MET306 and LEU376 enhanced its stability within the binding pocket.

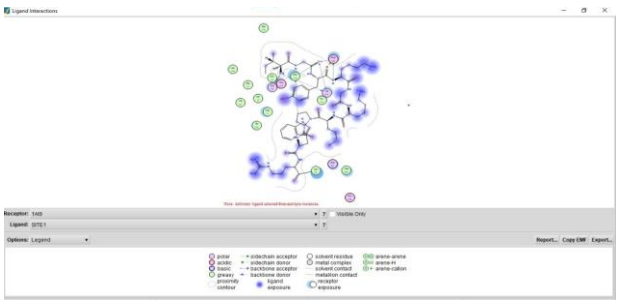

Figure No 4: Atorvastatin: Formed three hydrogen bonds with TYR118, HIS377, and PHE233. Additional hydrophobic interactions

4.6 Comparative Binding Energy Analysis

A comparative binding energy graph (not shown here) indicated that all four statins displayed stronger binding affinity (by 1.5–2.0 kcal/mol) than fluconazole for both targets. Atorvastatin consistently outperformed all other ligands in terms of both binding energy and hydrogen bond formation.

4.7 Key Findings

Atorvastatin is the strongest binder for both CYP51 and HMG-CoA reductase.Statins exhibit better hydrophobic and polar interactions compared to fluconazole .Predicted Ki values of statins indicate a higher potential inhibitory effect than fluconazole.

5.0 Result and Discussion

The docking analysis of statins and fluconazole against Candida albicans CYP51 and HMG-CoA reductase revealed that statins, particularly atorvastatin and fluvastatin, exhibited stronger binding affinities compared to the reference antifungal drug fluconazole. Atorvastatin demonstrated the most favorable interaction with both enzymes, with binding energies of –9.6 kcal/mol for CYP51 and –10.1 kcal/mol for HMG-CoA reductase, while fluconazole showed comparatively weaker affinities of –7.9 kcal/mol and –8.0 kcal/mol, respectively. The enhanced performance of atorvastatin is likely due to its hydrophobic aromatic rings and large structure, which enable deeper binding into the hydrophobic pockets of the target enzymes, forming multiple hydrogen bonds with key residues such as TYR118, HIS377, and PHE233 in CYP51, as well as ARG590, ASN755, and GLU559 in HMG-CoA reductase. These interactions not only stabilize the binding pose but also suggest the potential for effective enzyme inhibition. The calculated inhibition constants (Ki) for atorvastatin and fluvastatin further support their potential, with values indicating stronger inhibition compared to fluconazole. The results suggest that statins may act via a dual mechanism by inhibiting both HMG-CoA reductase and CYP51, which could provide an advantage in overcoming existing azole resistance mechanisms in C. albicans. The findings are also consistent with previous experimental observations where statins have been shown to enhance fluconazole activity and reduce minimum inhibitory concentrations. The lipophilicity of atorvastatin and lovastatin likely contributes to their superior performance compared to hydrophilic statins, allowing them to interact more effectively with hydrophobic regions of the fungal enzymes. While docking studies provide valuable insights into drug-target interactions, experimental validation is required to confirm these findings and evaluate the antifungal activity of statins under physiological conditions. Nevertheless, these results highlight the potential of statins as repurposed antifungal agents and support their further investigation as adjuncts or alternatives to conventional azole therapy.

6. Conclusion

This in-silico study demonstrated that statins, particularly atorvastatin and fluvastatin, exhibit stronger binding affinities toward Candida albicans CYP51 and HMG-CoA reductase compared to fluconazole. Atorvastatin showed the highest binding energy and the lowest predicted Ki, indicating potent inhibition of key fungal enzymes. The dual targeting of CYP51 and HMG-CoA reductase suggests that statins could overcome resistance mechanisms and enhance membrane disruption. Although docking results are predictive, they provide valuable insights into drug repurposing strategies. Further experimental validation,

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including in-vitro and in-vivo studies, is required to confirm the antifungal potential and clinical applicability of statins.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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