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ORIGINAL ARTICLE

In Silico Analog Design for Terbinafine Against *Trichophyton rubrum*: A Preliminary Study

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Abstract The diseases caused by dermatophytes are common among several other infections which cause serious threat to human health. It is evident that enzyme squalene epoxidase is responsible for prolonged dermatophyte infection and it is appealing to note that this enzyme is also responsible for fatty acid synthesis in these groups of fungi. In the present study, terbinafine drug which targets enzyme squalene epoxidase has been explored to design its various novel analogues. The present study suggests that many more prominent drug analogues could be constituted which may be crucial towards designing new drug candidates. In the present study, we have designed a series of such analogues viz. [(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)(naphthalen-1-ylmethyl)amine, N-[8-({[(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)amino}methyl)naphthalen-1-yl]-2-(sulfoamino) acetamide, {[4-(dihydroxyamino)-8-({[(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)amino}methyl)naphthalen-1-yl]sulfanyl}methanol and (R)-{[4-({[(2E,6R)-6,7-dimethyloct-2-en-4-yn-1-yl](methyl)amino}methyl)-5-[(hydroxysulfamoyl)amino]naphthalen-1-yl]amino}sulfinic acid. Moreover, further by molecular docking approach the binding between enzyme and designed analogues was further analysed. The present preliminary report suggested a considerably good docking interaction score of −338.75 kcal/mol between terbinafine and squalene epoxidase from *Trichophyton rubrum*. This preliminary study implies that few designed candidate ligands can be effectual towards the activity of this enzyme and can play crucial role in pathogenesis control of *T. rubrum.*

Keywords Dermatophytes · Molecular docking · Squalene epoxidase · *Trichophyton rubrum*

Introduction

Trichophyton rubrum is a major dermatophyte causing several skin diseases which infects superficial layers of skin feeding on keratinous regions [1]. There are several reports about some commercially available drugs to control the infection caused by this fungus. In a recent report it is described that marine microorganisms can be crucial source for such drugs [2]. The major concern about drug action against this fungi is changes in target site that are caused by mutations in active site of amino acid sequence corresponding to the protein which is responsible for interaction of the target with this drug [3].

Squalene epoxidase is a prominent enzyme produced by *T. rubrum*, it is responsible for the fatty acid synthesis in dermatophyxtes and plays key role in sterol biosynthesis [4]. Moreover, it is evidenced that Squalene epoxidase leads to synthesis of ergosterol following a cascade of biosynthetic pathways which are essential to maintain integrity and functionality of fungal cells. Due to this property, it acts as major target for the drugs which are designed to inhibit dermatophytosis [5]. terbinafine, chemically written as N,6,6-trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine is commercially used drug for treating dermatophytosis [6]. It inhibits activity of the enzyme

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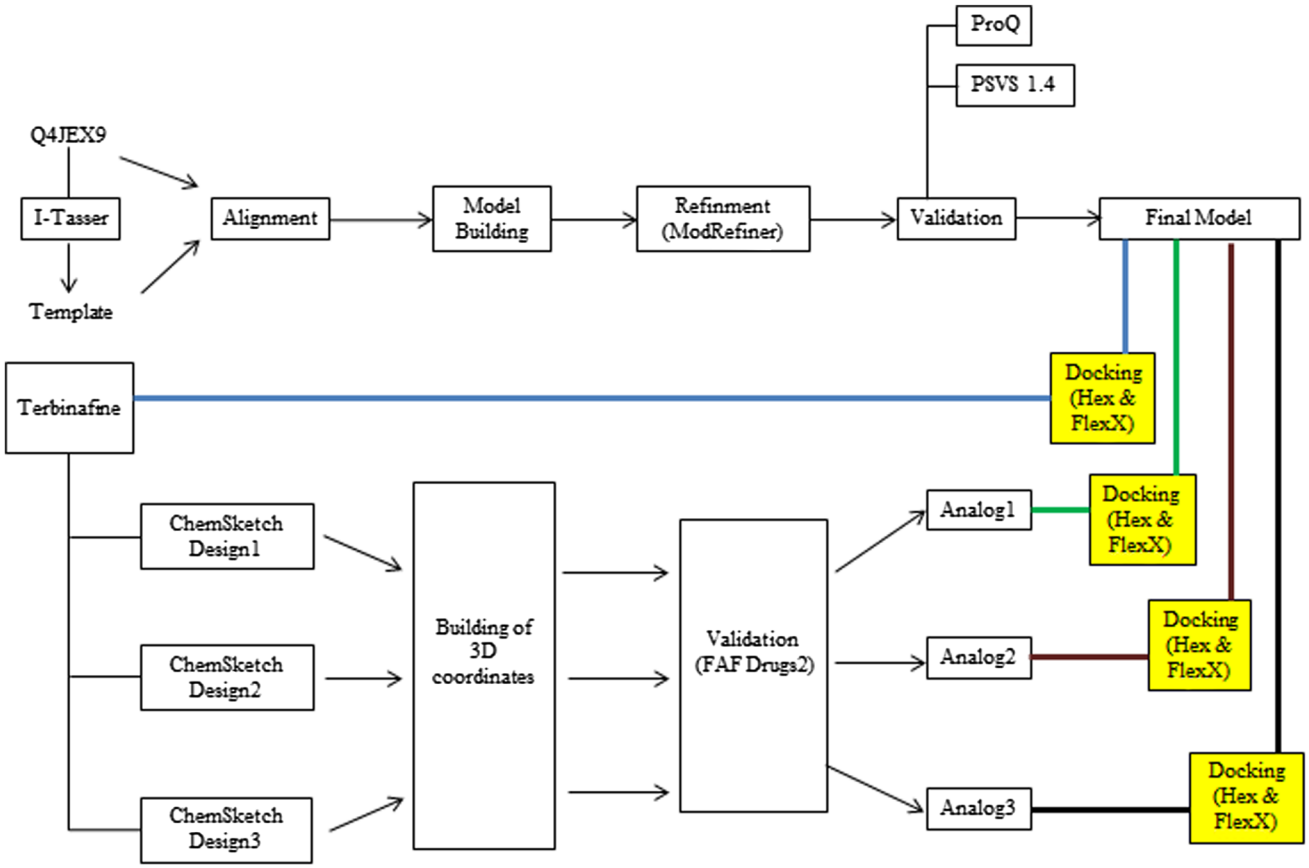

Fig. 1 Schematic representation of the work flow presented in this study

squalene epoxidase and thus pausing ergosterol synthesis which leads to the death of fungi. It is obvious that the drugs available to inhibit dermatophytes are not being efficient in long run, so there is necessity to design drugs that can encumber the activity of dermatophytes [7]. A report on associated employ of terbinafine and tramadol illustrated that it reduces μ-opioid receptor-related analgesia and augmented risk of serotonergic effects and it should be avoided [8].

We understand that there are several studies in which antifungal analogs were designed to improve their inhibitory action against dermatophytes which targets the function of squalene epoxidase. In 1999 and 2008, Gokhale et al. and Kharkar et al. carried out comparative molecular field analysis (CoMFA) of fungal inhibitors that act against squalene epoxidase of *Candida albicans, Aspergillus fumigatus, and Trichophyton mentagrophyte*, and simultaneously designed few compounds that show Minimum Inhibitory Concentration (MIC) with less toxicity [9, 10]. In 2008–2009, three different studies by Francis et al., Che et al., and Wang et al. [1, 11, 12] respectively were conducted and in these studies speculating design and synthesis of antifungal agents was suggested. However, Che et al. [11] designed and synthesized a series of azoles drugs against *C. albicans* whereas Francis et al. [12] modified the structure of 1-benzylamino-2-phenyl-3-(1H-1,2,4-triazol-1-yl)propan-2-ols to act better against *C. albicans* [11]. Furthermore, Wang et al., designed, synthesized 2-(2,4-Difluorophenyl)-3-(methyl-(3-phenoxyalkyl)amino)-1-(1H-1,2,4-triazol-1-yl)propan-2-ols against *C. albicans* [1]. In 2014, computational docking study of cytochrome P450 14alpha-demethylase (CYP51) in *C. albicans* was carried out with a series of fluconazole analogs [13] which resulted in synthesis of few analogs that were proposed to be better inhibitors than fluconazole. Though structure of Squalene epoxidase enzyme shares similarity with other fungi and mammals its structure in *T. rubrum* is different as a stretch of 32–34 residues that are present in *Sachharomyces cerevisiae*, *C. albicans* and *Neospora* are not present in *T. rubrum* [14–16]. Though studies of anti fungals against fungal squalene epoxidase were presented till now, insight of the structure and binding of squalene epoxidase in *T. rubrum* with its inhibitors is not illustrated yet. In this study, details about the structure and residues involved in binding of squalene epoxidase of *T. rubrum* are provided. Figure 1 shows the schematic representation of the work flow presented in this study and

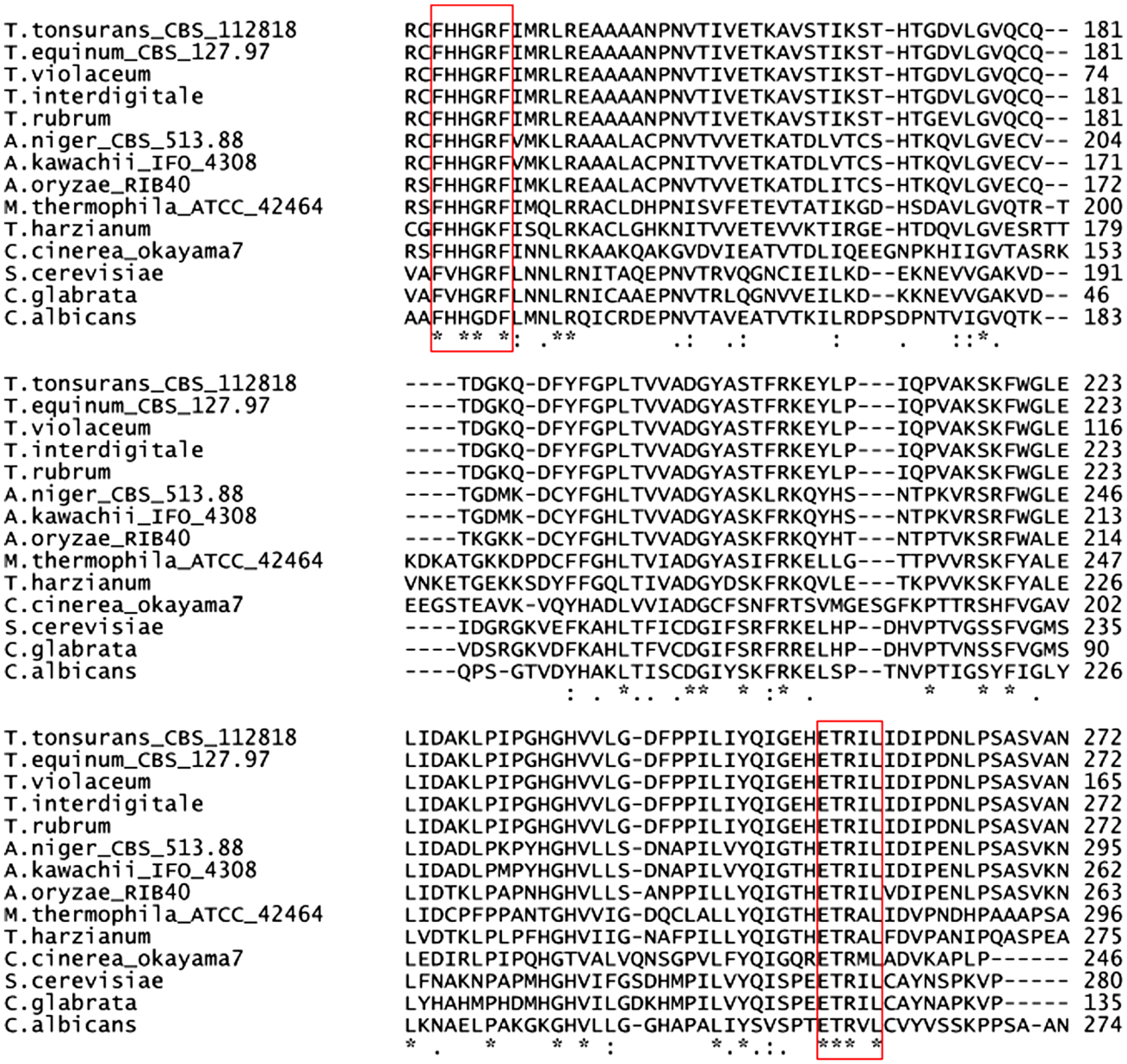

Fig. 2 Multiple sequence alignment to locate the conserved sequence in the enzyme

Table 1 The names and docking results of the analogs compared to that of terbinafine using Hex and FlexX

Name	IUPAC name	Hex score	FlexX score	Interacting residues (predicted by FlexX)
Original	[(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)(naphthalen-1-ylmethyl)amine	−338.75	−7.6749	25, 27, 31, 63, 156
Analog1	N-[8-({[(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)amino}methyl)naphthalen-1-yl]-2-(sulfoamino)acetamide	−400.79	−12.9380	27, 31, 63
Analog2	{[4-(dihydroxyamino)-8-({[(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)amino}methyl)naphthalen-1-yl]sulfanyl}methanol	−376.16	−13.7826	25, 27, 30, 31, 62, 63, 64, 155
Analog3	(R)-{[4-({[(2E,6R)-6,7-dimethyloct-2-en-4-yn-1-yl](methyl)amino}methyl)-5-[(hydroxysulfamoyl)amino]naphthalen-1-yl]amino}sulfinic acid	−369.98	−16.1865	25, 27, 29, 31, 63, 64

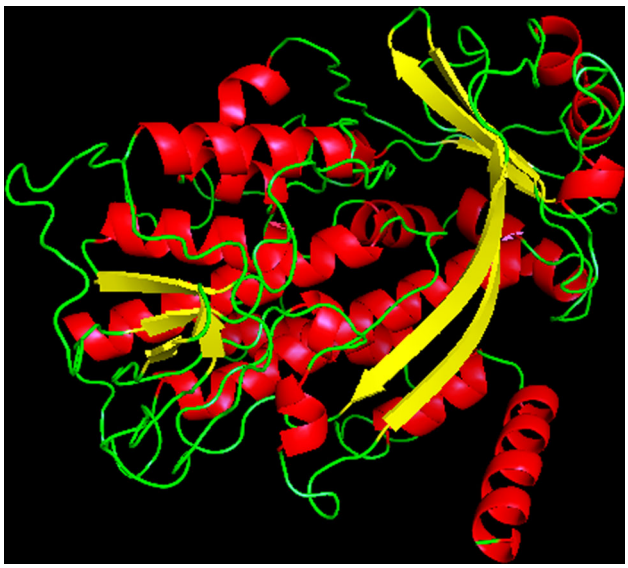

Fig. 3 The 3D model of squalene epoxidase generated by ITasser

Fig. 2 provides phylogenetic tree and multiple sequence alignment of active sites of squalene epoxidase among *T. rubrum* and 15 other fungi. Further, there are few studies have also reported about the perceptive enzyme substrate interactions which are also helpful in understanding the role of enzymes [17, 18].

Despite the fact that few studies proposed the activity of terbinafine against squalene epoxidase, the 3D structure and binding activities of squalene epoxidase were not elaborated. Furthermore, failure of long lasting inhibition of this enzyme by antifungals has raised the need for new drugs which can bind more firmly with this prominent enzyme. In our study we presented a tertiary model of squalene epoxidase enzyme from *T. rubrum* and tried to study its interaction with terbinafine like compounds. In this process, following in-silico methods, we designed few analogues of terbinafine that can bind well with squalene epoxidase better than terbinafine. These designed analogues were also tested for drug-like properties. Therefore, the present work might be helpful in further developing drug candidates that can inhibit the squalene epoxidase activity under in vitro and in vivo environments.

Materials and Methods

Protein Structure Prediction

Amino acid sequence of squalene epoxidase of *T. rubrum* (Q4JEX9) was obtained from UniProt Protein Database (http://www.uniprot.org/uniprot/) and this was used as a target to generate 3D structure for this enzyme [19]. In addition, an automated protein modelling server I-Tasser which automatically searches for best templates to build an efficient protein model was preferred for the present study [20]. Further, the protein model was refined using ModRefiner and was appraised for its quality using Protein Quality Predictor (ProQ) [21]. The overall evaluation of different parameters of the generated model was completed using Protein Structure Validation Server (PSVS 1.4) [22].

Ligand Preparation

Structure of drug terbinafine (DB00857) is obtained from DrugBank (http://drugbank.ca/) [23], an open drug database. ChemSketch package (ACD/ChemSketch Freeware, version 11.00) from ACD/Labs (http://www.acdlabs.com/home/) software is used to view and modify the structure of terbinafine. Marvin Sketch 5.10.3 tool of ChemAxon software (http://www.chemaxon.com) is also used for the same purpose. Analogs designed by modifying the structure of terbinafine and named as Analog 1,2 and so on. Building of 3D coordinates and optimization of these designed analogues was done using Avagadro software [24]. Physico-Chemical and ADMET properties of these designed structures are estimated by FAF Drugs2 of Mobyle (http://bioserv.rpbs.univ-paris-diderot.fr/FAF-Drugs) [25].

Molecular Docking

The evaluation of interactions between squalene epoxidase with terbinafine and designed analogs, docking was performed using Hex6.3 software [26] and FlexX software [27]. In addition to this, the improved docking strategy by using the concept of 'designed analogues ligands' was taken into consideration. After this, the binding competence of the commercially available terbinafine analogues

Table 2 The demonstration of Lipinski's rule of 5 for the Analogs

Name	Mol. wt.	No. of H-bond donors	No. of H-bond acceptors	logP	No. of flexible bonds	State based on ADME-Tox
Terbinafine	301.36	1	5	3.5	5	Accepted
Analog1	443.56	3	7	1.13	8	Accepted
Analog2	400.53	3	5	4.65	7	Accepted
Analog3	494.63	5	9	3.55	10	Accepted

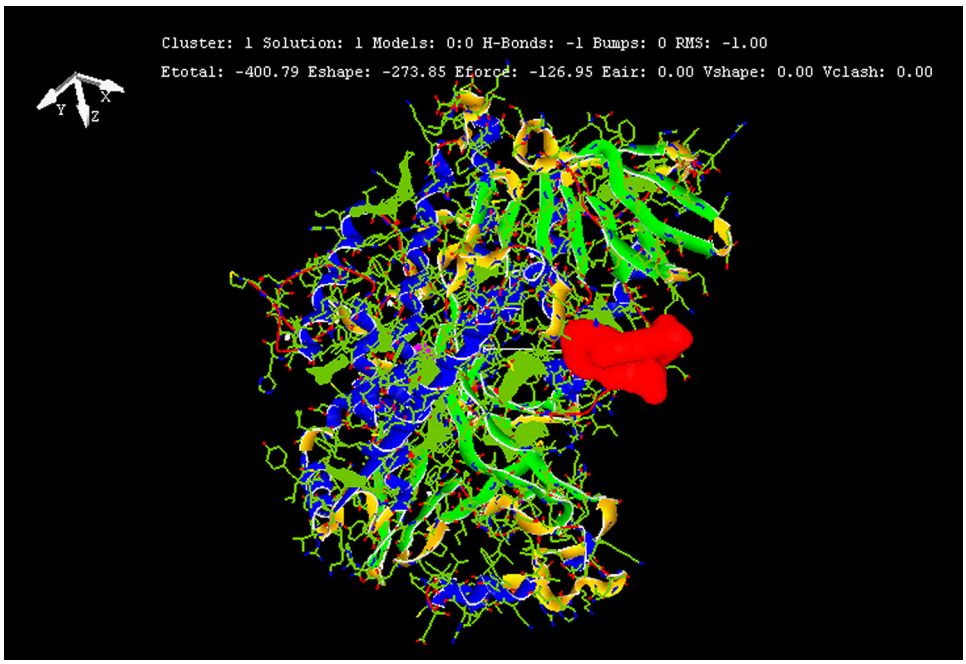

Fig. 4 Docking of the Analog1 with squalene epoxidase using Hex

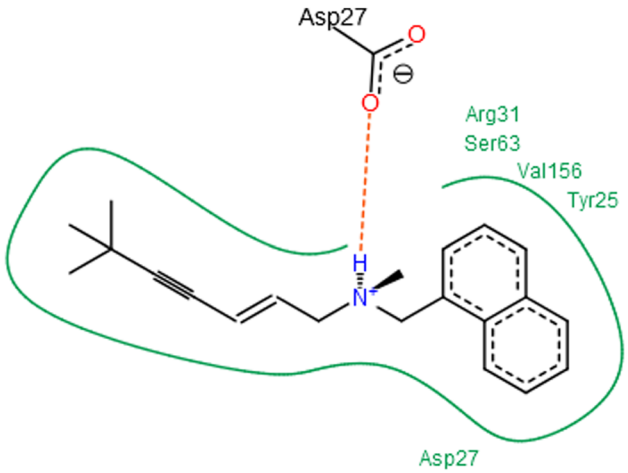

Fig. 5 Best pose of terbinafine generated by FlexX, with the interacting aminoacids

was compared to these designed analogues. This was achieved by using Hex6.3 and FlexX softwares. The commercially available terbinafine analogues are represented in Table 1.

Results and Discussion

Tertiary model for squalene epoxidase protein built by I-Tasser server and refined by Mod-Refiner server showed 90.0 % of residues in the most favoured regions, 6.6 % of residues in additionally allowed regions, 1.5 % of residues

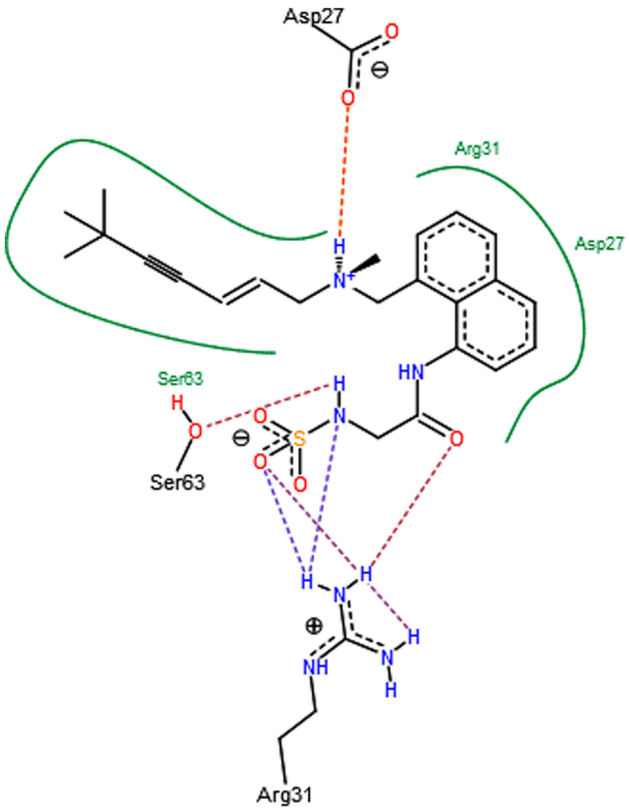

Fig. 6 Best pose of the Analog1 generated by FlexX, with the interacting aminoacids

in generously allowed regions, 2.0 % of residues in disallowed region, LGscore of 3.932 and MaxSub of 0.264. LGscore and MaxSub are two parameters used by ProQ to

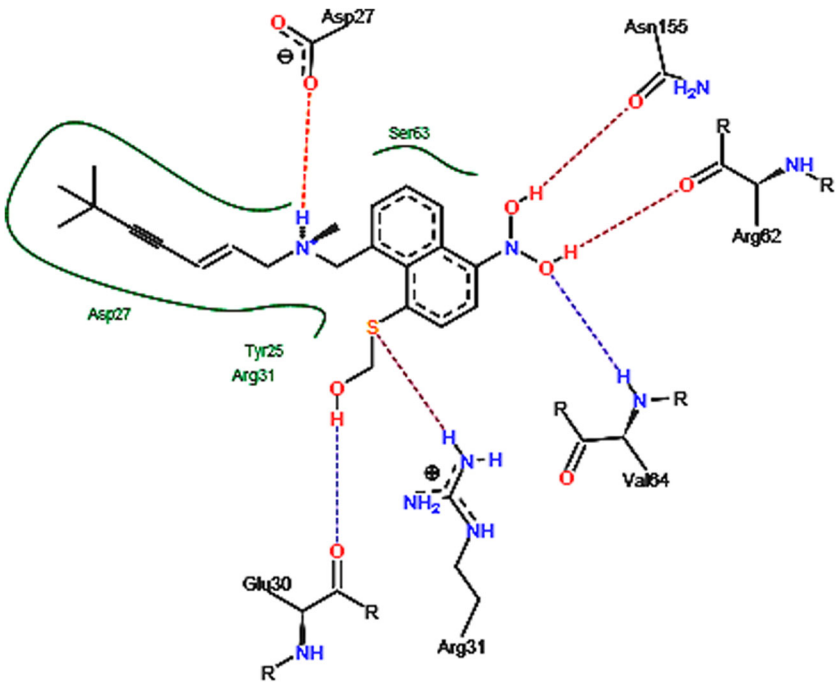

Fig. 7 Best pose of the Analog2 generated by FlexX, with the interacting aminoacids

determine quality of the proteins, where LGscore is negative log of a *P* value ranging between 1.5 for good models and five for very good protein models. MaxSub ranges between zero for significant models and one for very significant models. These results showed that the model generated is of better quality. 3D structure of enzyme squalene epoxidase generated by I-Tasser server is represented in Fig. 3.

Ligands designed by ChemSketch and Marvin Sketch were checked for their drug likeliness taking its properties into consideration as per the Lipinski's rule of five. Formula Weight, logP value, Number of H-Bonds and Number of H-Acceptors of the designed analogs were calculated using online tool FAF Drugs2 of Mobyle. Scores of these five properties for drug likeliness test are exhibited in Table 1. ADME-Tox properties (Adsorption, Distribution, Metabolism, Excretion and Toxicity) of the drug candidates have been analyzed based on the five parameters namely molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, logP and number of flexible bonds in the compound. Acceptance level of the drug candidate is measured on the basis of its permeability and also ratio of octanol solubility to water solubility referred as liphophilicity. More the molecular weight less would be the permeability of the compound. Same as the prior, more the number of h-bond donors or acceptors less would be the permeability. Therefore, accepted level of drug likeliness of the compounds were considered as molecular weight <500, number of hydrogen bond donors less than 5, number of hydrogen bond acceptors <10 and logP value <5. 10 or <10 flexible bonds or rotatable bonds in a compound indicate good oral bioavailability [28]. As all the results are satisfying the basic rules for drug likeliness, these compounds were considered as ligands to study their interaction with the enzyme squalene epoxidase.

Docking

Docking scores of original drug compound terbinafine with squalene epoxidase was compared with docking scores of the designed analogues. Docking scores of these analogues and original drug after interaction with squalene epoxidase are shown in Table 2 along with the residues that are predicted to be involved in the binding. From Hex docking scores it is found that E-Total (kcal mol) of Analog1 (−400.79), shown in Fig. 4, Analog2 (−376.16) and Analog3 (−369.98) were better than that of terbinafine (−338.75). FlexX results were also found to be supporting the docking results obtained by Hex as the FlexX E-Scores (kcal/mol) of Analog1 (−12.9380), Analog2 (−13.7826) and Analog3 (−16.1865) are better than that of the original drug (−7.6749). Figures 5, 6, 7 and 8 obtained from the FlexX docking results of squalene epoxidase with terbinafine analogs 1, 2 and 3 respectively, showed that residues 25, 27, 31 and 63 of squalene epoxidase were involved in interacting strongly with the ligands, whereas residues 29, 30, 31, 62, 64, 155 and 156 also contribute in docking.

From FlexX results it was shown that residues Asp at 27th position along with Arg at 31st, Ser at 63rd, Val at 156th and Tyr at 25th positions are responsible for the strong binding of squalene epoxidase with terbinafine.

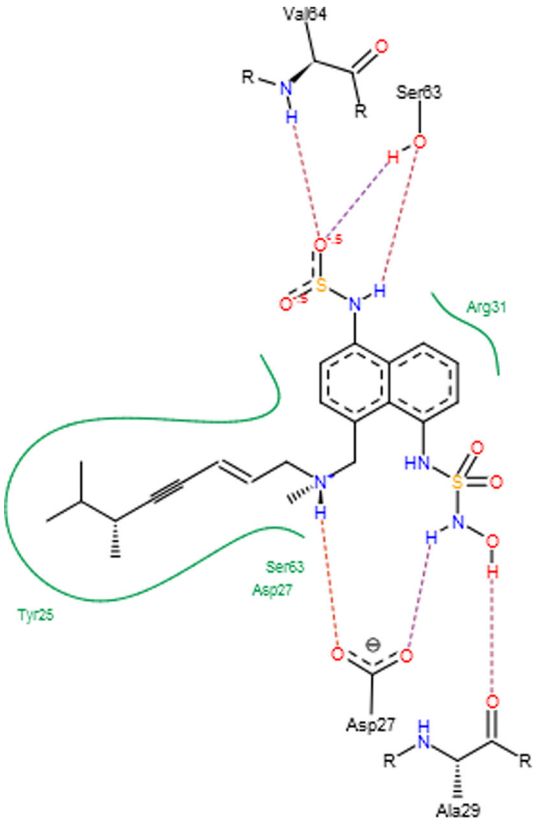

Fig. 8 Best pose of the Analog3 generated by FlexX, with the interacting aminoacids

Residues Asp at 27th position along with Arg at 31st and Ser at 63rd position of squalene epoxidase favoured binding with analog 1 whereas residues Asp at 27th, Arg at 31st, Ser at 63rd positions favoured binding with analog2 and residues Asp at 27th, Arg at 31st, Ser at 63rd, Tyr at 25th position along with Ala at 29th, Val at 64th positions favoured binding with analog3. From all the above observations, it can be concluded that the residues Asp27, Arg31, Ser63, Tyr25, Ala29 and Val64 residues play an important role while determining the bonding of squalene epoxidase with terbinafine and its analogs.

Speculation from docking results indicate the presence of another methyl group and sulphur functional groups namely sulfoamino, sulfanyl and hydroxysulfamoyl in the ligands which was absent in the native and was cause of efficient binding with the enzyme. These additional functional groups were also checked for their acceptance levels, by inspecting their presence and function among already existing drugs. Presence of methyl group is known to influence the drug-like properties of small molecules. Sulphur functional groups have property of easily penetrating into the skin and are known to treat adverse skin conditions. Derivatives of sulfoamino, sulfanyl and hydroxysulfamoyl with sulfinic acid are present in the analogs 1,2 and 3 respectively which were missing in the original terbinafine molecule. Sulfoamino is used among several drugs namely Fondaparinux, which is an antithrombotic drug. Derivatives of sulfanyl are used in the designing antifungal drugs following mechanism of Michael addition between α,β-unsaturated carbonyl compounds and mercaptans. In 1978, Steven et al. [29] used hydroxysulfamoyl derivatives as a prominent sulphur analog of L-asparagine to act against L-asparagine synthetase. These structural analyses of the designed analogs support our proposal to use these analogs for further experimentation in designing inhibitors of squalene epoxidase.

Structural analysis of squalene epoxidase, structural as well as functional insight into the designed analogs provides several clues regarding interaction of *T. rubrum* squalene epoxidase with its inhibitors. Taken together our study of structure based drug design against squalene epoxidase might be considered while designing novel dermatophyte inhibitors in an in-vivo system.

Conclusion

It is evident that, in silico studies provides insights in drug designing and this leads to analysis of the compounds behaviour. The structural modification of commercially available terbinafine may be a useful tool towards its effective binding to the respective re-designed analogues. We understood that interaction of the squalene epoxidase with above designed analogs are showing effective binding energy values which shows a prominent clue improved efficiency of terbinafine. This illustrate that different analogs of the present drug could open the avenues to search for the better compound which might work more effectively than the present drug. Furthermore, drug likeliness tests scores for these restructured also add more authenticity to the work related to the analogs designing. Thus, the present preliminary report also provides a scope to improve the efficient binding between terbinafine analogues and squalene epoxidase from a dermatophyte *T. rubrum*.

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