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Research Article

Molecular Docking Based Virtual Design of Polysubstituted Triazoles as Cytochrome P-450 14-Alpha-Sterol Demethylase (Cyp51) Inhibitor

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Abstract

Computational ligand docking methodology, AutoDock 4.0, based on Lamarckian genetic algorithm was employed for virtual screening of a compound library with 13 entries including reference compound as fluconazole with the enzyme Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51), a potential antifungal drug target. Considering free energy of binding as a criteria of evaluation, a total of 12 compounds were predicted to be potential inhibitors of Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) and 10 compounds displayed greater binding affinities than fluconazole as Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) Inhibitor. Compound 1a & 1b were the most potent in inhibiting the Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51), in silico. Putative interactions between Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) and inhibitors were identified by inspection of docking-predicted poses. This understanding of protein–ligand interaction and value of binding energy imparts impetus to the rapid development of novel Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51).

Keywords: Polysubstituted triazole, cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51), molecular docking

Introduction

Azole antifungal agents are useful drugs and are widely used for the treatment of topic or inner mycoses, in particular AIDS-related mycotic pathologies.[1-4] It is well-known that azole derivatives block ergosterol biosynthesis, causing its depletion and accumulation of lanosterol and some other 14-methylsterols.[5-7] Such sterols alter membrane fluidity with concomitant reduction in the activity of membrane-associated enzymes, increased permeability, and inhibition of cell growth and replication.[8-9] The intermediate step of ergosterol biosynthesis is the demethylation of lanosterol performed by 14-α-lanosterol demethylase (P-450-14$_{DM}$, CYP51), a member of the enzyme cytochrome P-450 dependent superfamily,[10,11] which catalyzes the removal of 14- α -methyl group of lanosterol. Crossover inhibition of CYP51 in different species is assumed to cause undesirable side effects and is one of the reasons for the search of better, more selective agents.

The problem of specificity is commonly addressed empirically by applying computer simulation techniques such as receptor fitting and receptor mapping. In the first approach, the development of structural models for the active site of enzymes (based on primary sequence analyses and modeling by homology) is exploited. The structures of a number of P-450-dependent enzymes belonging to prokaryotic microorganisms, such as P-450cam from Pseudomonas putida,[12] P450eryF from Saccaropolyspora erythrea,[13] and P-450ter from Pseudomonas sp.,[14] have been elucidated, while only the crystal structure of a CYP51 cytochrome, a soluble orthologue from Mycobacterium tuberculosis (MTCYP51), is available until now.[15] Despite a limited sequence identity (generally 10-30%), all cytochromes P-450 show very similar secondary and tertiary structures; consequently, homology modeling studies have been performed in the past few years, starting from 3D structures of the abovementioned prokaryotic enzymes.[16-18]

Docking-based drug design by use of structural biology remains one of the most rational approaches in drug discovery process. The structured knowledge of the binding capabilities of the active site residues to specific groups on the agonist or antagonist leads to proposals for synthesis of very specific agents with a high probability of biological action.[19] Two of the most commonly used methodologies in structure-based CADD are docking and molecular mechanics.[20]

Molecular docking is a very popular method introduced to investigate molecular association and is particularly useful in the drug discovery field to study the binding of small molecules (ligands) to macromolecules (receptor).[21] This involves two key components, namely the search algorithm and scoring function; former positions the molecules in orientations and conformations

within the active sites, while latter one determining if the orientation chosen by search algorithm is most energetically favorable.[22] A rigorous search algorithm would exhaustively elucidate all possible binding modes between ligand and receptor. The essence of GA is the evolution of a population of possible solution via genetic operators to final population, optimizing a predefined fitness function.[23] Autodock 4.0 uses GA as a global optimizer combined with energy minimization as a local search method.[24]

Materials and methods

Materials

The macromolecule, Cytochrome P-450 14-Alpha-Sterol Demethylase (Cyp51) model was built by using AutoDock Tools-1.4.6 and MGL Tools-1.5.4 packages (The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037) running on LINUX, (FEDORA-2008)

Ligand receptor modeling

Acd/3d viewer Version 8.0 for microsoft windows was used to draw the 2D structures of different ligands. Ligands were further refined and cleaned in 3D by addition of explicit hydrogens and gradient optimization function of Prodrg server. All the structures were written in pdb format. Input molecules files for an AutoDock experiments must confirm to the set of atom types supported by it. AutoDock requires that ligands got partial atomic charges and AutoDock atom types for each atom; it also requires a description of the rotatable bond in the ligand. This set consists of united atom aliphatic carbons, aromatic carbons in cycles, polar hydrogens, hydrogen-bonded nitrogen, and directly hydrogen-bonded oxygen among others, each with partial charges. Therefore, pdbqt format was used to write ligands, recognized by AutoDock.

Torsional degree of freedom (TORSDOF) is used in calculating the change in the free energy caused by the loss of torsional degree of freedom upon binding. In the AutoDock 4.0 force field, the TORSDOF value for a ligand is the total number of rotatable bonds in the ligand. This number excludes bonds in rings, bonds to leaf atoms, amide bonds, and guanidinium bonds.

It consists of several steps. First, the 3D crystal structure of Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) (Fig. 1); PDB entry 1H5Z was downloaded from Brookhaven protein data bank (PDB; http://www.rcsb.org/pdb), and subsequently sequence identity was determined by following the process provided in the website as follows. http://blast.ncbi.nlm.nih.gov/Blast.cgi. As a result of the search more than 30% similarity was noticed, therefore homology modeling was not performed with the enzyme under study. The details of the percent similarity has been shown in the following Table 1 and loaded to python molecular viewer.

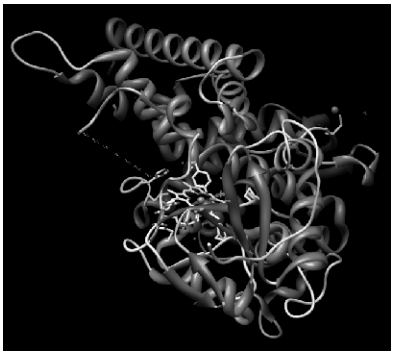


Fig.1: Native structure of Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) (PDB ID: 1H5Z)

Table 1: Sequence alignment output of Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51)

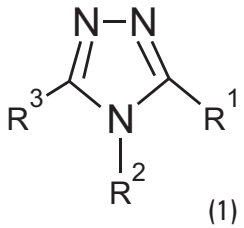
Accession	Description	Max score	Total score	Query coverage	E value	Max identity
NP_000777.1	lanosterol 14-alpha demethylase isoform 1 precursor [Homo sapiens]	243	243	95%	3e-77	33%
NP_001139624.1	lanosterol 14-alpha demethylase isoform 2 [Homo sapiens]	230	230	89%	3e-73	34%

The nonbonded oxygen atoms of waters, present in the crystal structure were removed. After assigning the bond orders, missing hydrogen atoms were added, then the partial atomic charges was calculated using Gasteiger–Marsili method (Gasteiger). United atom charges were assigned, non-polar hydrogens were merged, and rotatable bonds were assigned, considering all the amide bonds as non-rotatable. The receptor file was converted to pdbqt format, which is pdb plus "q" charges and "t" AutoDock type. (To confirm the AutoDock types, polar hydrogens should be present, whereas non-polar hydrogens and lone pair should be merged, each atom should be assigned Gasteiger partial charges).

Molecular docking studies

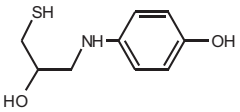
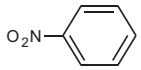
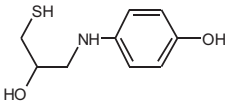
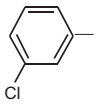
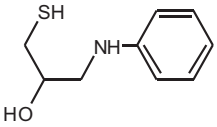
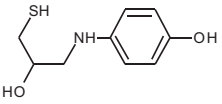
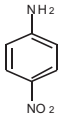
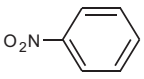
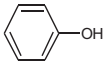
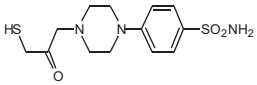
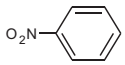
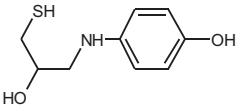
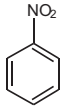
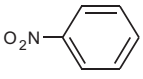
Prior to actual docking run, AutoGrid 4.0 was introduced to pre-calculate grid maps of interaction energies of various atom types. In all dockings, a grid map with 100*100*100 points, a grid spacing of 0.675 A° (roughly a half of the length of a carbon–carbon single bond) were used, and the maps were

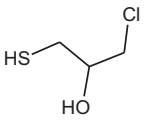
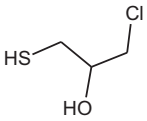
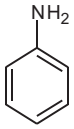
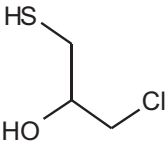
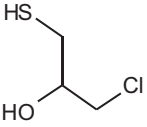
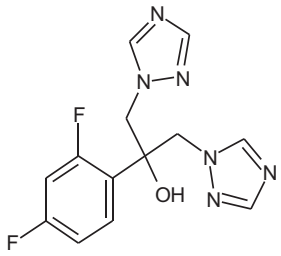
centered on the ligand binding site. In an AutoGrid procedure, the protein is embedded in a 3D grid and a probe atom is placed at each grid point. The energy of interaction of this single atom with the protein is assigned to the grid point. An affinity grid is calculated for each type of atoms in the substrate, typically carbon, oxygen, nitrogen, and hydrogens as well as grid of electrostatic potential using a point charge of 1 as the probe. Autodock 4.0 uses these interaction maps to generate ensemble of low energy conformations. It uses a scoring function based on AMBER force field, and estimates the free energy of binding of a ligand to its target. For each ligand atom types, the interaction energy between the ligand atom and the receptor is calculated for the entire binding site which is discretized through a grid. This has the advantage that interaction energies do not have to be calculated at each step of the docking process but only looked up in the respective grid maps. Since a grid map represents the interaction energy as a function of the coordinates, their visual inspection may reveal the potential unsaturated hydrogen acceptors or donors or unfavorable overlaps between the ligand and the receptor. Of the three different search algorithms offered by AutoDock 4.0, the Lamarckian Genetic algorithm (LGA) based on the optimization algorithm was used, since preliminary experiments using other two (Simulated annealing and genetic algorithm) showed that they are less efficient, utilizes Lamarckian notation that an adaptations of an individual to its environment can be inherited by its offspring. For all dockings, 40 independent runs with step sizes of 0.2Å for translations and 5Å for orientations and torsions, an initial population of random individuals with a population size of 150 individuals, a maximum number of 2500000 energy evaluations, maximum number of generations of 27,000, an elitism value of 1, and a number of active torsion of 8 were used. AutoDock Tools along with AutoDock 4.0 and AutoGrid 4.0 was used to generate both grid and docking parameter files (i.e., gpf and.dpf files) respectively.



(1)

Table 2: Docking output of compounds 1(a-l) and reference compound, fluconazole

Cmpnd (1)	R^1	R^2	R^3	Free energy of binding (Kcal/mole)	Surrounding residue
a.		$-NH_2$		-8.59	PRO 215
b.		$-CH_3$		-8.26	GLN 374, LEU 378.
c.		$-NH_2$		-8.15	PRO 422, ARG 443.
d.				-8.02	LYS154
e.	SH	$-NH_2$		-7.91	LYS 232, GLU 234.
f.	SH	$-NH_2$		7.73	PHE 88,LEU 99, GLU 97, MET 248.
g.		$-NH_2$		-7.62	ASP 161,GLY 162, GLU 141,ARG 163.
h.				-7.56	GLU 141, GLY 162, ARG 163, ASP 161.

Cmpnd (1)	R^1	R^2	R^3	Free energy of binding (Kcal/mole)	Surrounding residue
I.		$-NH_2$		-7.48	GLN 302, LEU 288, GLU 287.
j.				-7.28	GLU 35, PRO 6, ARG 7.
k.		NH_2	$-CH_3$	-6.96	GLY 329, PHE 331, ASP 68, LYS 323, GLU 330, LYS 328.
l.		NH_2	$-CH_2-CH_3$	-5.98	LEU 185, ASP 182, TYR 184.
FLUCONAZOLE(Referene)				-7.04	GLY 83, PHE 82, THR 79, LYS 73.

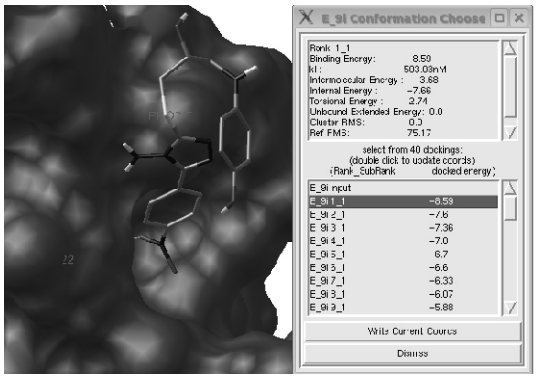


Fig.2: Molecular surface view of compound 1a docked within the binding pocket of the enzyme 1H5Z

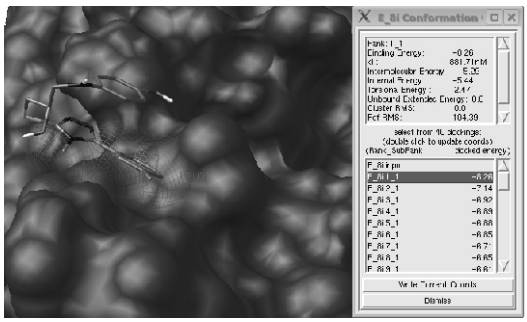


Fig.3: Molecular surface view of compound 1b docked in the binding pocket of the enzyme1H5Z

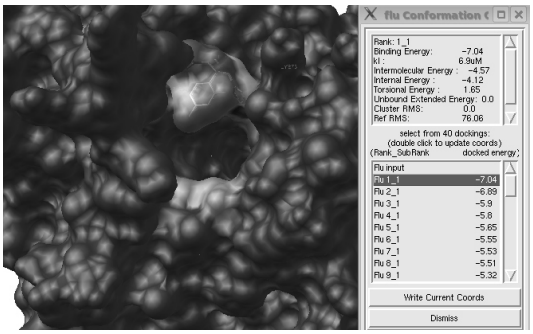


Fig.4: Molecular surface view of reference compound, fluconazole docked in the binding pocket of the enzyme 1H5Z

Conclusion

The results of LGA docking experiments of the Polysubstituted Triazoles using AutoDock 4.0 and AutoGrid 4.0 are summarized in Table 2. For each docking experiment, the lowest energy docked conformation was selected from 40 runs. The successful completion of docking experiment took 2 hours, on a 2.0 GHz Intel (R) core 2 duo machine with 3.0 GB of RAM and LINUX (FEDORA-2008) operating system.

In order to evaluate accuracy of docking, binding energy and numbers in cluster was used. 10 molecules showed better

inhibition potential than fluconazole as Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) Inhibitor with binding energy -7.04 kcal/mole. The chemical structures of all the 13 molecules including the reference are shown in the Table 2. Modeling and docking analysis revealed the nature of the active site and some key interactions that enabled the binding of Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) Inhibitor to the active site. Among all molecules (12) screened, the docking interactions of compound 1 with PRO 215 appeared to be in close proximity and explains the high Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) selectivity observed. Docking poses and binding interactions of and the first two highest binding energy compound (1, 2) and fluconazole are shown in Fig. 2-4.

This study contributes molecular insight into the binding process, which is of great pivotal importance for designing new ligands interfering with Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) and shows that new wave of flexible ligand docking program like Auto dock can produce unbiased docking of Cytochrome P-450-14-Alpha-Sterol Demethylase (Cyp51) inhibitors in the enzyme active site. The binding interactions revealed from docking poses provide the clues for the design of new molecules thus giving insight on structural requirement for designing more potent analogs.

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