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Structure-based designing of sordarin derivative as potential fungicide with pan-fungal activity

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ABSTRACT

Fungal infections have become a significant problem for immunosuppressed patients. Sordarin, a promising fungicidal agent, inhibits fungal protein synthesis by impairing elongation factor-2 (eEF2) function. Intriguingly, despite high sequence similarity among eEF2s from different species, sordarin has been shown to inhibit translation specifically in certain fungi while unable to do so in some other fungal species (e.g. *Candida parapsilosis* and *Candida lusitaniae*).

The sordarin binding site on eEF2 as well as its mechanism of action is known. In a previous study, we have detailed the interactions between sordarin and eEF2 cavities from different fungal species at the molecular level and predicted the probable cause of sordarin sensitivity.

Guided by our previous analysis, we aimed for computer-aided designing of sordarin derivatives as potential fungicidal agents that still remain ineffective against human eEF2. We have performed structural knowledge-based designing of several sordarin derivatives and evaluated predicted interactions of those derivatives with the sordarin-binding cavities of different eEF2s, against which sordarin shows no inhibitory action. Our analyses identify an amino-pyrrole derivative as a good template for further designing of promising broad-spectrum antifungal agents. The drug likeness and ADMET prediction on this derivative also supports its suitability as a drug candidate.

1. Introduction

Immunosuppressed patients are more prone to get systemic fungal disease. Immunosuppression occurs due to cancer, AIDS, transplantation, broad spectrum antibiotics and glucocorticoid therapy, in premature infants, peritoneal dialysis or haemodialysis [1,2]. These fungal diseases are mainly treated by the azoles, polygenes and echinocandins, but therapeutically satisfactory results cannot always be achieved by these antifungal drugs due to the resistance in some fungal species, their toxicity, and limited ways of administration. These drugs act on the plasma membrane of cell by binding or blocking the synthesis of ergosterol which will cause the inhibition in the fungal growth [3].

Sordarin, a known antifungal agent, may be a molecule with better prospects compared to others because it targets one of the most vital cellular processes in fungi, protein synthesis, by impairing the function of an essential translation factor, the eukaryotic elongation factor 2 (eEF2). Eukaryotic elongation factor 2 (eEF2) is a member of the GTPase superfamily of proteins which is known to assist in the tRNA translocation step of protein synthesis. Sordarin is a known antifungal agent which targets eEF2 so as to inhibit protein translation either by blocking eEF2-mediated translocation of tRNAs from A and P sites to P and E sites of the ribosome [4], or by interfering with eEF2-dependent ribosome splitting [5]. Interestingly, sordarin does not hinder GTP hydrolysis. Upon GTP hydrolysis, structural reorientation of the C-terminal half (domains III, IV and V) relative to the N-terminal half (domains G', I and II) occurs within eEF2 promoting its release from the ribosome. Sordarin inhibits the domain rearrangement required for eEF2 release by binding to a pocket within domains III, IV and V [6,7] (Supplementary Fig. S1A). It is apparent that sordarin's activity is directly related to its ability to bind inside the eEF2 cavity.

Sordarin has a remarkable selectivity of action which makes it ineffective against human eEF2. It is, however, also not equally effective against all fungal species. Certain fungal species are highly resistant towards the drug (Supplementary Fig. S1B). This remark-

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able selectivity of the drug has been attributed to some species specific alterations within a stretch of amino acids (termed as the 'sordarin specificity region (SSR)', residues 517–524 in yeast) at the entrance of the binding cavity of eEF2 [8]. In human, almost all the SSR residues are found to be different with respect to their yeast counterparts (Supplementary Fig. S1B).

Our aim was to decipher the structural basis underlying the species selectivity of sordarin while targeting translation and to design some modified versions of the drug with broader spectrum antifungal activity. In order to evaluate the compatibility of the drug and the protein cavity, we have introduced several 'deterministic parameters' in a previous study [9] which could successfully describe the molecular basis of selectivity of sordarin binding to different fungal eEF2s. Our analysis revealed that although the fungal eEF2s are globally highly conserved at the sequence level (more than 80% conserved across fungal species) as well as in structural folding, the local environment of the binding cavity interiors differs significantly in some eEF2s altering drug-eEF2 interaction patterns. The effects collectively act as a key factor in determining the selectivity of the drug binding. The study inferred that subtle local level structural changes due to amino acid substitutions in the SSR region of eEF2s from certain species (e.g. *C. parapsilosis* and *C. lusitaniae*) make the eEF2 cavity incompatible both physically and chemically toward sordarin and consequently render them insensitive to the drug. We predicted that favourable interactions between sordarin and an aromatic residue at position 521 of the SSR stabilize the drug in the cavity and that lack of this interaction results in sordarin insensitivity. Further, we identified some nonconserved residues within the human eEF2 cavity apart from those within the SSR, which were also found to contribute substantially towards sordarin resistance in human.

Guided by the knowledge gained from this earlier study, structure-based design of sordarin derivatives with broad-spectrum antimycotic activity is the aim of the present study. Our analyses showed that the C61 position of sordarin is the closest neighbour of residue 521 within the SSR region of eEF2 and we have chosen this position as the site for modification. We computationally modified the sordarin structure by introducing different functional groups at C61 position and assessed the effect on predicted drug-protein interactions using the 'deterministic parameters'. Functional groups that might augment the possibility of sordarin's favourable interaction with any residue at position 521 irrespective of species were inserted at C61 position. Based on the assessment we have proposed one derivative that might act as a potent fungicide with 'pan-fungal' inhibitory action. Interestingly, preliminary predictions of drug-likeness and computational ADMET predictions suggest the derivative could be a good template for a drug candidate.

2. Methods

2.1. Modification of protein and ligand structures

The eEF2 models for all the fungal species used in this study were generated using a crystal structure of sordarin-bound yeast eEF2 (RCSB PDB code 1N0U) as the template for homology modelling (described in details in Ref. [9]). Substitution of conserved residues in yeast eEF2 coordinate at different positions and modifications on the sordarin structure with different substituent were done using Accelrys Discovery Studio 4.0 software.

Models of seven sordarin derivatives (Supplementary Fig. S2) were prepared by attaching different groups, e.g. long aliphatic chain, aromatic/heterocyclic ring structure (good for stacking interaction), and others being part of known antifungal/antimicrobial agents (Fig. 2B).

2.2. Approach for assessing the effects of the derivatives

For every derivative prepared, we first analyzed its predicted binding with the S.cerevisiae eEF2 model with $_{Tyr}521^{Ser}$ and $_{Ser}523^{Asn}$ substitutions. This was the first step to check whether the derivative is engaged in favourable contacts with Ser521 and Asn523 (residues present in eEF2s of sordarin-resistant fungal species *C. parapsilosis* and *C. lusitaniae*). Next and perhaps the most important step was to examine the insensitivity of the drug towards human eEF2. Once a derivative successfully passed the two tests we moved on to analyze its binding likeliness for eEF2 from different sordarin-resistant fungal species.

2.3. Docking

The flexible docking procedure [10] within the "Affinity" module of Insight II was used to get an accurate description of ligand (sordarin and its derivatives) recognition by each of the eEF2 structures (without imposing any initial bias from the crystal structure). 'Affinity' is a suite of programs for automatically docking a ligand to a receptor by a combination of Monte Carlo minimization followed by Simulated Annealing procedure (Affinity user guide. San Diego, USA: Accelrys Inc.; 1999) as described previously [9].

The resulting set of complexes following docking had the ligand bound to the cavity in various poses. To select the final structure from all the top-ranked poses for each case we have used the root mean square deviation (rmsd) with respect to the sordarin pose in the x-ray structure 1N0U as done earlier [9]. Additionally, we also introduced %ASA (percent accessible surface area) as another selection criteria. The pose selection procedure is detailed in Section 2.4.

2.4. Selection of the final ligand poses

As described previously, root-mean-square deviation (RMSD) of the different poses of sordarin and all its derivatives were calculated by comparing with that of sordarin in crystal structure 1N0U. For the derivatives (since they are much bulkier), the extra group was excluded and sordarin backbone of the various poses was considered while calculating the RMSD. Calculation of the rmsd was carried out in the Discovery Studio version 4.0 (RMSD value of ≤ 1 was considered to be acceptable).

For all those poses that met the criterion that the RMSD to the crystal structure should be <1 Å, another selection criterion was imposed. Sordarin-bound yeast eEF2 crystal structure is available (1N0U). We have compared the percentage of solvent exposed surface area of the molecules with that of yeast eEF2-bound sordarin in 1N0U. ASA for the ligands was obtained from 'StrucTools' server (http://helixweb.nih.gov/structbio/basic.html) and %ASA was calculated by using the following formula:

$$\%\text{ASA} = (\text{Total surface area of the ligand}/\text{Accessible surface area of protein-bound ligand}) \times 100$$

The pose having the least %ASA value (preferably not exceeding the %ASA value for sordarin in 1N0U) was considered reasonable over others. The ligand pose fulfilling both the criteria was selected for further analyses.

2.5. Analysis of ligand–protein contacts

As described in our previous study [9], the analysis of ligand–protein contacts was done using LPC/CSU server (http://ligin.weizmann.ac.il/cgibin/lpccsu/LpcCsu.cgi) [11,12]. This server