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Research Article

Insilico Design of Few Allicin Derivatives

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Abstract

In search for a good lead, the contribution of natural products cannot be ignored. In our present study, considering the therapeutic importance of allicin, we have designed a series of 22 compounds based on the pharmacophoric features of allicin. Prior directing the study towards synthetic chemistry, as medicinal chemists were often confronted with painfully disappointing results and the realization dawned that the chemical space was too vast to be systemically explored experimentally. We have screened all the 22 compounds through molecular docking. In an effort to develop novel antifungal, the target enzyme chosen for the study is cytochrome P450 enzyme lanosterol-14-α-demethylase of Candida albicans. Binding mode analysis between docked compounds and the protein were analyzed using AutoDock 4.0. The conformer of compounds SB6, SB7, SB8 and SB9 were found in close proximity with the active site residue and their binding energy were comparable to the reference standard, allicin.

Keywords: Allicin, autodock, *Candida albicans*, 1EA1

Introduction

Virtual screening via in silico design has recently emerged as a robust technique complementing traditional HTS technologies. Virtual screening can be broadly defined as the use of computational analysis of a database of chemical structures to identify possible drug candidates for a specific biological target, preferentially an enzyme or receptor. This eventually makes the discovery process smoother in terms of time and economy. Thus, researchers are continually seeking to develop novel methodologies for the efficient discovery of new chemical scaffolds for further optimization. Nature has provided us with a huge affluence of intricate molecular scaffolds unparalleled in function and diversity. In the recent past, natural products have gained a considerable attention in the field of computer assisted drug design. The emerging use of computer-aided high-throughput molecular docking methodologies to identify bioactive natural products against important therapeutic targets further boosted the process to be executed incessantly. The natural products have been refined over evolutionary time scales for optimal interactions with macromolecules. Virtual screening studies were performed with various docking methods on natural products [1]. Our present study aims at exploring the bonding skeleton of allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities, including antibacterial; against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of Escherichia coli; antifungal activity, particularly against Candida albicans; antiparasitic activity, including some major human intestinal protozoan parasites such as Entamoeba histolytica and Giardia lamblia; and antiviral activity [2]. C. albicans, a systemic fungus, is a major cause of morbidity and mortality in patients immunocompromised as a result of AIDS, cancer chemotherapy, radiotherapy, organ transplantation, or bone marrow transplantation. First line treatment involves severe side effects especially in immunocompromised patients or those who receive repeated dosing to treat recurrent infections.

Allicin has also been shown to increase oxidative stress, reduce glutathione levels, and inhibit biofilm formation in C. albicans [3-4]. In addition, it was recently suggested that allicin could increase the activity of Cu^{2+}, which is a known promoter of antimicrobial activity; allicin works by accelerating the production of endogenous reactive oxygen species (ROS) [5].

Although, allicin has marked antimicrobial effects, it has limited clinical applications, because the minimal inhibitory concentration (MIC) of allicin is relatively high [6].

The therapeutic diversity of allicin leaves an immense scope for optimization and it also directs us to proceed with a virtual screening prior delivering it to the bench chemist. Since the biomolecular mechanism of antifungal drugs have already been established, which further ease the selection of biological target as the crystallographic structure of the enzyme lanosterol-14-α-demethylase of Candida albicans (PDB entry; 1EA1) is shown in Figure 1.

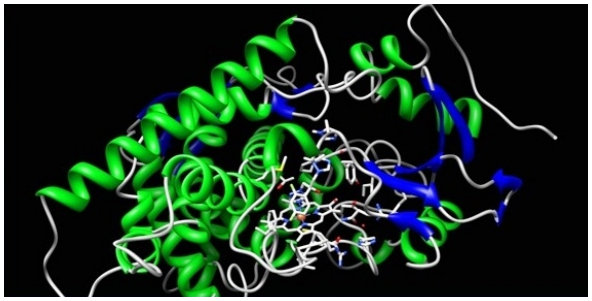
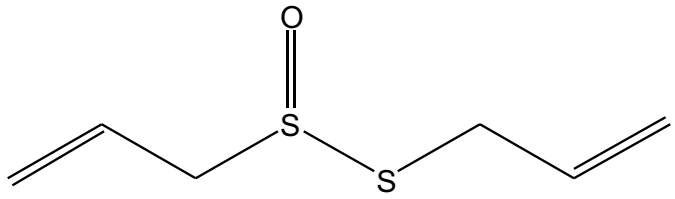


Figure 1: 3D crystal structure of chimeric 1EA1

Computational methods amalgamated with ever rising number of protein structures shift the research paradigm towards macromolecule based drug design, driven by binding mode analysis aided by molecular docking has drawn a considerable attention in drug discovery [7-8]. Molecular-docking methodologies ultimately seek to predict the best mode by which a given compound will place itself within the binding site of a macromolecule. Docking, as a result, usually involves two independent steps: (1) positioning the ligands in orientations and conformations and (2) the scoring of the ligand's

pose such that the ranking typically is an arbitrary reflection of how well a ligand is expected to bind to its complementary residues within the binding sites of the receptor. The re-emergence of such in silico-based screening methods is of practical importance for lead-compound generation in drug discovery. The Docking output has now been proved essential tools that enable computational chemists to rapidly screen large small molecules library and thereby identify promising candidate compounds for further experimental processing.

All sampling methods are guided by a function that evaluates the fitness between the protein and ligand. A rigorous search algorithm would exhaustively elucidate all possible binding modes between ligand and receptor. Autodock 4.0 uses GA as a global optimizer combined with energy minimization as a local search method. Our present study is directed towards developing novel lanosterol-14-α-demethylase inhibitors considering the molecular framework as diallyl thiosulphinate of a natural compound, commonly known as allicin.



Materials and Methods

Materials

FEDORA-8 (LINUX), SYBYL 7.1, AutoDock Tools- 1.5.4, the crystallographic structure of the enzyme Mycobacterium tuberculosis-CYP51(PDB entry code 1EA1), Chimeric 1EA1.

Methods

Chimeric 1EA1 modeling

The enzyme model was developed by using AutoDock Tools- 1.5.4 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 8.0. The crystallographic structure of the enzyme Mycobacterium tuberculosis-CYP51 in Figure 2 was also obtained from the Brookhaven Protein Data bank (PDB entry code 1EA1; http: //www.rcsb.org/pdb) [9]. As the enzyme was procured from Mycobacterium tuberculosis, few residues of it has been mutated to develop an enzyme which is having a higher homology between the same enzymes of Candida albicans. Missing atoms in the crystal structure were added and the structure was optimized and displayed in python molecular viewer. 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 [10]. Kollman [11] united atom charges were assigned, non-polar hydrogens were merged, and rotatable bonds were assigned, keeping 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).

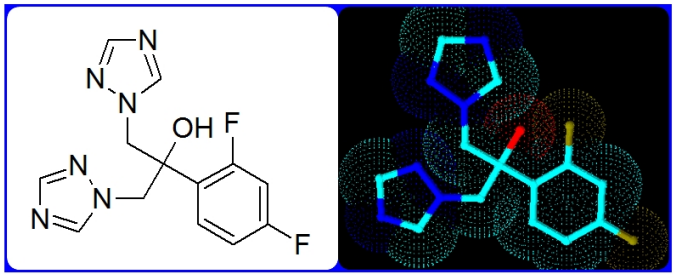


Figure 2. Stereoview of the co-crystallized ligand fluconazole

Validation of the Docking Protocol in Autodock

The most useful method of evaluating the accuracy of a docking procedure is to determine how closely the least energy conformation predicted by the scoring function resembles an experimental binding mode. In our present study for the enzyme 1EA1, the validation of the docking method was carried out by redocking fluconazole into the active site of chimeric 1EA1. The structures of the newly synthesized compounds were drawn and optimized using PRODRG online server [12] and saved in PDB format. Autodock was able to reproduce the experimental binding conformation of fluconazole within a minimal root-mean-square deviation (RMSD) of 1.599. This indicated the reliability of the docking method in reproducing the experimentally observed binding mode for COMT.

Ligand Receptor Modeling

All the 2D structures were drawn and refined in prodrg. 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. Input molecules files for an Autodock experiments must confirm to the set of atom types supported by it. 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.

Molecular Docking Studies

AutoGrid 4.0 [13] was introduced to pre-calculate grid maps of interaction energies of various atom types in all dockings, a grid map with 126*126*126 points, a grid spacing of 0.900 Å (roughly half of the length of a carbon–carbon single bond) were used, and the maps were centered on the macromolecule. 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 studied for each type of atoms in the substrate, typically carbon, nitrogen, oxygen, and hydrogens as well as grid of electrostatic potential using a point charge of +1 as the probe [14-15]. Autodock 4.00 [16-17] 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 (discredited) Lamarckian notation that an adaptations of an individual to its environment can be inherited by its offspring. For all dockings, 50 independent runs with step sizes of 0.2 Å for translations and 5 Å for orientations and

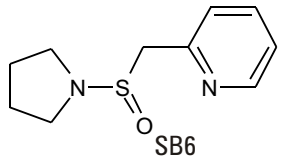
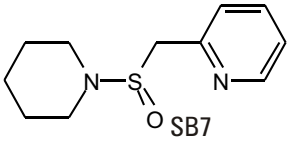
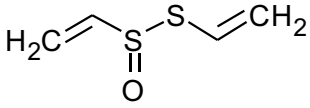
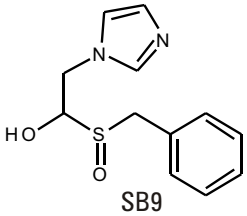
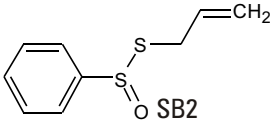
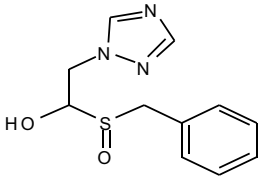
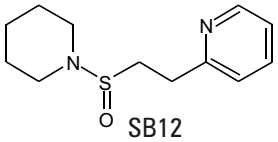
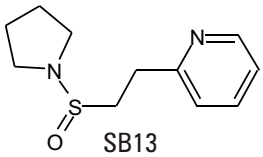
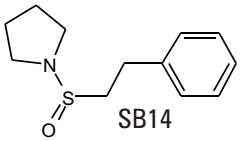
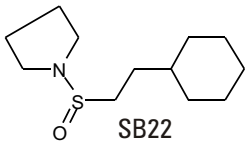
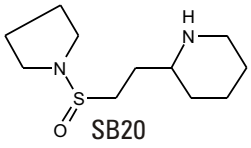
torsions, an initial population of random individuals with a population size of 150 individuals, a maximum number of 2.5*106 energy evaluations, maximum number of generations of 27,000, an elitism value of 1, and a number of active torsion of 5 were used. AutoDock Tools along with AutoDock 4.0 and Auto-Grid 4.0 was used to generate both grid and docking parameter files (i.e., gpf and.dpf files) respectively.

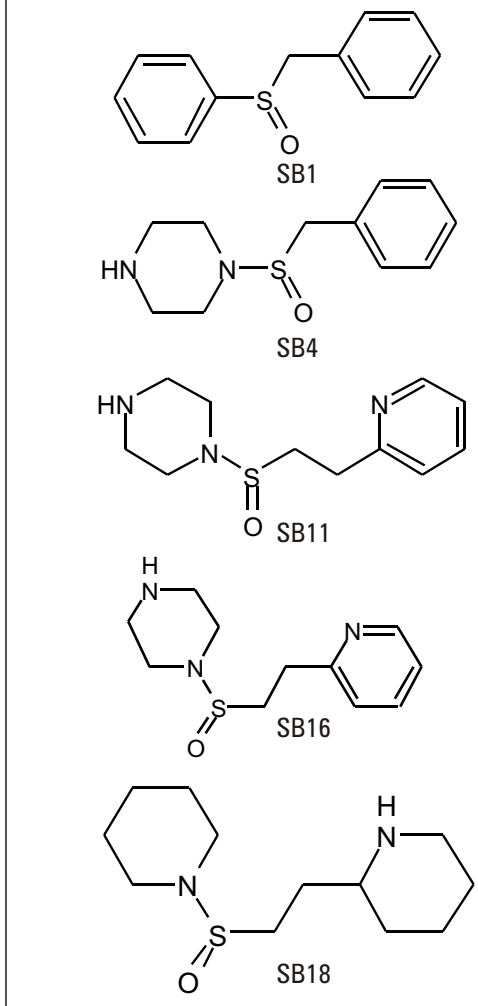
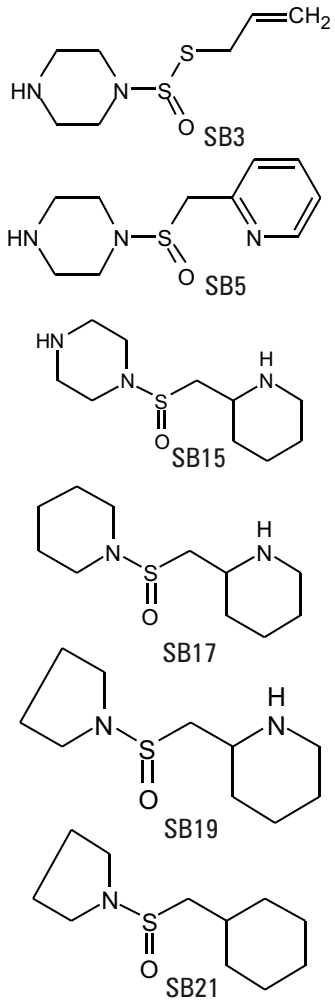
The molecular docking studies were carried out using Autodock 4.0.1 [18-22] installed on a single machine running on a 3.4 GHz Pentium 4 processor with FEDORA-8 (LINUX) as the operating system. The X-ray co-crystal structure (Protein Data Bank entry code 1EA1) of Lanosterol-14-α-demethylase with inhibitor as fluconazole was used as the initial structure for the computational studies. All the residues within 7 Å core from fluconazole were used to define the active site. The starting conformations for docking studies were obtained using molecular dynamics with simulated annealing by heating at 1000 K for 1 ps and then cooling at 200 K for 1ps as implemented in SYBYL 7.1. The AutodockTools package version 1.5.4 was employed to generate the docking input files and to analyze the docking results. For the docking, a grid spacing of 0.900 Å and 126*126*126 number of points was used. The grid was centered on the allosteric site, using the TPF 470 crystallographic position as reference. The AutoGrid program generated separate grid maps for all atom types of the ligand structures plus one for electrostatic interactions. Autodock generated 50 possible binding conformations, i.e., 50 runs for each docking by using Lamarckian Genetic Algorithm (LGA) searches. A default protocol was applied, with an initial population of 150 randomly placed individuals, a maximum number of 2.5*105 energy evaluations, and a maximum number of 2.7*104 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used.

Results and Discussion

The binding mode of the highest active test compounds 2-[(pyrrolidin-1-ylsulfinyl)methyl]pyridine(SB6), 2-[(piperidin-1 ylsulfinyl)methyl]pyridine(SB7), S-ethenyl ethenesulfinothioate(SB8) and 1-(benzylsulfinyl)-2-(1H-imidazol-1-yl)ethanol(SB9) with respect to distance from HEM and binding energy was investigated by docking studies. Ligand structures were drawn and optimized using PRODRG online server and saved in PDB format. In order to understand the binding mode of these compounds, Autodock 4.0.1 program was used to dock test compounds SB1-SB22 (Table 1) into the active site of cytochrome P450 enzyme 14- sterol demethylase of *Candida albicans* (Candida P450DM) (Chimeric 1EA1).

Table 1. The structures of different cytochrome P450 enzyme inhibitors

Type of Inhibitors	Structures of the inhibitors	
Potent inhibitors	 SB6	 SB7
	 SB8	 SB9
Moderate inhibitors	 SB2	 SB10
	 SB12	 SB13
	 SB14	
	 SB22	 SB20

Type of Inhibitors	Structures of the inhibitors	
Weak inhibitors		

Since no experimental structural information on the active site of the target enzyme Candida P450DM is available. A persual of the literature showed that high homology exists between the mycobacterium P450DM and Candida P450DM. Following the method of Rossello et al [23], the chimeric enzyme for the Candida albicans (CACYP51) was constructed from that of mycobacterium P450DM (MT CYP51) extracted from the protein data bank (PDB entry code 1EA1). The residues that were arranged in a range of 7Å from fluconazole were substituted with those of Candida P450DM is shown in Figure 3. Substitutions were made by replacement of the residues Pro77, Phe78, Met79, Arg96, Met99, Leu100, Phe255, Ala256, His258, Ile322, Ile323 and Leu324 by Lys77, His78, Leu79, Leu96, Lys99, Phe100, Met255, Gly256, Gln258, His322, Ser323 and Ile324, which were thought to be necessary for the ligand-receptor interaction.

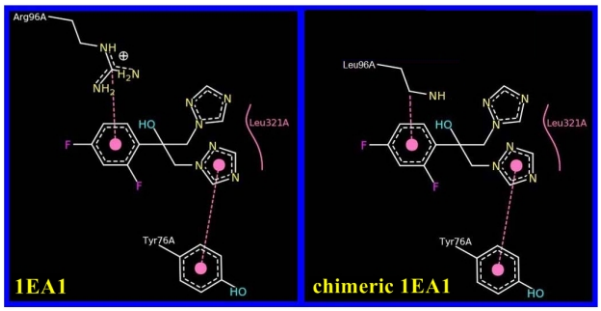


Figure 3: Binding mode interaction view of 1EA1 and chimeric 1EA1

Initially, the docking of fluconazole in the active site of chimeric 1EA1 was performed to ensure the validity of docking calculations, reliability and reproducibility of the docking parameters for our study is shown in Figure 4. Autodock successfully reproduced the

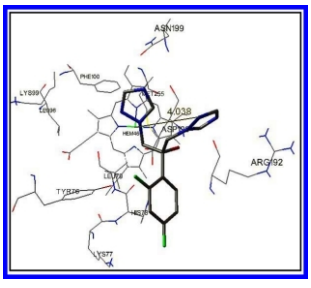


Figure 4: Redocked mode of co-crystallized ligand fluconazole in the active site of cytochrome P450 enzyme 14--sterol demethylase of *Candida albicans*(Candida P450DM) (chimeric 1EA1). Ligand is shown as stick model and the amino acid residues interacting with the ligands are shown as line model

experimental binding conformations of fluconazole in the binding pocket of chimeric 1EA1 with an acceptable root-mean-square deviation (RMSD) of 1.602 Å (<3Å).

As the bio-molecular mechanism of inhibition of cytochrome P450 enzyme, lanosterol-14-α-demethylase by any natural product has not been established, it has been assumed that the natural product, (here we have considered Allicin) may have some similarity with respect to their mechanism of inhibition with that of existing antifungal azoles. Hence, it was thought worthwhile to investigate the binding mode of the highest active test compounds, 2-[(pyrrolidin-1 ylsulfinyl)methyl]pyridine(SB6), 2-[(piperidin-1-ylsulfinyl)methyl]pyridine(SB7), S-ethenyl ethenesulfinothioate(SB8) and 1-(benzylsulfinyl)-2-(1H-imidazol-1-yl)ethanol(SB9) in the active site of chimeric enzyme of cytochrome P450-14-α-sterol demethylase from *Candida albicans* (Candida P450DM)(chimeric 1EA1). All the ligand molecules taken for molecular docking study were designed considering the pharmacophoric features of allicin is featured in Figure 5.

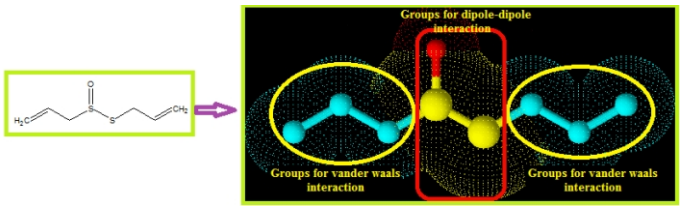
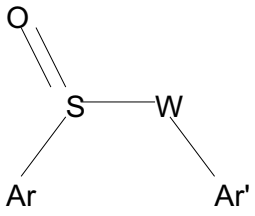


Figure 5: 2D structure and pharmacophoric features of allicin

Following the molecular features of allicin, with respect to molecular fragments and their relative position, the base component thus generated assumes the following form:



Thus, the estimated binding free energy and predicted inhibitory constant values for fluconazole was -6.44 kcal/mol and 20.26 μM. The estimated binding free energy and predicted inhibitory constant values for the highly active test compounds SB6, SB7, SB8 and SB9 were -2.94 kcal/mol and 6.96 mM, -3.02 kcal/mol and 6.10 mM, -3.17 kcal/mol and 4.77 mM and -3.58 kcal/mol and 2.36 mM respectively. It was worth mentioning that the experimental results of the highest active test compounds correlated well with the estimated binding free energy and predicted inhibitory constant values in that the highest active test compounds showed inhibitory activity comparable to reference stadard allicin. Binding mode analysis of the potent inhibitors in the active site of chimeric 1EA1 showed that the test compounds were very close to the porphyrin ring, HEM 460, similar as that of the co-crystallised ligand fluconazole. Docking poses of both the test compounds suggest the presence of active site residues ARG95, LEU 96, LYS99, PHE165, PHE169, CYS 394, HIS 318, ARG 427 as in case of fluconazole.

In order to evaluate the accuracy of docking study, binding energy and the closeness of distance between the essential biomolecular fragments and the ligand under study have been prioritized. Compound SB9 had shown promising binding energy, even superior to the reference standard, allicin. The structural details of all the 22 compounds are shown in the Table 2.

Table 2. Predicted computational details of compounds considered for the study

Compound code/ Conformer No	Ar	W	Ar´	Distance (Å)	Binding Energy	Inhibition constant(KI)	Docking rank
SB1/50	phenyl	CH_2	phenyl	15.52	-1.91	39.57 mM	21
SB2/27	phenyl	S	allyl	12.36	-2.18	25.18 mM	20
SB3/33	piperazinyl	S	allyl	15.24	-3.66	2.06 mM	7
SB4/15	piperazinyl	CH_2	phenyl	18.83	-4.12	961.76 μM	4
SB5/18	piperazinyl	CH_2	2-pyoliridyl	18.19	-4.11	976.78 μM	5
SB6/32	pyrrolidinyl	CH_2	2-pyridyl	5.39	-2.94	6.96 mM	15
SB7/34	piperidinyl	CH_2	phenyl	4.52	-3.02	6.10 mM	14
SB8/12	vinyl	S	vinyl	3.70	-3.17	4.77 mM	13
SB9/2	1-hydroxy-2-(1-imidazolyl) -ethyl	CH_2	phenyl	4.19	-3.58	2.36 mM	8
SB10/15	1-hydroxy-2-(1,2,4 -trizole-1yl)-ethyl	CH_2	phenyl	7.33	-2.76	9.55 mM	17
Sb11/-	piperazinyl	$(CH_2)_2$	2-pyridyl	-	-	-	-

Compound code/ Conformer No	Ar	W	Ar´	Distance (Å)	Binding Energy	Inhibition constant(KI)	Docking rank
SB12/48	piperidyl	$(CH_2)_2$	2-pyridyl	6.66	-1.85	44.41 mM	22
SB13/19	pyrrolidinyl	$(CH_2)_2$	2-pyridyl	11.46	-2.57	13.02 mM	18
SB14/7	pyrrolidinyl	$(CH_2)_2$	Phenyl	13.64	-3.35	3.52 mM	10
SB15/50	piperazinyl	CH_2	2-piperidyl	24.08	-3.21	4.46 mM	12
SB16/26	piperazinyl	$(CH_2)_2$	2-piperidyl	18.76	-4.44	561.17 μM	2
SB17/12	piperidyl	CH_2	2-piperidinyl	18.08	-4.43	562.2 μM	3
SB18/40	piperidyl	$(CH_2)_2$	2-piperidinyl	16.70	-3.3	3.82 mM	11
Sb19/-	pyrrolidinyl	CH_2	2-piperidinyl	-	-	-	-
SB20/13	pyrrolidinyl	$(CH_2)_2$	2-piperidinyl	12.80	-3.91	1.35 mM	6
SB21/16	pyrrolidinyl	CH_2	cyclohexyl	16.90	-2.82	8.53 mM	16
SB22/42	pyrrolidinyl	$(CH_2)_2$	cyclohexyl	13.42	-2.34	19.33 mM	19
Allicin	reference standard			4.15	-3.55	2.5 mM	9
Fluconazole (TPF 470)	co-crystallized ligand			4.038	-6.44	20.26 μM	1

such a way that every structure under study must avail the essential chemical component responsible for van der waals interaction and dipole dipole interactions. Synthetic possibilities of all the compounds were taken care of prior going for the main course.

All the 22 compounds excluding the reference as allicin were screened, the docking interactions of 2-[(pyrrolidin-1-ylsulfinyl)methyl]pyridine(SB6), 2- [(piperidin-1-ylsulfinyl) methyl] pyridine (SB7), S-ethenyl-ethenesulfinothioate(SB8), 1-(benzylsulfinyl)-2-(1H-imidazol-1-yl) ethanol (SB9) with the active site residues, like Tyr 76, Leu 96 and Leu 321 appeared to be in proximity and explains the higher selectivity to the enzyme. The docking poses of highest active compounds were indeed very close to the ferrous ion of HEM 460, the main protagonist in drug receptor interaction especially for antifungal azoles. It further confirms the possibilities of ionic interaction. Docking poses of all the potent inhibitors and allicin are shown in Figures 6 – 10.

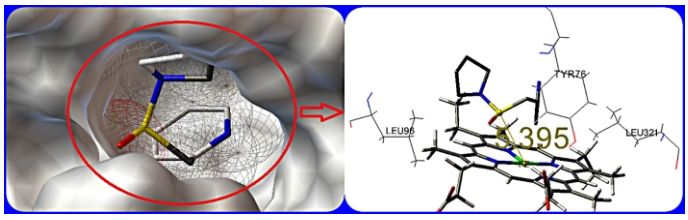


Figure 6: Molecular surface and stereo view of the docking predicted pose of bioactive conformer of compound 6 within the active site of chimeric 1EA1

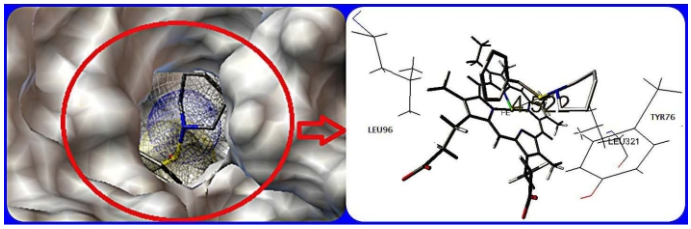


Figure 7: Molecular surface and stereo view of the docking predicted pose of bioactive conformer of compound 7 within the active site of chimeric 1EA1

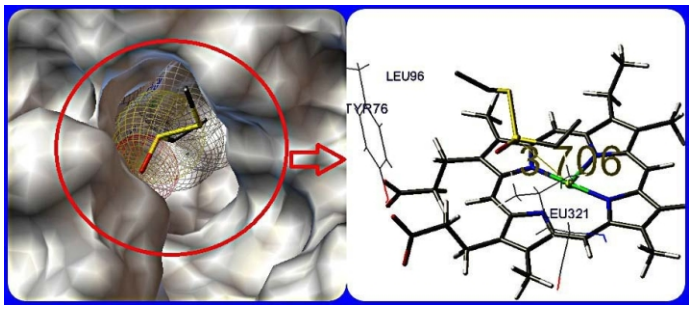


Figure 8: Molecular surface and stereo view of the docking predicted pose of bioactive conformer of compound 8 within the active site of chimeric 1EA1

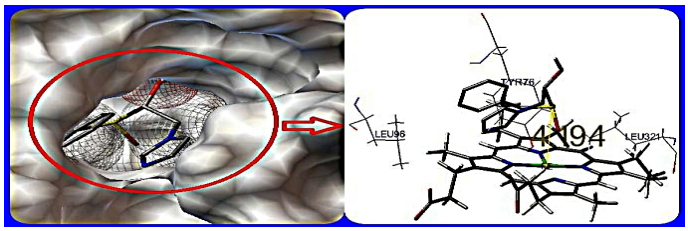


Figure 9: Molecular surface and stereo view of the docking predicted pose of bioactive conformer of compound 9 within the active site of chimeric 1EA1

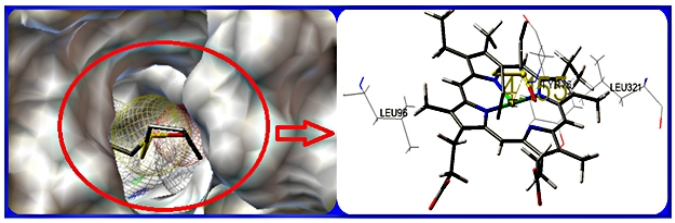


Figure 10: Molecular surface and stereo view of the docking predicted pose of bioactive conformer of the reference standard, allicin within the active site of chimeric 1EA1

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