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CYP51: A Potential Target

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Abstract

CYP51 has been emerged as a potential therapeutic target in the management of microbial infections. In recent years, inhibitions of CYP51 enzyme in treatment of microbial diseases have successfully declined the rate of mortality and morbidity especially in immune-compromised patients. CYP51 has now been considered as established therapeutic target for the treatment of fungal infections, leishmanial disease and many more. There is still a lot to unfold within the structure of CYP51 which could help the researchers to make it a more versatile target in the management of complex diseases.

Keywords: Carboxaldehyde; Microsomal; NADPH; Anti-fungal; Anti-leishmania; Anti-trypanosoma; CYP51

Introduction

Study of sterol biosynthetic pathway has been a topic of interest since thirty years [1-6]. During 1984, this enzyme was precisely purified from the well known yeast *Sacharomyces cerevisiea* and was placed within CYP51 family, a section reserved for fungal sequences [7]. This study initiated a series of purification of the enzyme from various other living organisms such as orthologous mammalian, *Sorghum bicolor* [8] and *Mycobacterium tuberculosis* [9].

Enzymes of CYP450 have been found to be associated with numerous important roles during the synthesis of cellular compounds. Building up of steroids, vitamin A, vitamin D and some lipid-like eicosanoid molecules have been governed by the catalytic action of cytochrome enzymes. From the electron transfer to the conversion of lanosterol to ergosterol, CYP family have found active. P450 family has been further divided into four major classes on the basis of the selective path for electron transfer from NADPH to the catalytic site. Activation of oxygen has been found common in major section of P450 enzymes [10]. When studied at molecular level, it was found that iron-Precoporphyrin IX (heme) with thiolate act as activation center during catalytic action.

Cytochrome P450 14α-demethylase play a prominent role during the catalysis of oxidative removal of 14α-methyl group of lanosterol and 24,25-dihydrolanosterol in mammals in order to obtain the product Δ14,15-desaturated intermediates in ergosterol (fungi), phytosterol (plants) and cholesterol (animals) biosynthesis. The process of catalysis includes three proceedings of mono oxygenation reactions which help to obtain 14-hydroxymethyl, 14-carboxaldehyde and 14-formyl derivatives along with the elimination of formic acid and introduction of double bonds at 14-15 position [11]. The reaction series proceeds in the presence of nicotinamide adenine dinucleotide phosphate reduced (NADPH) cytochrome P450 reductase, a P450 redox partner and microsomal electron transferring protein, NADPH and molecular oxygen. The product so formed may get accumulated within female and male gonads. Endoplasmic reticulum or inner mitochondrial membrane has been found as a locating region for the cytochrome P450 enzymes [12]. CYP51 actively participate in the synthesis of meiosis-activating sterols within oocytes and testis [13,14]. Specificity and specialization of the enzyme to form double bond has been found to evolve early in the eukaryotic lineage. Studies illustrated that the enzyme lack in insects and nematodes which are considered as sterol heterotrophs. It has been observed that the evolution have not effected the structural fold of CYP51 from the single cell microorganism to that of the multi-cellular organism. Researchers are still searching the possible reason for its homology behavior in all biological kingdoms.

CYP51 has been found as a vital target for the development of anti-fungal, anti-leishmania, anti-trypanosoma and also for the design of cholesterol-lowering drugs. Recent research have

focused to design the respective drugs which have high selective ability in order to distinguish the enzyme from disease causing organism and that of human CYP51.

CYP51: Structural insight

Geometry of the substrate is of prime importance to make sure that the reaction is preceded with favorable orientation. The catalyst to work efficiently must have homologous configuration with the substrate along with a few amino acid residues conservation. The library of CYP51 genes have been increasing swiftly in past recent years where it was found that the invariant amino acid residues diminished from 40 to 29 [15]. Out of various sequences, it was coined up that the glycine majorly in *M. tuberculosis* has been found vital within the CYP51 structure thereby directing its flexibility and functionality.

Structure of CYP51 mainly composed of six regions which includes substrate recognition site enclosing heme-coordinating cystein [16,17]. With high conservation of structures in CYP family, CYP51 have marked specific substrate recognition site. From the view of the crystal structure, it can be said that SRS1 and SRS4 act as a significant region within CYP51 structure. SRS1 has been located at the upper surface of the binding cavity. With few substitutions at B'helix, the catalytic activity can be degraded easily in *M. tuberculosis* and human CYP51 [18]. *M. tuberculosis* is 26-29% identical to that of fungal enzymatic structure. Extensive research has been performed to study the effect of mutations in specific regions of the enzyme. Such as, if alanine is substituted in the region, there is declination in enzyme expression where as no major difference has been observed in its activity. It has been observed that B'C loop within the CYP51 structure of *M. tuberculosis* have open conformation which is absent in all other known P450 structure. Molecular modeling of the enzyme showed that the substrate in *M. tuberculosis* approach from the top instead of front. With the study of eukaryotic enzymatic structure one can find the position of B'C loop and also the possible approach of the substrate.

Another important region, SRS4 was found to be located in the region of C-terminal of enzymatic I-helix within the binding cavity [19]. The region has been specified with the presence of triplet -HT/sS-. Out of the triple residues, first and last are conserved in entire CYP family. The middle residue may vary such as many of the enzymes within filamentous fungi has been found with S as middle residues thus distinguishing itself from other sequences of biological kingdom. Further study of existing motifs within the enzyme could unfold numerous enzymatic gene sequences which could be used for therapeutics in an efficient way. Moreover, mutations could also be made for the improvement of existing sequences or for the evaluation of designed drugs within different organisms.

CYP51: In therapeutics

CYP51 has been found out as a potential target mainly in unicellular microorganism where it inhibits the organism growth thereby curing the respective infectious disease. In plants, if the enzyme is targeted it badly effect its growth as well as the development whereas animals may result with declined growth of cholesterol synthesis. Although the enzyme has been considered as a proficient target in different fields but currently plays a major role in treatment of clinical and agricultural mycoses. This area of study was previously neglected but elevation in number of mortality and morbidity pose a serious concern worldwide. Infectious disease is common in immuno-compromised patients such as HIV, cancer patients and many more. Since past few decades, innumerous work has been performed toward this area in order to develop novel and potential anti-microbial agents. The urge for the development of drugs keep on increasing due to high resistance ability of microbes, low bio-availability and high toxicity.

Designing and synthesis of azoles and its derivatives have emerged as the class of well known CYP51 inhibitors. During 1960, clotrimazole, miconazole and econazole were developed as fungicidal having imidazole as main nucleus [20]. The mechanism of action caused increased concentration of 14α-methyl sterols along with arrest of ergosterol synthesis [21]. The action leads to the lowering of fungal cell growth. Activity of azoles is directed by the formation of the coordination bond with heme iron atom within the enzymatic binding cavity. This binding behavior was analyzed and is considered as one of the major reason for high toxic behavior of the existing anti-fungal drugs.

With the augmentation of so many problems related to azoles, researchers have moved towards the development of potent agents with new nucleus devoid of azoles. Computational drug designing have played a vital role in this. Structure based pharmacophore modeling was employed rigorously for the designing of potent lead compounds which could synthesized further and could be evaluated in less period of time. During the pharmacophore generation, structure of the receptor is available and ligand needs to be devised. For the development of anti-fungal drugs, crystal structure of CYP51 (*C. albicans*) and MTCYP51 was employed as a base during structure base drug designing. The modeling techniques illustrated that we can develop and evaluate a series of non-azole anti-fungal agents with low toxicity and no interaction with heme iron atom.

Recent advances with potential target CYP51

Extensive work has been performed considering CYP51 as target. Researchers are focused to develop agents which can combat resistance ability of fungus and also could enhance the bio-availability. In 2017, Silva et al. [22] have analyzed the potency of two class of drugs; VNI and VFV. Both the drugs where tested on Swiss mouse models against chagas disease using both the animal genders. Studies illustrated high potency of VFV against T. cruzi strain in comparison to VNI. Zhang et al [23], have studied the plant extract; hydro alcoholic extracted from Flos Rosae Chinensis. The extract illustrated successful results against *C. albicans* with high potency and also it was efficient

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in promoting the effect of fluconazole, a resistant drug against *C. albicans*. Results were further confirmed by *in vivo* studies [22]. Schell et al, have demonstrated the anti-fungal activity of VT-1161 and VT-1129 which was tested against two fungal strains; *Candida glabrata* and *C. krusei*. Results of study showed promising result assuring the potency of the drugs towards these two fungal strains [24].

A quest towards the designing and development of novel antifungal agents targeting CYP51 enzyme lead to the discovery of potential lead compounds using ligand based pharmacophore modeling by Singh et al. [25] in 2016. Modeling results were confirmed by in vitro and gastrointestinal studies. The study was preceded using four fungal strains; *C. albicans, C. parapsilosis, C. tropicalis* and *A. niger*. Moreover, docking studies were also performed in order to illustrate a view of receptor-ligand interaction [26]. Pyrazoline and isooxazoline derivative has been designed and synthesized by Bano et al. [27] in 2015, to target CYP51 enzyme and inhibit the fungal growth [28].

In 2015, a step towards green chemistry was made by Anusha et al. [29] they have synthesized a series of novel 6-(adamantan-1-yl)-2-substituted-imidazo [2,1-b] 1,3,4 thiazoles which were studies to combat Mycobacterium tuberculosis focusing on inhibition of CYP51 enzyme. The results were performed at a mechanistical level with efficient results [27]. Structure based drug designing was used as a query to synthesize a series of 4-aminopyridyl-based inhibitors of *Trypanosoma cruzi* CYP51 in 2013 by Choi et al. [30]. Designed compounds illustrated good stability as well as selectivity for the targeted enzyme. Results of the study were found significant for changes disease which further could be evaluated [28]. In 2011, Same et al., have performed antifungal activities using a synthesized series of 2,5-dimercapto-13,4-thiadiazole derivatives and also evaluated the receptor-ligand interactions of these compounds with CYP51 enzyme. Results demonstrated the successful inhibition of CYP51 and good anti-candida activity of the compounds [29,30].

Conclusion

Recent studies have shown the importance of CYP51 in controlling the growth of deadly mycoses and also cancerous cells. This target has been profoundly studied and used for the development of novel agents to slow down the mortality and morbidity rate for various diseases. The structure of the enzyme is yet to be explored for different organisms which could help to use the enzyme for further eradication of other complex diseases.

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