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REVIEW ARTICLE OPEN ACCESS

Current Promising Therapeutic Targets for Aspergillosis Treatment

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Abstract

Aspergillosis is a fungal disease caused by different species of *Aspergillus*. They live in soil,dust and decomposed material. Number of *Aspergillus* species found till now is about 300 and more are still to be identified. Only few *Aspergillus* species can cause human disease and the most common species for human infection is *Aspergillus fumigatus*, which is a ubiquitous airborne saprophytic fungus. Severity of the disease ranges from an allergic response to life-threatening generalized infection. They grow optimally at 37°C and can grow upto 50°C. The fungal conidia are being constantly inhaled by humans and animals everyday normally gets eliminated by innate immune mechanism. Due to increasing number of immunocompromised patients, severe and fatal Aspergillosis cases have augmented. Currently, available antifungal drug for the treatment of Aspergillosis act on these three molecular target are 14 alpha demethylase for Azoles, ergosterol for Polyene and β-1,3-glucan synthase for Echinocandin. These antifungal drug show high resistance problem and toxicity. So, it is high time to develop new drugs for treatment with reduced toxicity and drug resistant problem. Synthesis of essential amino acid is absent in human as they obtain it from their diet but fungi synthesis these amino acid. Thus, enzymes in this pathway acts as novel drug target. This article summarizes promising drug targets presents in different metabolic pathway of *Aspergillus* genome and discusses their molecular functions in detail. This review also list down the inhibitors of these novel target. We present a comprehensive review that will pave way for discovery and development of novel antifungals against these drug targets for Aspergillosis treatment.

Keywords: Aspergillosis, Novel Targets, *Aspergillus fumigatus*, Antifungal, Pathways, Enzymes, Fungi, Inhibitors

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INTRODUCTION

In 1729, Pier Antonio Micheli who was both biologist and priest in Italy, named a mold "Aspergillus" as it looked like a holy water sprinkler (Aspergillum). The genus Aspergillus has approximately 184 species. A total of 40 among them are known to cause human or animal infections. *Aspergillus fumigates* is reported as most frequent species for causing human infections.

The physician George W. Fresenius was the first who described the species "fumigatus" in 1863. Aspergillosis occurs worldwide having estimated life-threatening infection per year at that location is >200,000 and thus having the mortality rate of 30-95% in the infected population[1]. *Aspergillus fumigatus* is an ubiquitous human fungal pathogen due to its effective dispersal in air, survival and growth in the wide range of environmental condition, swift adaptability to host environment, physical characteristics that allow conidia to reach distal airway[2]. Aspergillosis includes a wide range of diseases, each related to a spectrum of abnormal immune responses within the host. It is broadly classified into three categories; allergic, chronic and invasive. The pathogen has eight chromosomes comprising 28.526 Mb genome length which codes for 9630 proteins. Due to severity of antifungal agents with the problem of side effect and drug resistance emergence, treatment of fungal infection is confined[3,4]. For the treatment of Aspergillosis, four different class of antifungal drug is used which includes- azoles, polyenes, echinocandins, and alyllamines[5]. Since 2006, no new types of antifungal drug have been revealed till now[6].

The drug targets with their metabolic pathway and functions

For developing drugs against Aspergillosis many target have been studied and still the hunt is on for discovering drugs with better efficacy and precision. Some of the drug targets have been already explored while other targets are still under investigation for designing inhibitors against them. Such targets have been described in detail along with their biosynthesis pathway,biological role, molecular function and their sub-cellular localisation. A drug target in pathogen must be essential for survival of pathogen and it shall have no significantly similar genes in host genome. The following point we should consider while finding the drug target-(1)Target must be essential for the survival of fungi.(2)Both human and fungi shares basic eukaryotic characteristics,so target or inhibitors should provide favourable therapeutic-toxic ratio.(3)Target of fungal pathogen is widely spread and it should be economically attractive[7].

We have summarized 33 novel therapeutic targets present in Aspergillus genome which may further facilitate the antifungal drug discovery.

Amino acid biosynthetic pathway

There are twenty amino acid in which only nine are essential for human,which they cannot synthesize in their body. Thus,some of the steps of the amino acid biosynthetic pathway was catalyzed by the enzymes which is absent in human. So,on targeting these enzymes we find some novel drug targets. In pathogenesis of A.fumigatus,biosynthetic pathways like biosynthesis of lysine, aromatic amino acids and sulfur-containing amino acids such as methionine and cysteine are determinants of its virulence[8]. Growth defect and reduced virulence factor is seen when there is some defect in amino acid biosynthetic pathway[9].

Biosynthesis of Histidine

Histidine biosynthesis pathway is present in bacteria,fungi and plant but it is absent in mammal. Due to this feature, it will be an attractive target for antifungal drug discovery. Histidine biosynthesis pathway is closely linked to the metabolism to purine and pentose rather than any other amino acid. Both bacteria and fungi have similar enzyme and intermediates in histidine biosynthesis pathway but having dissimilar operon and controlling genes[10]. The novel target in this pathway are as follow-

Imidazole glycerol phosphate dehydratase

In, histidine biosynthesis pathway, imidazole glycerol-phosphate (IGP) synthesizes imidazoleacetol-phosphate (IAP)in the presence of Imidazole glycerol-phosphate dehydratase (IGPD; EC 4.2.1.19)[11]. In Aspergillus nidulans, erasure of the IGPD encoding gene, named hisB, was known to cause histidine auxotrophy. The IGPD activity containing protein is monofunctional. Removal of the gene encoding imidazole glycerol-phosphate dehydratase (HisB) results in histidine auxotrophy, resistance declines to both starvation and overabundance of different heavy metals, including iron, copper and zinc, which assume an essential part in antimicrobial host defence. Experimentally, it resulted to decrease pathogenicity in pulmonary infection, systemic infection,corneal infection of murine and larvae of wax moth[12].

Histidinol dehydrogenase

The terminal step in the biosynthesis of histidine is catalysed in the presence of enzyme histidinol dehydrogenase. HisD gene encode the enzyme Histidinol dehydrogenase[13]. This enzymes belongs to the family of oxidoreductase[14].

L-histidinol + 2NAD↔L-histidine + 2NADH + 2H

In Fungi, histidinol dehydrogenases are multifunctional catalysts, their c-terminal domain which catalyzes three different steps of histidine biosynthesis[15]. HisD gene is absent

Table 1. List of novel drug target of Aspergillus fumigatus and their metabolic pathway

	Novel Drug Target	Metabolic Pathway	
1	Imidazole glycerol phosphate dehydratase	Biosynthesis of Histidine	Amino acid Biosynthetic pathways
2	Histidinol dehydrogenase	Biosynthesis of Histidine	
3	ATP phosphoribosyl transferase	Biosynthesis of Histidine	
4	Phosphoribosyl AMP cyclohydrolase	Biosynthesis of Histidine	
5	Tryptophan synthase	Biosynthesis of Tryptophan	
6	Anthranilate synthase	Biosynthesis of Tryptophan	
7	Anthranilate Phosphoribosyl transferase	Biosynthesis of Tryptophan	
8	Ketol Acid Reducto-Isomerase(KARI)	Biosynthesis of Valine,leucine and Isoleucine	
9	Dihydroxy acid dehydratase	Biosynthesis of Valine,leucine and Isoleucine	
10	Glutamate N-acetyltransferase	Biosynthesis of Arginine	
11	Homoserine dehydogenase	Biosynthesis of Threonine, Methionine and Lysine	
12	Orotate phosphoribosyl transferase 1	Pyrimidine biosynthetic pathway	Other Pathways
13	Dihydroorotate dehydrogenase(DHODH)	Pyrimidine biosynthetic pathway	
14	Mannitol-1-phosphate-5-dehydrogenase	Mannitol biosynthesis	
15	1,3 beta glucan synthase(G.S.)	Biosynthesis of Starch and sucrose	
16	N-myristoyltransferase(NMT)	Lipid Biosynthesis	
17	Phosphatidyl ethanolamine N-methyl transferase	Lipid Biosynthesis	
18	Ras protein(RasB)	Signal Transduction pathway	
19	Thiroredoxin reductase	Thioredoxin pathway	
20	Chrosimate mutase	Shikimate pathway	
21	3-deoxy-7-phosphoheptulonate synthase	Shikimate pathway	
22	Phosphomevalonate kinase	Isoprenoid/Mevalonate pathway	
23	ACP(Acetyl transferase)	Biosynthesis of fattyacid	
24	Fatty acid synthase	Biosynthesis of fattyacid	
25	Hsp90	Hsp90-calcineurin pathway	
26	Calcineurin	Hsp90-calcineurin pathway	
27	Trehalose-6-phosphate synthase	Trehalose Biosynthetic pathways	
28	Trehalose phosphate phosphatase (Alpha-alpha-trehalose phosphate synthase)	Trehalose Biosynthetic pathways	
29	2-methyl isocitrate lyase	Propionate metabolism	
30	CYP51A	Sterol Biosynthesis Pathway	
31	Urate oxidase(Uricase)	Purine degradation pathway	
32	Riboflavin(VITAMIN –B2)	Vitamin Biosynthetic pathway	
33	3,4 dihydoxy-2-butanone 4 phosphate synthase	Vitamin Biosynthetic pathway	

in mammals,thus it can be used as target for herbicide development also[13].

ATP phosphoribosyl transferase

It catalyse the first step in the biosynthesis of Histidine. This pathway is present only in bacteria, fungi, and plants. ATP-phosphoribosyltransferase (ATP-PRT) is the first enzyme of this pathway. ATP-phosphoribosyltransferase (ATP-PRT) form N' -5' -phosphoribosyl-ATP (PR-ATP) on condensation with ATP. There are two form of ATP-PRT,one is long having two catalytic domain and a C-terminal regulatory domain and the other is short where regulatory domin is missing. This pathway is, therefore, an promising potential target for the developing non-toxic antifungals[16].

Phosphoribosyl AMP cyclohydrolase

Phosphoribosyl-AMP cyclohydrolase (EC:3.5.4.19) involved in catalyzing the third step of the histidine biosynthetic pathway which requires Zn2+ ions. In which hydrolysis of phosphoribosyl-AMP takes place. It additionallyin volved in incorporating various histidine biosynthesis bifunctional proteins[17].

Biosynthesis of Tryptophan

Microorganisms like fungi commonly synthesize tryptophan from shikimic acid or anthranilate[19].

Tryptophan synthase

In the biosynthesis of tryptophan, tryptophan synthase an essential enzyme catalyse

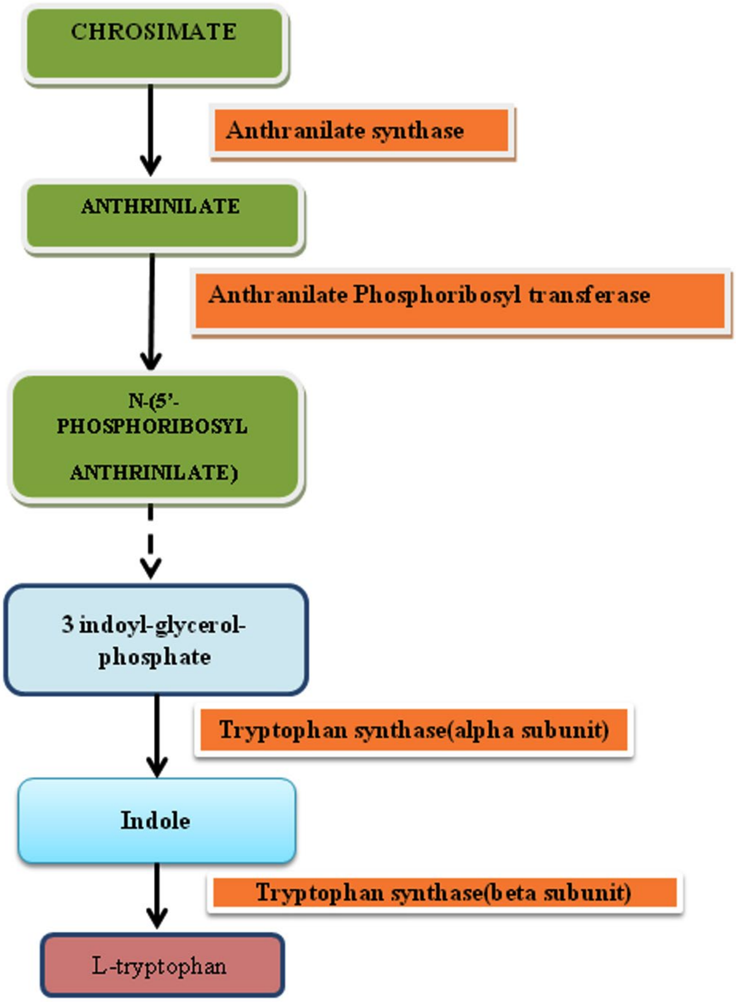

Fig. 1. Biosyntheis of Tryptophan.

the last step of the pathway in which indole is converted into L-tryptophan. Tryptophan synthase represents as excellent target for the development of new antifungal agents as genes of tryptophan biosynthesis pathway is absent in human[20].

Anthranilate synthase

Anthrinilate is intermediate of tryptophan biosynthesis and it is produce by microorganism which is used by the industry for the synthesis of dyes, perfumes and pharmaceutical compounds.

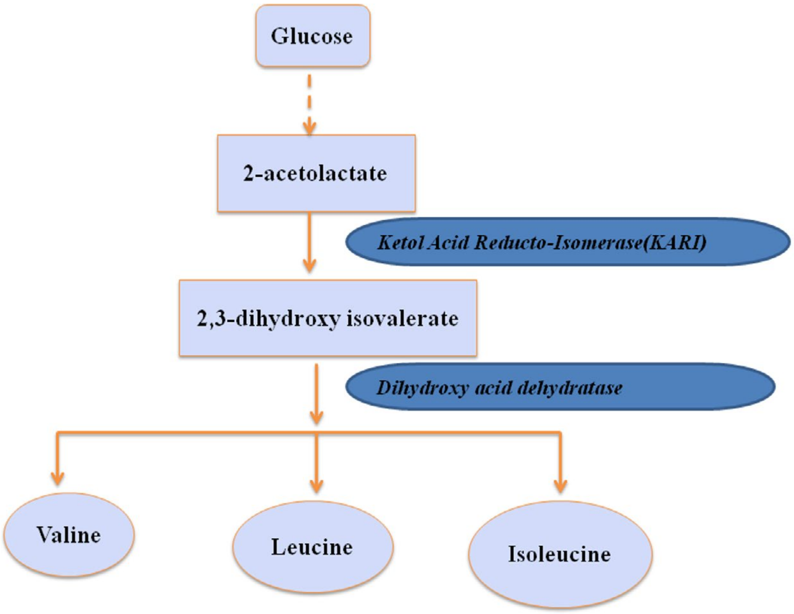

Fig. 2. Biosynthesis of Valine,leucine and Isoleucine.

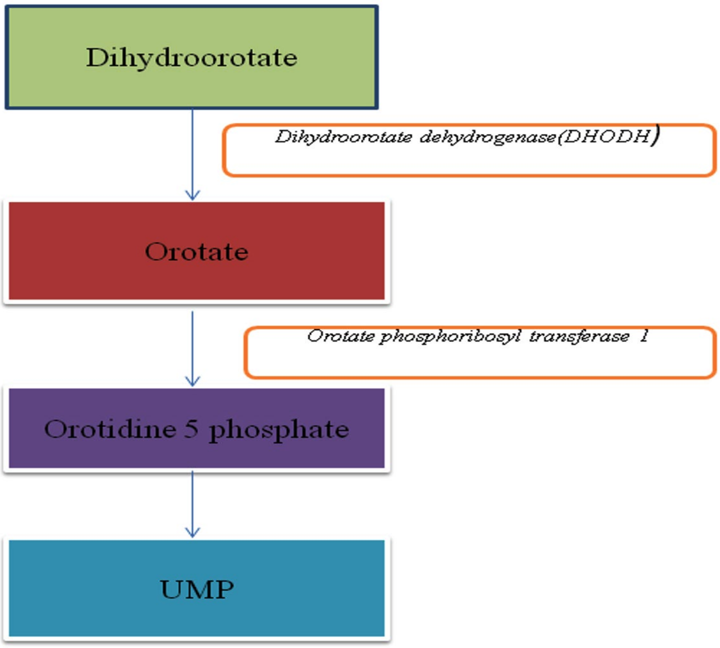

Fig. 3. Biosynthesis pathway of pyrimidine.

Many prokaryotics microorganisms was engineered to overproduce anthrinilate but on engineering eukaryotic microorganisms production of anthrinilate was less[21].

Anthranilate synthase (EC 4.1.3.27) is involved in catalyzing the reaction which prompts the biosynthesis of tryptophan from either chorismate and ammonia or chrosimate and glutamine[22]. In every single microbial species, anthranilate synthase (AS) is an oligomer of nonidentical subunits assigned AS alpha-subunit (ASI or part I) and AS beta-subunit (ASII or segment II). In a few living beings, the subunits are related and give an αβ dimer and in others an α2 β2 tetramer. In prokaryotes like blue-green algae and bacteria, the trpE gene is encoded by AS subunit while in eukarya like plant and fungi, the TRP2 gene is encoded by ASA1/ASA2 but fl-subunit of the AS enzyme complex contains another enzyme of tryptophan biosynthesis, other genes, besides trpG and trp3 and ASB can encode the multifunctional subunit[23]. Anthranilate synthase (AS) is responsible for synthesis of anthranilate which is common precursor of many compounds, from chorismate and Gln. Along with chrosimate and Gln, when concentration of ammonia is high then Anthranilate synthase speed up the synthesis step of anthranilate from chorismate in which ammonia acts as an amino donor. Tryptophan cause feedback inhibition of the enzyme[24]. Thus, on inhibition, this enzyme may put an end to the normal growth and production of pathogen. The reported inhibitors of this enzymes are Acivicin, anthranilic acid, Chanoclavine and Elymoclavine[23].

Anthranilate Phosphoribosyl transferase

Anthranilate phosphoribosyltransferase (TrpD) enzyme is member of the family of glycosyltransferases, specifically the pentosyltransferases. It is known for catalyzing the transfer of a phosphoribosyl group to anthranilate which eventually leads to the generation of phosphoribosyl anthranilate. It is involved in tryptophan biosynthesis. This enzyme participates in the phenylalanine, tyrosine and tryptophan biosynthesis and two-component system – general. It has been reported that anthrnilic acid, 3-hydroxyanthranilic acid, n-(5-phospho-d-ribosyl) anthrnilic acid and pyrophosphate are inhibitors of this enzyme. Inhibition of TrpD will be critical for fungal survival as it will stop Tryptophan synthesis[23,25,26].

Biosynthesis of Valine, leucine and Isoleucine

In comparison to fungi, humans are unable to synthesize these branched chain amino acids (Valine, leucine and Isoleucine). These biosynthesis pathway has three steps till the production of 2-ketoisovalerate which later breakdown for the synthesis of these branched chain amino acid -valine, leucine, and isoleucine[27].

Ketol Acid Reducto-Isomerase(KARI)

Ketol-acid reductoisomerase (KARI) is an enzyme which is involved in the biosynthesis of chain amino acids, Valine, leucine, isoleucine, Pantothenate and CoA in Aspergillus. Metabolization of isoleucine and valine biosynthesis in Aspergillus is done through threonine moiety. For KARI to catalyze a reaction, Mg++ is required as cofactor and NAPDH as a coenzyme. An interruption in this pathway influences the survival of the Aspergillus under the condition of threonine limitation. The interesting fact about this enzyme is its involvement in the biosynthesis of leucine, isoleucine, and valine which is an essential amino acid for a human. In human, this enzyme is not

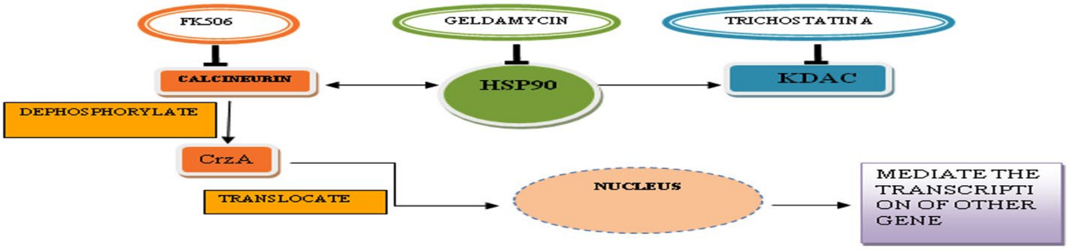

Fig. 4. Hsp90-calcineurin pathway.

be able to amend the amino acid metabolism on inhibiting but in a pathogen, inhibition occurs when these amino acids are not available[23,28]. Thus, the KARI is selected as a putative antifungal target. They reported N-hydroxy-n-isopropy loxamate, P-chloromercuribenzoic acid, 2-methyllactic acid and 2-oxo-p-hydroxy isovalerate as inhibitors of KARI[23].

Dihydroxy acid dehydratase

The dehydration and tautomerization reaction of two 2,3dihydroxycarboxylic acids (occurs naturally) to the corresponding 2-kaectoids was the third step in the branched chain amino acid biosynthesis which was catalyzed by dihydroxy acid dehydratase. This branched chain amino acid biosynthetic pathway synthesize valine, leucine, CoA, and isoleucine. Thus,the intermediates present in the biosynthesis of valine, leucine, CoA, and isoleucine were used as the drug target. These enzymes were not present within the human host. Therefore, the side effect on the host will be minimum. There are no reported inhibitors[23].

Biosynthesis of Arginine

Arginine is converted into NO (nitric oxide) in the presence of enzyme Nitric oxide synthase. Nitric oxide is necessary for fungal growth and development. It also acts as signalling molecules[29].

Glutamate N-acetyltransferase

In the biosynthesis of ornithine, transacetylation reaction occurs between N2-acetylornithine and glutamate which is catalyzed by the enzyme Glutamate N-acetyltransferase. It uses acetyl acceptors in the form of arginine, glutamine, and lysine. This protein produce an high intracellular concentration of arginine for biosynthesis of clavam biosynthesis inspite of primary metabolism. It is also involved in the pathway of clavulanate biosynthesis, which is part of Antibiotic biosynthesis[30,31].

Biosynthesis of Threonine, Methionine and Lysine

Homoserine dehydogenase

This enzyme belongs to the family of oxidoreductase. The biosynthesis of threonine, isoleucine, and methionine from aspartic acid is catalysed by homoserine dehydrogenase. It is absent in human thus,it can be a antifungal target with high specificity and few side effect[32,33].

Pyrimidine biosynthetic pathway

Synthesis of uracil monophosphate(UMP) is essential for nucleic acid synthesis. Using pyrimidine, some cell also make UMP. Pyrimidine is also required for cell proliferation and adaptation to cell stress[34].

Orotate phosphoribosyl transferase 1

Orotate phosphoribosyltransferase (OPRTase) is the among the top ten phosphoribosyl transferases enzyme, which is required in both de novo and salvage pathway of nucleotide synthesis. This enzyme is also require for histidine and tryptophan formation. Orotate phosphoribosyltransferase (OPRTase) also play essential role in the biosynthesis of pyrimidine nucleotides. De novo pyrimidine nucleotide synthesis pathway leads to the synthesis of the pyrimidine ring earlier to attachment of the ribosyl group. When orotate reacts with ribose-5-phosphate donor 5-phosphoribosyl-1-pyrophosphate (PRPP) it synthesizes orotidine-5-phosphate (OMP) and pyrophosphate in the presence of OPRTase. It signifies essentiality of OPRTase for de novo pyrimidine biosynthesis[35,36].

Dihydroorotate dehydrogenase(DHODH)

DHODH is involved in denovo synthesis of pyrimidine and catalyzes the fourth step of pyrimidine biosynthesis pathway in which it converts dihydroorotate to orotate[37]. On the basis of the difference in amino acid sequence, there are two classes of DHODH. Class II DHODH is found in most of the fungi (including *A.fumigatus* and *C.albicans*) animals, plants, gram-negative bacteria and archaebacteria[38]. They are omnipresent FMN (flavin mononucleotide) flavoenzymes which are involved in both basic and applied area of research. Teriflunomide is known to inhibit DHODH in humans[39].DHODH is widely used as a potential drug target in infectious disease, rheumatology, oncology and is a up-and-coming model for the evolution study for enzymatic catalysis[40].

Mannitol biosynthesis

Mannitol-1-phosphate-5-dehydrogenase

The NAD-dependent reduction of mannitol-1-phosphates from fructose-6-phosphate is catalyzed by Mannitol dehydrogenase. On dephosphorylation, mannitol-1-phosphates forms Mannitol in the presence of Mannitol-1-p-phosphatase, which is the irreversible step[41]. Mannitol 2-dehydrogenase then catalyzes the NAD-dependent reduction of mannitol to fructose,which again on phosphorylation form fructose-6-phosphate. Thus,this cycle will run in this direction where Mannitol-1-phosphate-5-dehydrogenase is present. Fungal mannitol serves as carbon source, as reservoir of reducing power, stress resilience and spores dispersal agent. Dehydrogenases are evolutionarily related, including mannitol 2-dehydrogenase (gene mtlK), mannitol-1-phosphate 5-dehydrogenase (gene mtlD) and mannonate oxidoreductase (fructuronate reductase) (gene uxuB)[42].

Biosynthesis of Starch and sucrose 1,3 beta glucan synthase(G.S.)

Fungal 1,3 glucan plays an important role in construction of cell wall and growth of cell. Its localization in plasma membrane is essential for its activity. This enzyme form fungal 1,3 b-glucans on polymerization of uridine 5-diphosphoglucose.Fks is a transmembrane protein is the catalytic subunit of GS complex. Fks is located in growing part of cell like cell tip and septum site. On inhibiting this enzyme,there is depletion in 1,3 b-glucans. The reported inhibitors of this enzymes are echinocandin and pneumocandin[43].

Lipid Biosynthesis

Lipids such as phospholipids, sphingolipids fatty acids, sterols are important component of cell membrane. They also act as signalling molecules by regulating cellular metabolism[44].

N-myristoyltransferase(NMT)

This enzyme catalyzes the process of adding up of myristic acid to the amino-terminal glycine residue in a number of eukaryotic proteins including protozoa, fungi and mammals and viral proteins. Cellular function, signal transduction cascade functions, and membrane targeting function of a protein is NMT dependent[45,46]. N-myristoylation is a crucial protein to all pathogenic fungi and plays an important role in cell morphology and cell wall integrity. This protein is absent from human cell. Thus NMT is a promising therapeutic target for antiviral, antiparasitics, antifungal and anticancer drugs[47-49]. Discovery of pyrazole sulphonamide compound as an inhibitor for NMT has been obtained after screening a library and under repressive condition, it has shown fungicidal activity[46].

Phosphatidyl ethanolamine N-methyl transferase

In the biosynthesis of phosphatidylcholine, the initial step of the methylation pathway was catalyzed by phosphatidyl ethanolamine N-methyl transferase in which phosphatidylethanolamine (PE) is converted to form phosphatidylcholine (PC). This protein is involved in the pathway of phosphatidylcholine biosynthesis, which is part of Phospholipid metabolism and it is one among two pathways for PC biosynthesis[50,51]. Methyltransferase show essential role in different cellular processes which includes biosynthesis, signal transduction, repair of protein, regulation of protein and gene silencing[52,53].

Signal Transduction pathway

Ras protein(RasB)

Ras protein was located in the plasma membrane as it is a low molecular weight monomeric G-protein. It is required for Ras network interaction which has a crucial role in fungal growth and its virulence. Ras protein induced by external stimuli work as a signal mediator for different downstream cascades and activates transcription factors and play role in different types of a cellular process like cell division, differentiation, growth, and survival.

In a human cell, Ras protein consists of three isoforms (HRas, KRas, NRas) but *Aspergillus fumigatus* only had two Ras homologs(RasA, RasB). The sequence of RasA is more similar to human HRas with homologs found in most eukaryotes and filamentous fungi only produce RasB. Both protein RasA and RasB alter virulence in *A.fumigatus* and other pathogenic fungi. Both of them exhibit discrete role but have some common roles in conidial germination, conidiogenesis, mitosis and mycelia growth. In regulating cell response to the various types of stresses from a wide range of effector protein, an important role is played by Ras-mediated signaling pathway. This pathway control host virulence in pathogenic fungi. Thus, this protein and their effector represent a potential target of intervention for novel antifungal therapy[54].

Thioredoxin pathway

Thiroredoxin reductase

Thioredoxin (Trx) and thioredoxin reductase (TrxR) are two enzymes of Thioredoxin systems. TrxR is responsible for the reduction of

Thireodoxin(Trx). Thioredoxins is tiny protein of 12–13 kDa and is distributed universally. Two categories of Thioredoxin reductase are found in all organisms, one having low molecular weight about 35–36 kDa subunits and the other one is having high molecular weight of 55–58 kDa. Prokaryotes, archaea, plants, and fungi has low molecular weight(TrxR) and high molecular weight (TrxR) is present in higher eukaryotes. High molecular weight TrxR contain an extra redox active site in the C-terminal from low TrxR. This active site is responsible for the interaction with the substrate. They are involved in the regulation of methionine biosynthesis,cell growth,gene transcription and apoptosis[55]. Number of inhibitors targetting this pathway have been identified which are used as antibacterial and anti-cancer drugs[56].

Shikimate pathway

The shikimate pathway is found in both microorganisms and plants but missing in animals[57]. In the formation of phenylalanine, tyrosine, tryptophan and some aromatic compounds,study of this pathway is essential.

Chrosimate mutase

Chorismate reside in a middle place in the shikimate pathway which connect primary and secondry metabolism in bacteria,plant and fungi[58].Conversion of chorismate to prephenate is done by Claisen rearrangement in the presence of chorismate mutase (CM). Enzymes other than chrosimate mutase which utilize chorismate are chorismate lyase, isochorismate synthase, anthranilate synthase, and p-aminobenzoate synthase. CMs exist in various forms both functionally and structurally.This pathway is also absent in human. Thus,chrosimate mutase can be a novel drug target for the development of antifungal drugs[51].

3-deoxy-7-phosphoheptulonate synthase

In shikimate pathway, DAHP is be responsible for biosynthesis of phenylalanine, tyrosine, and tryptophan. It regulates the amount of cabon entering the pathway[59]. When phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate (E4P) condense,it synthesizes 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP). This conversion is catalyzed by DAHP synthase. The enzyme is a target for negative-feedback regulation by pathway intermediates or by their end products[60].

Isoprenoid/Mevalonate pathway

Mevalonate pathway is also known as Isoprenoid pathway or HMG-CoA reductase pathway. This pathway consist of seven reactions which starts with acetyl CoA and finally produces isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Statin inhibit this pathway and thus, no production of mevalonate5-phosphate[61,62].

Phosphomevalonate kinase

In the biosynthesis of sterol (fungal ergosterol), mevalonate is the key metabolite and is phosphorylated by mevalonate kinase. This enzyme convert mevalonate 5-phosphate and ATP to mevalonate 5-diphosphate and ADP[61].

Biosynthesis of fatty acid

ACP(Acetyl transferase)

The pathway of fatty acid biosynthesis has been well elucidated in both prokaryotes and eukaryotes[63]. In fungi, synthesis of fatty acid synthesis is divided into different compartment having large multifunctional enzymes: the fatty acid synthases (FASs). There are three different system for the synthesis of fatty acid by de novo are studied. Usually type I fatty acid synthase system (FAS I) was used by eukaryotic and prokaryotic while FAS II was used by bacteria and FAS III was used by some parasite. The fungal fatty acid synthase complex consist of two polyfunctional protein. Due to essential role of fatty acid biosynthesis, this pathway makes the FAS systems potential targets for novel antifungals[64,65].

Fatty acid synthase

Fatty acids (FAs) is synthesized in the presence of fatty acid synthase (FAS) from acetyl CoA[66]. In *Aspergillus nidulans*, there are two different form of fatty acid synthase. One of them is for the metabolism of primary fatty acid and the other is for secondry metabolism. Primary FAS requires long chain fatty acid for growth and secondry FAS grows normally[67]. FAS catalyse the synthesis of palmitate from acetylCoA and malonylCoA in the presence of NADPH. Fatty acid are phospholipid part of the cell membraneand play important role in intracellular communication as lipid messenger[68].

Hsp90-calcineurin pathway

Calcineurin, lysine deacetylases (KDAC), other client proteins and Hsp90 as a center form a complex network. This complex network, in response to stress occurs due to some antifungal compound coordinates the compensatory repair mechanism of the cell wall.

Hsp90

Hsp90 is also termed as heat shock protein, stress protein or molecular chaperons. They are a group of conserved protein which prevents misfolding and aggregation of the protein. They initiate adaptive response under stress condition[69]. In *Aspergillus fumigatus*, Hsp90 consists of 706 amino acids. They are approx 60-65% homologous to human and about 75% with yeast. It is highly conserved and found in all living cells[70]. Hsp90 control the activity of its linked protein calcineurin in repairing mechanism of the cell wall and in stress response[69,71]. It is vital in bringing out molecular responses to ecological changes, morphogenesis, resistance occurs due to antifungal compound, and fungal pathogenicity[72].

Studies suggests that suppressed Hsp90 causes drastic changes in *A. fumigatus* conidiation, germination, and hyphal growth . The Hsp90 inhibitor like geldanamycin, when used alone, show restricted antifungal activity. It can not be used for treating fungal disease due to its cross-reactivity against the human proteins and lack of its fungal specificity. The Hsp90 inhibitor geldanamycin when used with FK506 show synergistic effect against echinocandin resistant strain and when it used with caspofungin it shows synergistic effect against the azole-resistant strains, achieving a fungicidal activity[73,74].

Calcineurin

Calcineurin is a Calcium and calmodulin-activated protein phosphatase enzyme which is present from fungi to mammals[75]. Calcineurin(CrzA) is serine/threonine-specific protein phosphatase. In fungi, morphology of cell and its virulence is controlled by CrzA. The activity of transcription factor, CRZ1 and its localization is to be in command of CrzA by dephosphorylating. In *A. fumigatus*, calcineurin activation is directly proportional to serum growth. When it inactivates,it decreases the virulence and provides decreased filamentation and thus, there is no growth[76]. In fungi, a critical signal transduction pathway which is essential for growth, morphology, stress response and pathogenicity is Calcineurin pathway. This pathway build a prospect for the development of new antifungal therapies by targeting it[77]. The function of Calcineurin is inhibited by cyclosporine A (CsA) and tacrolimus (FK506) on binding with their particular intracellular receptor, the immunophilins cyclophilin A (CyA) and FK506-binding protein (FKBP12)[78]. Calcineurin is essential for fungal development, stress responses, and virulence[79]. CrzA of *Aspergillus fumigatus* has approx 14.9% identity with human protein NFAT, minimizing the cross-reactivity possibility with human target, and thus it can be a good antifungal target[80].

Trehalose Biosynthetic pathways

Trehalose biosynthetic pathways arises as novel drug target for antifungal drug. Trehalose acts as energy reservoir and under stress condition it produces ATP. It also protect plasma membrane from degradation[7]. The main feature of this pathway is its direct link with glycolysis and the presence of two enzyme which is trehalose-6-phosphate synthase (Tps1), and trehalose phosphate phosphatase (Tps2)[81]. Trehalose pathway is absent in human and thus, on targeting, we may get some novel drug target.

Trehalose-6-phosphate synthase

Trehalose is a disaccharide form of glucose and it is present in bacteria, plant, insect and fungi.

In yeast and fungi,trehalose act as reserve carbohydrate other than glycogen. It also play important role in various cellular process like glycolysis, germination and sporulation. It is absent in human thus, it can be considered as novel antifungal target[82,83]. In stress condition, trehalose helps protein to uphold their native conformation. Trehalose protects cell from a number of unfavourable conditions such as heat, desiccation, freezing[84], hydrostatic pressure[85], nutrient starvation, enviormental stress and several abiotic stresses[86].

Trehalose phosphate phosphatase (Alpha-alpha-trehalose phosphate synthase)

Trehalose, which is a sugar moiety is used as a source of carbon, is essential for survival of conidia. It protects cells against a variety of environmental stresses including desiccation, dehydration, heat, cold and oxidation[87]. The enzyme alpha-alpha-trehalose phosphate synthase catalyzes the trehalose formation reaction. Firstly, trehalose 6-phosphate is synthesized from UDP glucose (UDPG) and glucose 6-phosphate (G-6-P).

Trehalose-6- Phosphatase then converts it into to free trehalose. This sugar is found in a wide variety of organisms. There are no reported inhibitors of Alpha-alpha-trehalose phosphate synthase[23].

Propionate metabolism

Propionate is used as source of energy and carbon in microorganisms. Methylcitrate cycle (MCC) is the main propionate metabolizing route in fungi. The cycle starts with propionate and finishes with the α-oxidation of propionate[88].

2-methyl isocitrate lyase

Methylisocitrate lyase is main enzymes of the cycle to catalyzes the reaction in which methylisocitrate breaks down into succinate and pyruvate. It shows that disruption of the methylisocitrate lyase leads to an inhibition of growth and reduced conidiation[89].

Sterol Biosynthesis Pathway

Sterol biosynthetic pathway of human is mostly similar to fungi, both of them result in two different molecules. Ergosterol is the major sterol present in fungal cell membranes and cholesterol is the major sterol found in mammals[90]. Studies suggest that Ergosterol is responsible to conserve mitochondrial DNA in fungi[91].

CYP51A

In eukaryotes, cytochrome P450 14a-sterol demethylases (CYP51) is the vital enzymes for sterol biosynthesis. CYP51 belongs to the superfamily of haem-containing enzymes, cytochrome P450. CYP51 is situated inside the superficial membrane of the endoplasmic reticulum and it catalyzes methyl group removal from carbon 14 position. Binding of Azole to CYP51 is non-competitive, which causes reduction in the final fungal sterol (usually ergosterol) and simultaneously cause accumulation of 14-methylated sterols which cause disruption of cell membrane by inhibiting fungal growth . Aspergillus species include two CYP51 isoenzymes, ie CYP51A, and CYP51B which is considered as a drug target[92,93]. The biosynthesis of ergosterol involves approximately 20 enzymes and includes the synthesis of squalene from mevalonate. At a genomic stage, the presence of multiple genes which encodes key enzymes having different 14-α sterol demethylases (Cyp51A and Cyp51B) and three C-5 sterol desaturases (Erg3) was confirmd by examining this pathway in *A. Fumigatus*. The fungal CYP51 (lanosterol demethylase) was inhibited by the azole class of antifungal drugs through competitive, reversible binding to the heme cofactor which was located in the enzyme active site[94].

Purine degradation pathway

The breaking down of purine to uric acid is conserved among different organisms but further degradationof this reaction is depend on the presence of enzyme in different organism.

Uric acid in the presence of uricase form allantonin, which is used as carbon and nitrogen source to plant and fungi[95,96].

Urate oxidase(Uricase)

Urate oxidase plays an important role in the degradation of purine pathway. It catalyses the oxidation of uric acid and produces allantoin. Uricase is not expressed in the genome of human and other higher animals and thus, uric acid was the end product of purine degradation. In fungi, purine degrades in its simplest form i.e. ammonia which causes a serious threat to human[97,98]. Fungal allantoin provide nitrogenous product to the host plant. Reportedly Amelide, 8-azaxanthine, Cyanurate, dicyandiamide, dithionite, glycerol, guanine,2-hydroxypurine, oxonic acid, sucrose and heavy metal ion act as inhibitor of urate oxidase[23].

Vitamin Biosynthetic pathway

According to Meir et al., inhibitors of these pathways inhibit many metabolic processes, which can leads to cell death[99].

Riboflavin(VITAMIN –B2)

The essential constituent of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) is Riboflavin and in *A.fumigatus* 238 flavoproteins is used as such. Deletion of this enzyme result in disturbance of many cellular process include iron homeostatis and vitamin auxotrophy[100].

3,4 dihydoxy-2-butanone 4 phosphate synthase

The riboflavin biosynthesis pathway is an essential pathway in most of the fungal pathogens[101]. Absense of this enzyme in human makes it attractive target for the discovery of antimicrobials against microorganisms that are unable to gain adequate riboflavin from their hosts. It catalyzes a stage in the biosynthesis of riboflavin. The conversion of Ribulose 5-phosphate into 3,4-dihydroxy-2-butanone 4-phosphate and formate involve different chemical reaction i.e. enolization, ketonization, dehydration, skeleton rearrangement, and formate elimination[102]. 3,4-Dihydroxy-2-butanone 4-phosphate synthase produces eight carbon atom to the xylene ring of the vitamin and thus form the building blocks. There are no reported inhibitors of 3,4 dihydoxy-2-butanone 4 phosphate synthase.

DISCUSSION AND CONCLUSION

Currently Broad-Spectrum Triazoles, Liposomal Amphotericin B, and Echinocandins are being used for Aspergillosis treatment, while increasing resistance against known antifungals is a major concern[103,104]. Recent advancements in sequencing technologies have helped in easily sequencing genomes of organisms. Availability of various microbial genomes and their different strains in public domain has facilitated the comparative genomics and mutant discovery. Increasing resistance in fungal pathogens like *Aspergillus fumigatus* against commonly used antifungal is a major concern. Despite of widespread life-threatening infections due to *Aspergillus fumigatus*, limited treatments options are available. On the other hand significant increase of drug-resistance is becoming a major challenge to treat fungal infections. There is an urgent need to discover novel of antifungal agents with optimum safety and efficacy[105,106]. However, broad spectrum antifungal drug development is relatively difficult as the fungal pathogens are more closely related to the host. The fungal pathogens are metabolically similar to their mammalian hosts at cellular level therefore offers few pathogen-specific targets[107,108]. In this review, we explained novel drug targets for Aspergillosis published within the last years. This review summarizes different drug targets in Aspergillus genomes and implications of their inhibition. This study will facilitate further antifungal drug discovery through exploration of new drug targets in fungal genomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AM conceived this project. SS, NSM and SK reviewed literature. All the authors contributed in writing the manuscript.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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