A Computational Approach of Benzimidazole Containing Pyrazoline-5-one Derivatives as Targeted Antifungal Activity

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Abstract
14α-demethylase enzyme is a primary target in treatment of fungal infections in organisms ranging from humans to plants, and development of more potent and selective 14α-demethylase inhibitors is an important biological objective. Molecular docking is routinely used for understanding drug-receptor interaction in modern drug design. Here we described the docking of benzimidazole containing pyrazoline-5-one derivatives as inhibitors of 14α-demethylase. The inhibitory activities against 14α-demethylase were investigated by molecular docking using the HEX docking software. These compounds docked into the active site of receptor (PDB code, 1E9X) using Hex docking tools software which showed good affinity for the enzyme when compared with the binding energies of standard drugs such as clotrimazole (-24.05) and griseofulvin (-36.57). Among all the designed compounds, the compound 7 shows more binding energy values (-59.85). Further we planned to synthesis these benzimidazole derivatives and screen for in-vitro anti fungal effect on different fungal organisms.

Keywords: Bioinformatics, 14α-demethylase, HEX docking, Lipinski's Rule.

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INTRODUCTION

Laboratory techniques for drug discovery are very time-consuming and expensive. Each candidate drug must be synthesized and assayed for activity on the target protein, as well as cross-reactivity with non-targets. There is therefore a great deal of interest in developing computational techniques to assist with this stage of drug development. Although they are still largely an area of research rather than production, a number of automated methods have emerged for identifying promising drug candidates. One such method is the docking of the drug molecule with the receptor. Docking approach starts with a database of known molecules and attempts to place each one in the binding pocket of the protein and, if successful, estimates the affinity of the binding using a scoring function.

In recent years, the widespread use of antifungal agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an increase in morbidity and mortality (Giulia et al. 2004). Thus, intense efforts in antifungal drug discovery are still needed to develop more promising and effective antifungal agents for use in the clinical arena (Vincent 1999). The important step in the biosynthesis of membrane sterols and steroid hormones is the oxidative removal of the 14α-methyl group from sterol precursors by sterol 14α-demethylase. Sterol 14α-demethylase is a cytochrome P450 (P450, CYP) heme thiolate containing enzyme involved in biosynthesis of membrane sterols, including cholesterol in animals, ergosterol in fungi, and a variety of C24-modified sterols in plants and protozoa in most organisms in biological
kingdoms from bacteria to animals (Aoyama 2005). 14α-
demethylase has been a therapeutic target for several
generations of azole antifungal agents including fluconazole,
voriconazole, itraconazole, ravuconazole, and posaconazole
(Sheehan et al. 1999). These drugs inhibit microbial growth
by disrupting biosynthesis of ergosterol, a major component
of fungal membrane. Protozoa share with fungi the
requirement of ergosterol and ergosterol-related sterols for
cell viability and proliferation (Roberts et al. 2003).
Inhibition of sterol biosynthesis has been proven to be
effective in trypanosomatids (Roberts et al. 2003; Urbina et
al. 1996; Maya et al. 2007) and Leishmania spp (Mishra et al.
2007), which cause such tropical diseases as African sleeping
sickness, Chagas disease, and leishmaniasis. Although
mammalian 14α-demethylase enzymes perform the same
catalytic reaction (Trzaskos et al. 1986) as their fungal and
protozoan orthologs (Aoyama 2005), they share relatively
modest overall sequence identity (within 30%) with them.

In literature review, some benzimidazole derivatives
showed potent anti fungal activity against pathogenic
organisms (Maxwell and Brody 1971; Ayhan-Kilcigil et al;
1999, Goker et al. 2006). In this study, we designed some
benzimidazole containing pyrazoline-5-one derivative as
targeted antifungal agents, based on molecular docking
between designed new inhibitors and 14α-demethylase using
HEX docking software.

MATERIAL AND METHODS

Material

For our present study we used bioinformatics tools,
biological databases like PDB (Protein Data Bank) and
software’s like Hex, ACD ChemSketch. Hex is an interactive
molecular graphics program for calculating and displaying
feasible docking modes of pairs of protein and DNA
molecules. Hex can also calculate protein-ligand docking,
assuming the ligand is rigid, and it can superpose pairs of
molecules using only knowledge of their 3D shapes (Ritchie
2003). It uses spherical polar fourier (SPF) correlations to
accelerate the calculations and its one of the few docking
programs which has built in graphics to view the result
(Ritchie et al. 2000).

The Protein Data Bank (PDB) is the single world wide
archive of structural data of biological macromolecules,
established in Brookhaven National Laboratories (Berman et
al. 2000). It contains structural information of the
macromolecules determined by X-ray crystallographic and
NMR methods. RASMOl [Raster Display of Molecules] is a
molecular graphics program intended for the structural
visualization of proteins, nucleic acids and small
biomolecules. The program reads in molecular coordinate
files and interactively displays the moleule on the screen in
variety of representations and color schemes.

Method

Computer – Aided Drug Design (CADD) is a specialized
discipline that uses computational methods to simulate drug–
receptor interactions. CADD methods are heavily dependent
on bioinformatics tools, applications and databases (Ambesi-
Impiombato and Bernardo 2006). The structure of sterol 14α-
demethylase (Figure 1) which is an essential target for novel
antifungal drug design was retrieved from protein data bank
(1E9X). All water molecules and ligands were removed from
the proteins for docking studies.

Using ChemSketch the structures of these drugs were
sketched. The docking analysis of these compounds with
1E9X was carried by using HEX docking software.

Docking allows the scientist to virtually screen a
database of compounds and predict the strongest binders
based on various scoring functions. It explores ways in
which two molecules, such as drugs and an enzyme sterol
14α-demethylase receptor fit together and dock to each other
well. The molecules binding to a receptor, inhibit its
function, and thus act as drug. The collection of drug and
receptor complex was identified via docking and their
relative stabilities were evaluated using molecular dynamics
and their binding affinities, using free energy simulations.
The parameters used in HEX for the docking process were;
• Correlation type – Shape only
• FFT Mode – 3D fast lite
• Grid Dimension – 0.6
• Receptor range – 180
• Ligand Range – 180
• Twist range – 360
• Distance Range – 40

The drug and its analogues were docked with the
receptor using the above parameters.
Lipinski's Rule of Five

Lipinski's Rule (Lipinski et al. 1997) of five is a rule of thumb to evaluate drug likeness, or determine 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 pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (ADME). It predicts high probability of success or failure due to drug likeness for molecules complying with 3 or more of the following rules (Sivakumar et al. 2010):

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as LogP less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130

These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures.

RESULTS AND DISCUSSION

Docking results between sterol 14α-demethylase (1E9X) receptor and designed benzimidazole derivatives containing pyrazolin-5-one moiety are reported in Table 1. Based on the literature it has been shown clearly that benzimidazole containing pyrazoline-5-one derivatives which can be a potent antifungal agent have been used to target sterol 14α-demethylase. The standard antifungal agents clotrimazole and griseofulvin on docking with 1E9X produce energy values of -24.05 (Figure 2) and -36.57 (Figure 3) respectively. The energy values were calculated using Hex. It was observed using RasMol that among all the designed compounds, the compound 7 containing ortho hydroxyphenyl group at 2nd position of benzimidazole is showing better binding nature, which resulted in a decrease in the energy value (Figure 4). In this study, we also calculated all five Lipinski parameters for all the designed compounds (Table 2).

<table>
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<tr>
<th>Compound docked</th>
<th>R</th>
<th>R’</th>
<th>E-value</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>-24.95</td>
</tr>
<tr>
<td>2</td>
<td>CH3</td>
<td>H</td>
<td>-53.15</td>
</tr>
<tr>
<td>3</td>
<td>-CH2C6H5</td>
<td>H</td>
<td>-40.39</td>
</tr>
<tr>
<td>4</td>
<td>-CH(OH)CH(OH)-COOH</td>
<td>H</td>
<td>-57.06</td>
</tr>
<tr>
<td>5</td>
<td>-CH=CH-C6H5</td>
<td>H</td>
<td>-44.57</td>
</tr>
<tr>
<td>6</td>
<td>-C6H4(o-COOH)</td>
<td>H</td>
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<td>H</td>
<td><strong>59.85</strong></td>
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<td>H</td>
<td>-30.43</td>
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<tr>
<td>9</td>
<td>H</td>
<td>-NO2</td>
<td>-25.22</td>
</tr>
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</tr>
<tr>
<td>11</td>
<td>-CH2C6H5</td>
<td>-NO2</td>
<td>-42.42</td>
</tr>
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<td>-CH(OH)CH(OH)-COOH</td>
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</tr>
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<td>16</td>
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</tr>
<tr>
<td>clotrimazole</td>
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<td>-</td>
<td>-24.05</td>
</tr>
<tr>
<td>griseofulvin</td>
<td>-</td>
<td>-</td>
<td>-36.57</td>
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</table>

Fig 2. Interaction and binding energy of clotrimazole with sterol 14α-demethylase (1E9X)
Fig 3. Interaction and binding energy of griseofulvin with sterol 14α-demethylase (1E9X)

Fig 4. Interaction and binding energy of compound 7 with sterol 14α-demethylase (1E9X)
Table 2 Lipinski properties of the docked ligands

<table>
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<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>Log P</th>
<th>H bond donor</th>
<th>H bond acceptor</th>
<th>Molar refractivity</th>
<th>Number of criteria met</th>
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<td>rule</td>
<td>&lt; 500</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>40-130</td>
<td>At least 3</td>
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<td>2.688</td>
<td>1</td>
<td>7</td>
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<td>360</td>
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<td>7</td>
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<tr>
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<td>7</td>
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<td>6</td>
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<td>2.636</td>
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<td>7</td>
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<td>10</td>
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<td>4.558</td>
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<td>1.531</td>
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<td>117,287</td>
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CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken sterol 14α-demethylase which is an essential target for novel antibacterial drug design. When the receptor (1E9X) was docked with Clotrimazole and griseofulvin and the energy values obtained were -24.05, -36.57 respectively. The present study also attempts a calculation of Lipinski’s rule of five to these derivatives to evaluate drug likeness, there was no violation of the rule determining drugs pharmacological activity in the body. When the designed benzimidazole containing pyrazolin-5-one derivatives were docked against the same receptor the energy values are grater than the standards for some derivatives. So it can be concluded that the designed compounds can be potent antifungal agent. In future research work we planned to synthesis these benzimidazole derivatives and screen for their in-vitro anti fungal activity.

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