Insilico docking for validation of drug leads on Superoxide dismutase of *Homo sapiens* and *Plasmodium falciparum*.

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Abstract

Superoxide dismutases (SODs) are metalloproteins which bring about dismutation of superoxides and prevent biological damage of cellular components during oxidative stress. The malarial parasite *plasmodium falciparum* has a well developed defense system which includes superoxide dismutase, to scavenge free radicals and combat oxidative stress during the erythrocytic stage of its life cycle. Alignment studies of the primary structure of *plasmodium falciparum* SOD (*Pf*Fe-SOD) have shown it to be structurally distinct from human cytosolic copper-zinc SOD1 (Cu-ZnSOD1). This feature makes it a potential target for therapy. A search for new targets as well as drugs is essential due to the phenomenon of drug resistance to chloroquine which is the drug of choice for treatment of malaria. A few potential inhibitors from the library of synthetic molecules have already been studied using recombinant *Pf*Fe-SOD by Soulere et al. Three of the fifteen effective lead molecules SP72, SP13 and SP59 show significant inhibition of recombinant *Pf*Fe-SOD. However, no attempt was made to investigate whether these lead molecules interact with the host Cu-ZnSOD1. In this study, the lead molecules showing significant inhibition were prepared using Marvin sketch. Cu-ZnSOD1 and *Pf*Fe-SOD was docked to these lead molecules and the energy values obtained. The results indicate almost equal affinity of the lead molecules to both, host and parasite, SOD. In conclusion, this study shows that compounds which are found to be quite effective inhibitors of recombinant enzyme *in vitro* need to be validated by alternate methods since, in the living system, compounds tend to deviate from their *in vitro* behavior in an unpredicted way. Computational tools such as insilico docking provide the scientist with an alternate base for validation of lead molecules.

Introduction

Generation of reactive oxygen species (ROS) results in oxidative stress which is responsible for the damage of cellular components [1]. Dismutation of superoxides can prevent such biological damage. Superoxide dismutases (SOD's), catalases and glutathione peroxidases are some of the enzymes which catalyse dismutation of superoxide free radicals [2]. The malarial parasite *Plasmodium falciparum* is exposed to oxidative stress during the erythrocytic stages of its life cycle [3]. The parasite has a well developed defense system with SOD and thioredoxin dependent peroxidases to scavenge free radicals generated in oxidative stress [4].

SODs are metalloproteins. Based on the metal cofactor that is associated with them SODs are classified as (i) Copper-Zinc SOD1 (Cu-ZnSOD1) exclusively found in eukaryotes (ii) Manganese dependent (Mn-SOD2) which is found in mitochondria and prokaryotes (iii) Iron dependent SOD (Fe-SOD) which is the most primitive form and associated with prokaryotes, protozoans, algae, anaerobic bacteria and some higher plants [5]. Studies of primary structure alignment of these SODs have shown the *Plasmodium falcifarum* iron superoxide dismutase (*Pf*Fe-SOD) is structurally similar to Mn-SOD2 and *Escherichia coli* Fe-SOD but distinct from human Cu-ZnSOD1 [6]. This confirmed the suggestion that *Pf*Fe-

SOD could be an ideal target for anti-malarials in the host erythrocytic phase of the parasite life cycle. Since erythrocytes do not have mitochondria and the related Mn-SOD2 isoform, the only interference is from the host Cu-ZnSOD1 which happens to be structurally distinct from the parasite *Pf*Fe-SOD. Therefore, inhibitors that are specific for the *Pf*Fe-SOD could contribute as new drug leads against the parasite and will be a step towards development of advanced chemotherapy for malaria [7]. A few potential inhibitors from the library of synthetic molecules have already been studied using recombinant *Pf*Fe-SOD and the results were confirmed using two different SOD assays. Selection of possible leads was based on their structural diversity and their ability to create interactions of stacking, polar or hydrophobic nature. Two strains of *Plasmodium falciparum, HB3* (which is sensitive to chloroquine, a first line drug of malaria) and *Dd2* (which is moderately resistant to chloroquine) were used to test the *in vitro* efficacy of the most effective leads among those tested on the parasite enzyme. Among the fifteen effective lead molecules SP72, SP13 and SP59 showed significant inhibition of *Pf*FeSOD. Since *Pf*FeSOD is a unique enzyme, these lead molecules could have anti-malarial activity. The IC_{50} values were in *μM* range and it was suggested by the authors that this work could be the first step in the search for an anti-malarial for *Plasmodium falciparum* with *Pf*Fe-SOD as target. However, no attempt was made in the study to check whether the molecules interact with and inhibit the host Cu-ZnSOD1 (from the erythrocyte cytosol). Our aim was to predict the ability of the prospective lead molecules to bind to both, the host Cu-ZnSOD1 and *Pf*Fe-SOD. Hence, it was of interest to try insilico docking of some of the lead molecules tested, on the 3D structures of SOD, both, from *Homo sapiens* (host) and the *Plasmodium falciparum* (parasite). This study involves insilico docking of lead molecules on both, the host and parasite SOD. Lead molecules which effectively inhibited the recombinant *Pf*Fe-SOD were selected. The results are suggestive of affinity of the lead molecules to both SODs based on their energy values. If the lead molecules were to bind to host Cu-ZnSOD1 as well, it could lead to oxidative stress in the host which may be lethal. In conclusion, these results suggest that insilico docking of the lead molecules could give a clue as to whether they bind to the host Cu-ZnSOD1 with lesser energy than *Pf*Fe-SOD which is suggestive of an easier interaction with the former. Secondly, potential lead molecules may be used for an inhibition assay with recombinant Cu-ZnSOD1 as well, before being tried as drugs against *Plasmodium falciparum*.

Materials and Methods

Computational bioinformatics tools have been employed in this study. Biological databases like PubMed and PDB (Protein Data Bank) and computational tools like Hex,

Marvin Sketch and MolConvert have been used. PubMed is a digital archive of biomedical and life sciences journal literature managed by NCBI (National Center for Biotechnology Information) which serves as a search engine for accessing the MEDLINE database of citations and abstracts of biomedical research articles [8].

PDB (protein data bank) is a structural archive for biological macromolecules which encompasses all the structural information of macromolecules as determined by X-ray crystallography, NMR studies, etc. [9]. HEX is an interactive molecular graphics program for calculating and displaying feasible docking models of protein - ligand pairs of molecules which are based on HEX algorithm. It explores the space of all possible binding modes and identifies the energy of particular molecular confirmation and then searches for a confirmation that minimizes the free energy of the system [10]. It uses spherical polar Fourier (SPF) correlation to accelerate the calculation and has inbuilt graphics to view the results [11].

Marvin Sketch is a chemical drawing tool which helps to quickly and easily draw molecules and chemical structures [12].

Molconvert is a tool which further converts the files in .mol format of Marvin Sketch to .pdb format which becomes accessible to hex algorithm [13].

Methodology

The 3D structures of the fourteen ligands from Soulere et al. [7] were drawn and the IUPAC names were assigned using the Marvin Sketch tool (Fig. 1). The structures generated as a .mol file were converted to .pdb file using tool MolConvert which makes the lead molecule accessible to docking. The three-dimensional structure of P*f*Fe-SOD and Cu-ZnSOD1 was retrieved from PDB. Taking P*f*Fe-SOD, as a receptor, the docking analysis was done with the individual lead molecules as ligand using the docking software Hex 4.2. Similarly, docking was carried out with human Cu-ZnSOD1 as the receptor with the same lead molecules. The relative stabilities were evaluated using molecular dynamics and their binding affinity using free energy simulations. The receptors and the lead molecules were docked using the following parameters.

|		
Display mode	-	polygons
Sort solutions by	-	energy
Correlation type	-	Shape only
FFT mode	-	3D Fast lite
Grid dimension	-	0.6
Receptor range	-	180
Ligand range	-	180
Twist range	-	360
Distance range	-	10

Results

The best fit interactions were found only in five (SP12, BB29, SG5281, DS88, SP72) of the fourteen molecules that were drawn in this study (Fig. 2). The results indicate that these ligands do not show much docking on the *Pf*FeSOD but seem to have docking sites or surface interactions on the human Cu-ZnSOD1 (Fig. 2).

It appears results of inhibition assays of the lead molecules with P*f*Fe-SOD do not show any correlation with respect to docking on the surface of the enzyme. Though F12, BB29, SP72 show no signs of docking on the 3D structure of *Pf*Fe-SOD, all the five ligands seem to be interacting with definite pockets on the surface of the 3D structures of the dimeric Cu-ZnSOD1.

Table 1: Percent inhibition of the lead molecules on recombinant PfFe-SOD and comparison of the total energy levels (E value)of the lead molecules after docking on PfFe-SOD and Cu-Zn SOD1.

| Lead molecule	Inhibition (%) of the lead molecule on recombinant PfFe-SOD	E value after docking the lead molecule on PfFe-SOD	E value after docking the lead molecule on Cu-ZnSOD1.
F12	100	-21.8	-201.1
BB29	100	0.0	-116.6
SG5281	100	-66.6	-279.9
SP72	97	0.0	-208.7
DS88	96	-114.0	-324.2

AB93 : Preferred **IUPAC` Name** = N-(cyclohexylmethyl)-N-[3-(4-{3-[(cyclohexylmethyl)amino] propyl}piperazin-1-yl) propyl]tetradecanamide
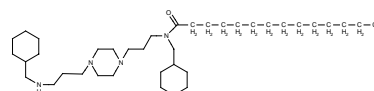
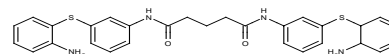**B31 :** Preferred **IUPAC Name** = (1-phenylethyl)benzene

DS88: Preferred **IUPAC Name**= 1,4,8-trichloro-10-(3 -{4-[3-(1,4,8-trichloro-10H-phenothiazin-10-yl) propyl] piperazin-1-yl} propyl)-10H-phenothiazine
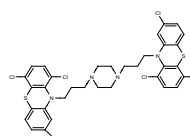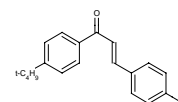

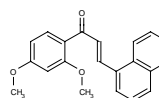**SG5281 :** Preferred **IUPAC Name**

=-{3-[(6-aminocyclohexa-2,4-dien-1-yl) sulfanyl]phenyl} -N'-{3-[(2-aminophenyl)sulfanyl]phenyl}pentanediamide

SP13 : Preferred **IUPAC Name** = (2E)-3-(4-bromophenyl) - 1-(4-tert-butylphenyl)prop-2-en-1-one
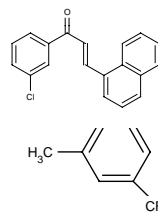

SP59: Preferred **IUPAC Name** = (2E)-1-(2,4- dimethoxyphenyl) -3-(naphthalen-1-yl) prop-2-en-1-one
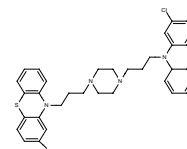
Insilico docking for validation of drug leads on Superoxide dismutase........
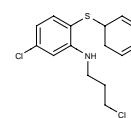
SP81 : Preferred **IUPAC Name** = (2E)-1-(3-chlorophenyl)-3-(naphthalen-1-yl)prop-2-en-1-one
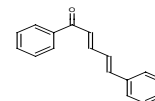
BB29 : Preferred **IUPAC Name** = 1-methyl-3-(trifluoromethyl)benzene

BB82: Preferred **IUPAC Name** = 2-chloro-10-(3-{4-[3-(8-chloro-10, 10a-dihydro-4aH-phenothiazin-10-yl)propyl] piperazin-1-yl}propyl)-10H-phenothiazine
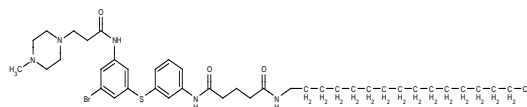
F12 : Preferred **IUPAC Name** = 5-chloro-N-(3-chloropropyl)-2-(cyclohexa-2,4-dien-1-ylsulfanyl)aniline
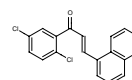
SP35 : Preferred **IUPAC Name** = (2E,4E)-1,5-diphenylpenta-2,4-dien-1-one

SG7881: Preferred **IUPAC Name**=
N-[3-({3-bromo-5-[3 (4-methylpiperazin-1-yl) propanamido] phenyl}sulfanyl) phenyl]-N'-hexadecylpentanediamide

SP72 : Preferred **IUPAC Name** = (2E)-1-(2,5-dichlorophenyl)-3-(naphthalen-1-yl)prop-2-en-1-one

SP112 : Preferred **IUPAC** Name = (2E)-3-(anthracen-9-yl)-1-(2,5-dichlorophenyl)prop-2-en-1-one
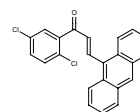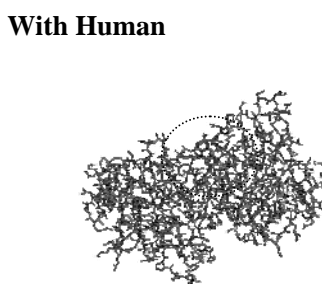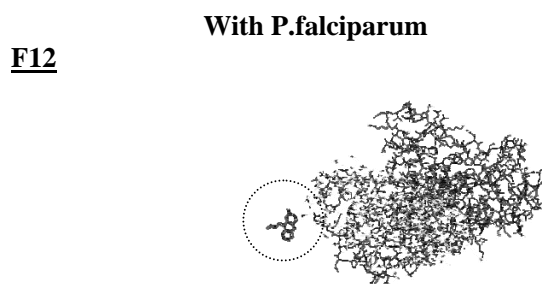
Fig. 1: *The structures of the drug lead molecules and their names according to the IUPAC nomenclature using Marwin Bean tool.*
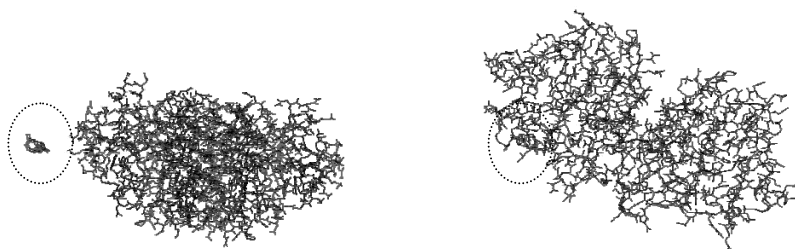
| | **With P.falciparum** | **With Human** |

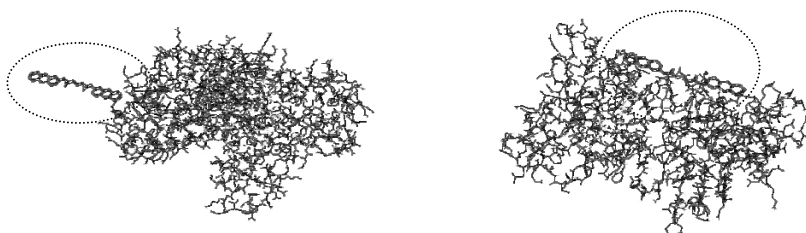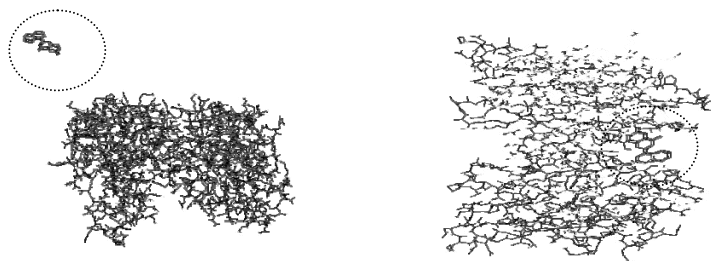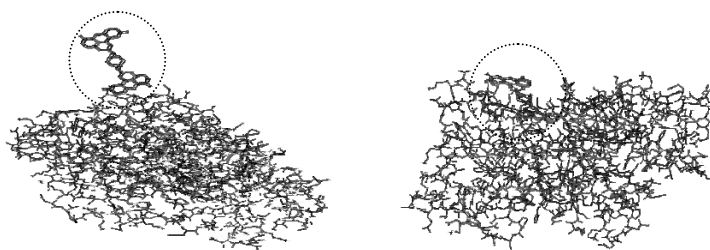**F12**

BB29

SG5281

SP72

DS88

***Fig. 2.* :** *Insilico docking of (a) F12, (b) BB29, (c) SG5281, (d) SP72 and (e) DS88 lead molecules on the 3D structures of PfFe-SOD and Cu-ZnSOD1. The ligands are encircledin..*

Discussion

Our earlier study has shown very little homology between Cu-ZnSOD1 of human and *Pf*Fe-SOD, which meant their sequences and structures are very distinct from each other [14]. This would probably be the best basis to target the *Pf*FeSOD for therapy of malaria falciparum caused by this parasite. Due to the phenomenon of drug resistance, a search for new leads against *Pf*Fe-SOD has shown that molecules SG5281, F12 and BB29 have 100% inhibition capacity at 50 μM concentration with the recombinant enzyme (7). While, other lead molecules like BB24, BB31, SP112, BB82, DS88, SG7861, SP13 and SP72

show lessor (80-100%) inhibition at that concentration. The best leads among all these were SG5281, F12 and BB29 since they inactivated the recombinant *Pf*Fe-SOD enzyme completely at 50 μM concentration. However, these leads behave differently in *in vitro* assays with two strains of parasite *plasmodium falciparum* cultures, *HB3* (chloroquine sensitive) and *Dd2* (moderately chloroquine resistant). Ten of the fourteen were inactive [7]. Of the three leads (SG5281, F12 and BB29) which inactivated the recombinant enzyme completely, two (BB29, F12) seem to be totally inactive, while, SG5281 value showed only 38% inhibition on *Dd2* strain. The authors speculate that this difference in the behaviour of the lead molecules

between recombinant enzyme and parasite cultures, maybe due to either decreased stability of molecules during assay or degradation of the molecules before they reach the target or absence of *in vivo* physiological conditions in the assay. Another reason for the altered behavior in cultures could be because the lead molecules may not have bound to the native enzyme. This difference in the behaviour of lead molecules with the recombinant enzyme and *in vitro* culture assays clearly indicates a need for lead molecules to be validated by alternate methods before they could be made / called as drug leads. Moreover, the IC_{50} values of these leads are in micromolar range as compared to the current drug of choice chloroquine which is in nanomolar range (21.2 nM and 54.9 nM for *HB3* and *Dd2* respectively) which is a thousand fold higher [7]. The study however, does not in any way indicate the effect of these lead molecules on the host Cu-ZnSOD1. *In vitro* inhibition assays of the lead molecules with the recombinant Cu-ZnSOD1 could also be done to check out possible interaction leading to inhibition. Although the results may be inconclusive as in the case of the *Hb3* and *Dd2* strain cultures of the parasite, it would probably throw some light on their effect on the enzyme.

A recent but alternate method to check out possible interaction between lead molecules and target enzyme is insilico docking. In recent times, the insilico approach to drug designing is gaining potential significance in enhancing the process of drug discovery [13]. This approach is an attempt to streamline the lead discovery process by moving as much of the *in vitro* experimentation as possible into the realm of computation, with the help of the ligand (lead molecule) docking and screening algorithms. Insilico docking of leads with 3D structures of target enzymes will throw light on whether the target enzyme has docking sites / pockets for the lead. This could be the possible alternate method for validation of drug leads in conjunction with the *in vitro* assays with recombinant enzymes and cultures of the parasite.

The results of the docking studies indicate that there are no docking sites on *Pf*Fe-SOD for these lead molecules which showed considerable inhibition with the recombinant enzyme. This could probably be the reason why these molecules did not show inhibition with the *plasmodium falciparum* cultures [14]. The E-values from the docking studies suggest that, the ligand–Cu-ZnSOD1 interaction would probably be more favorable than ligand-*Pf*Fe-SOD interaction. Even though the *in vitro* SOD inhibition assay of the ligands with recombinant *Pf*Fe-SOD showed inhibition (96-100%) of the enzyme, the insilico docking studies and the lower E values clearly show a better interaction with the host Cu-ZnSOD1. Taking this into consideration, it would be advisable to check the IC_{50} value of lead molecules with recombinant Cu-ZnSOD1 as well. In addition, an insilico docking evaluation of the possible ability of the lead molecules to dock on the surface of the host / parasite SOD could also be used to screen the molecules. An unnecessary interaction of the lead molecule with Cu-ZnSOD1 would decrease its activity *in vivo* and this could lead to oxidative stress.   In conclusion, this study shows that compounds which are found to be quite effective inhibitors *in vitro* (such as in assays with recombinant target enzymes / parasite cultures), need to be validated by alternate methods. Often it so happens that in the living system, compounds tend to deviate from their *in vitro* behavior in an unpredicted way. Thus computational tools such as insilico docking provide the scientist with an alternate base for validation of lead molecules since most of them could cause oxidative stress in the host which could be lethal.

Abbrevations

Superoxide dismutase -SOD; *Plasmodium falcifarum* iron superoxide dismutase - *Pf*Fe-SOD; human cytosolic copper-zinc superoxide dismutase - Cu-ZnSOD1; Mitochondrial manganese superoxide dismutase - Mn-SOD2

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