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CLINICAL MICROBIOLOGY - REVIEW

# Lysine acetylation as drug target in fungi: an underexplored potential in *Aspergillus* spp.

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## Abstract

In recent years, the intensification of the use of immunosuppressive therapies has increased the incidence of invasive infections caused by opportunistic fungi. Considering that, the spread of azole resistance and amphotericin B (AmB) inefficiency against some clinical and environmental isolates has been described. Thus, to avoid a global problem when controlling fungal infections and critical failures in medicine, and food security, new approaches for drug target identification and for the development of new treatments that are more effective against pathogenic fungi are desired. Recent studies indicate that protein acetylation is present in hundreds of proteins of different cellular compartments and is involved in several biological processes, i.e., metabolism, translation, gene expression regulation, and oxidative stress response, from prokaryotes and eukaryotes, including fungi, demonstrating that lysine acetylation plays an important role in essential mechanisms. Lysine acetyltransferases (KATs) and lysine deacetylases (KDACs), the two enzyme families responsible for regulating protein acetylation levels, have been explored as drug targets for the treatment of several human diseases and infections. Aspergilli have on average 8 KAT genes and 11 KDAC genes in their genomes. This review aims to summarize the available knowledge about *Aspergillus* spp. azole resistance mechanisms and the role of lysine acetylation in the control of biological processes in fungi. We also want to discuss the lysine acetylation as a potential target for fungal infection treatment and drug target discovery.

**Keywords** Drug resistance · Lysine acetylation · KATs · KDACs · *Aspergillus* spp.

## Introduction

The increase in the number of immunocompromised individuals and the development of mechanisms of resistance to the main antifungal drugs make fungal infection a serious clinical problem worldwide, with an estimate of one and a half million deaths per year [1, 2]. In addition, fungal infections are neglected by social and political communities [3], which creates a worse scenario. The current therapy to treat fungal diseases remains unsatisfactory, and significant investment in research is required to develop novel therapeutic alternatives [4, 5]. Further efforts in the development of new antifungal drugs and/or a combination of drugs are urgent and must be investigated to improve both human health and agricultural production [2]. Epidemiological studies report high azole resistance among *Candida* and *Aspergillus* species [6]. There are more than 250 *Aspergillus* species, around 40 of which are reported to cause infection in humans [7], such as *Aspergillus fumigatus* and *Aspergillus flavus*, the most common pathogens to which humans are exposed daily [8, 9].

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In recent years, studies have tried to explore the pathogenesis and treatment of diseases from the epigenetic perspective. Epigenetic mechanisms, including histone modifications that directly affect chromatin structure, such as methylation, phosphorylation, and acetylation, have been widely explored for the development of new treatments for cancer and inflammatory, immunological, and neurodegenerative diseases [10], but they have been less explored for fungal infections. Among epigenetic mechanisms, such as DNA methylation, modification of chromatin by proteins, the polycomb/trithorax system, and modulation of gene expression by microRNA, protein acetylation is one of the most studied epigenetic mechanisms, a dynamic posttranslational modification (PTM) present in hundreds of proteins, and the most notable modification on histones at lysine residues [11]. Not only histones can be acetylated but also several non-histone proteins involved in numerous biological processes, i.e., metabolism, translation, gene expression regulation, and oxidative stress response, have also been reported as acetylated in prokaryotes and eukaryotes, including fungi [12, 13].

In this review, we will explore the mechanisms of azole resistance in aspergilli, protein acetylation in fungi, especially in the *Aspergillus* genus, and describe the potential use of inhibitors of lysine acetyltransferases and lysine deacetylases as an antifungal therapy strategy.

## Targets for antifungal therapy and known resistance mechanisms in *Aspergillus* spp.

Azoles are the first choice of therapy for treating invasive aspergillosis [14]. The azole class comprises agents such as itraconazole, voriconazole, posaconazole, and, more recently, isavuconazole [15, 16] that are active against the *Aspergillus* species. Over the last few years, the incidence of secondary resistance to azole has increased among *Aspergillus* species, especially *A. fumigatus* [17–19], the one that causes aspergillosis most frequently [8, 20]. Although azole resistance has been widely reported [17, 21], the overall frequency of resistance in *Aspergillus* spp. is underestimated, mainly because most medical centers do not perform susceptibility testing routinely.

*A. fumigatus* azole resistance can be mediated by *cyp51A* and non-*cyp51A*-dependent mechanisms [22]. *A. fumigatus* strains with secondary azole resistance may show various mutations in the *cyp51A* gene [23, 24], which encodes the 14-α-lanosterol demethylase enzyme from cytochrome P450, the main target of these compounds [25]. These microorganisms develop resistance through two routes of resistance: (1) due to long-term therapy with azole derivatives, the patient route; and (2) due to the contact of these microorganisms with azolic compounds used in agriculture, the environmental route [18, 26–28].

In patients with chronic pulmonary diseases receiving long-term azole therapies, *A. fumigatus* may undergo multiple genetic changes during infection, including changes that confer resistance to these compounds [29]. Resistance mechanisms involving point mutations in the *cyp51A* gene can be found in laboratory cultures. These mutations can generate amino acid substitutions in G54, G138, G448, or M220, which are primarily located close to the opening of one of the two access channels of the protein binder, preventing the attachment of most of the azole molecules and thus reducing the interaction between drug and microorganisms [26, 30–32]. Similarly, the substitution in L98 is located at a highly conserved loop-like region, and modifications in this region affect the antifungal agent in the binder access channel [26, 33].

The environmental route of resistance is caused by tandem repeat (TR) mutations in the *cyp51A* promoter region, which causes overexpression of the protein and leads to a higher amount of antifungal agent required to prevent enzyme activity. The insertion of 34, 46, 53, or 120 base pairs into the *cyp51A* promoter region, combined or not with nonsynonymous mutations, TR34/L98H, and TR46/Y121F/T289A, may confer diverse degrees of azole resistance [34–37]. However, in vivo resistance development has primarily been associated with nonsynonymous mutations in *cyp51A*-inducing amino acid substitutions of hot spots (e.g., G54, G138, M220, and G448) or non-*cyp51A*-mediated mechanisms, but not with tandem repeats [37]. Although *A. fumigatus* is not phytopathogenic, many fungicides whose structures are similar to those of clinical compounds act against these microorganisms. Thus, the hypothesis is that *A. fumigatus* may develop resistance to azole compounds due to contact with molecules used in agriculture to protect plants against fungal pathogens [38, 39].

Although less characterized, azole resistance in *A. fumigatus* has also been attributed to the non-*cyp51* mutations. Genes involved in efflux pump play a role in azole resistance. *A. fumigatus* multidrug resistance pumps have been described in several studies and have been shown to be associated with increased resistance to itraconazole [40–42]. The deletion of the *cdr1B* gene encoding ATB-binding cassette (ABC) transporter, which is dependent on the transcriptional factor *AtrR* [43], resulted in azole-sensitive phenotypes [43, 44]. Another example of non-*cyp51* mutation is the amino acid substitution in the HapE (P88L) subunit of the CCAAT-binding complex that resulted in increased *cyp51A* expression [45, 46]. In addition, the deletion of a sterol element-binding protein in *A. fumigatus* (*SrbA*) showed decreased levels of the *cyp51A* and *cyp51B* expression, as well as hyper-sensitivity to azoles [47, 48]. Recent studies suggest that a substitution (R243Q) in AfCox10 causes azole resistance in *A. fumigatus* [49].

Azole resistance in *A. fumigatus* imposes the need to use alternative antifungal agents for the treatment of aspergillosis, such as amphotericin B (AMB) and echinocandins [14]. AMB used to be adopted as the first line of choice in the treatment of the disease, but it was replaced by compounds belonging to

the class of azoles due to its high toxicity [50]. Moreover, echinocandins are increasingly being used as a prophylaxis for patients at high risk of developing invasive fungal diseases, as well as in the therapy of patients with known or probable invasive aspergillosis who do not respond to conventional therapy. However, resistance to echinocandins was reported in *Candida* species and in *A. fumigatus* due to a mutation in the *FKS* genes, which encode the β-(1,3)-glucan synthase enzymes [51, 52].

## Lysine acetylation in fungi

The accurate regulation of the protein function is crucial for the organization and functioning of biological networks. Among the various regulatory processes, reversible PTMs provide a sophisticated apparatus to control the protein function. An important advantage of PTMs is that they can be regulated at a much faster rate and with a lower energy cost than protein turnover [53].

Multiple PTMs are well characterized, including phosphorylation, glycosylation, ubiquitination, methylation, and acetylation. Protein acetylation occurs by adding an acetyl group to the $N^{\varepsilon}$-amino group of lysine residues, eliminating the positive charge of this amino acid. This modification can result in alterations in the function of proteins by influencing their catalytic activity, their ability to interact with other proteins, or their subcellular localization [12, 53].

Lysine acetylation was first described for the N-terminal domains of histones in which it regulates chromatin structure and gene transcription [54]. However, the repertoire of acetylated lysines (Kac) has been expanded in the last 10 years with the inclusion of thousands of non-histone proteins in several organisms, such as bacteria, protozoans, worms, plants, mammals, insects, and fungi [55–62].

Several recently published studies have described the set of lysine-acetylated proteins, called acetylome, of different fungi species, including nonpathogenic fungi such as *Saccharomyces cerevisiae* and human pathogenic species such as *Candida albicans* and *A. fumigatus* [61–63]. These acetylomes revealed thousands of Kac sites of hundreds of proteins from different cellular compartments involved in several biological processes. The complete list of fungi acetylomes is shown in Table 1.

The most acetylated proteomes identified were those from *Trichophyton rubrum* mycelia (23.3%), *Yarrowia lipolytica* (22.1%), *S. cerevisiae* (19.6%), *Cryptococcus neoformans* (19.60%), and *A. fumigatus* (23.90%) [62, 64, 73, 74]. In *S. cerevisiae* and *Y. lipolytica*, the two nonpathogenic fungi, most of the acetylated proteins identified are involved in the regulation of glucose/amino acid metabolism and lipid metabolism, respectively [64, 74]. The *T. rubrum* acetylome revealed several acetylated proteins involved in metabolism and protein synthesis, but higher levels were observed in the mycelia in the growing stage compared with the conidial stage, which represents a quiescent state [73].

Among pathogenic species, several proteins associated with pathogenicity are acetylated. For example, in *Phytophthora sojae* and *Fusarium graminearum*, the two plant pathogens, some virulence factors and enzymes responsible for the production of secondary metabolites related to pathogenicity are acetylated [65, 68]. On the other hand, the acetylome of human pathogen *C. albicans* revealed acetylated proteins involved not only with glycolysis and oxidative phosphorylation but also

**Table 1** Acetylomes from different fungi species

| Organism | Number of Kac sites | Number of Kac proteins | Pathogenic | Proteome size | % Kac | Ref |
|---|---|---|---|---|---|---|
| *Saccharomyces cerevisiae* | 2878 | 1059 | No | 5907 | 19.6% | [64] |
| *Phytophthora sojae* | 2197 | 1150 | Yes (plants) | 26,469 | 6.0% | [65] |
| *Botrytis cinerea* | 1582 | 954 | Yes (plants) | 10,364 | 5.8% | [66] |
| *Histoplasma capsulatum* | 775 | 456 | Yes (human) | 9214 | 4.9% | [67] |
| *Fusarium graminearum* | 577 | 364 | Yes (plants) | 13,334 | 2.7% | [68] |
| *Candida albicans* | 1073 | 477 | Yes (human) | 9038 | 5.3% | [69] |
| *Candida albicans* | 2048 | 926 | Yes (human) | 6040 | 15.30% | [62] |
| *Aspergillus flavus* | 1383 | 652 | Yes (plants/human) | 12,818 | 5.2% | [70] |
| *Beauveria bassiana* | 463 | 283 | Yes (arthropod) | 10,363 | 2.7% | [71] |
| *Magnaporthe oryzae* | 1551 | 704 | Yes (plants) | 12,791 | 5.5% | [72] |
| *Trichophyton rubrum* (conidia) | 386 | 285 | Yes (human) | 10,005 | 2.8% | [73] |
| *Trichophyton rubrum* (mycelia) | 5414 | 2335 | Yes (human) | 10,005 | 23.3% | [73] |
| *Yarrowia lipolytica* | 3163 | 1428 | No | 6454 | 22.1% | [74] |
| *Cryptococcus neoformans* | 3535 | 1461 | Yes (human) | 7441 | 19.60% | [62] |
| *Aspergillus fumigatus* | 5238 | 2312 | Yes (human) | 9662 | 23.90% | [62] |

with histone acetylation, including H3K56ac, which is associated with the virulence of *C. albicans* [63, 75]. In addition, the acetylome network of *C. neoformans*, *A. fumigatus*, and *C. albicans* revealed that 40% of the pathogenicity-associated factors are acetylated, indicating that their functions are potentially influenced by Kac [62]. Indeed, the acetylome of the etiological agent of histoplasmosis, *Histoplasma capsulatum*, includes some virulence factors, such as calmodulin and DnaK, that are important for calcium intracellular intake during fungal infections [67].

*A. flavus*, a mostly saprophytic soil fungus, was the first specie from the *Aspergillus* genus with the acetylome described [70]. A total of 1383 Kac sites were detected in 652 proteins, with proteins related to secondary metabolite biosynthesis, i.e., enzymes with a predicted function in aflatoxin biosynthesis. Moreover, several transcriptional factors and proteins related to DNA repair mechanisms were acetylated. The acetylome of the *A. fumigatus* Af293 and *A. fumigatus* azole-resistant strains is under investigation in our group.

## Control of lysine acetylation in fungi

Protein acetylation levels are controlled by the activity of two enzyme families: lysine acetyltransferases (KATs), called "the writers," and lysine deacetylases (KDACs), "the erasers." KATs catalyze the addition of an acetyl group to the ε-amino group of a lysine residue, while KDACs do the opposite, removing the acetyl group from these proteins.

The KATs are grouped on the basis of their structural homology and catalytic mechanism. The KAT families are divided into three broad groups: GNAT (Gcn5-related N-acetyltransferases), MYST (MOZ, Ybf2/Sas3, Sas2, Tip60), and p300/CBP (protein of 300 kDa and CREB-binding protein) [76–78]. Other KAT enzymes have been identified, such as Rtt109 [63], transcription factor (TAFII250) [79], and nuclear receptor coactivators (SRC and CLOCK) [80–82]. The GNAT, MYST, and p300/CBP families are the most studied, and various crystallographic structures of their relatives have been reported [83]. The MYST family is identified only in eukaryotic cells, while the GNAT family is present and conserved in all domains of life [84]. Additionally, p300/CBP is metazoan-specific, while Rtt109 is fungal-specific [85].

Lysine deacetylases, also called histone deacetylases, have been classified into two groups: histone deacetylase $Zn^{2+}$–dependent family (classes I, II, and IV) and nicotinamide adenine dinucleotide ($NAD^{+}$)–dependent family (class III). In *S. cerevisiae*, three classes are present: (1) *class I*, represented by RPD3, HOS1, and HOS2; (2) *class II*, HDA1 and additionally HOS3, which is a fungal-specific KDAC [86]; (3) *class III* (sirtuins) [87, 88]. The eighteen KDACs found in humans are classically divided into four classes based on phylogenetic analysis and sequence homology concerning yeast protein sequences [89]. A complete description of fungal KATs and KDACs was previously reviewed [13].

## KATs and KDACs in *Aspergillus* spp.

Nutzmann and colleagues (2011) identified 40 genes encoding putative acetyltransferases in *A. nidulans*. Studies performed with the *A. nidulans gcnE* knockout strain showed that *gcnE* plays a minor function in the primary metabolism [90, 91]; however, this gene regulates development by inducing conidiation genes and activating specific gene clusters required for the biosynthesis of secondary metabolites [92, 93]. The orthologue gene in *A. flavus* (*AflgcnE*) is essential for growth and development. In addition, these results show that *AflgcnE* is also essential for cell wall integrity, genotoxic stress resistance, aflatoxin biosynthesis, and pathogenicity in maize seeds [94].

Esa1, a MYST family member, is the catalytic subunit of the NuA4 complex that specifically acetylates histone H4 [95]. The acetylation of histone H4 lysine 12 (H4K12) plays a role in the activation of secondary metabolite gene clusters in *A. nidulans* [96]. The gene expression data showed that a H4 acetyltransferase (MYST3) histone may play a role in the epigenetic control of aflatoxin gene transcription in *A. parasiticus*, in response to willow bark volatile exposure [97].

Rtt109 is a fungal-specific KAT that acetylates the histone H3K56 to promote gene activation and genome stability [98] and is essential for pathogenicity in *C. albicans* [99]. Although Rtt109 has been widely characterized regarding its function and structure, the role it plays in *Aspergillus* spp. is yet to be determined.

The reversible modification of lysine acetylation performed by KDACs is present in all organisms. About eleven KDACs are predicted in the *Aspergillus* spp. genome on average, with five zinc-dependent members and six sirtuins (Fig. 1 and Online Resource 1).

*RpdA* (class I), a *S. cerevisiae RPD3* orthologue, is essential for cell viability in *A. nidulans* and *A. fumigatus*, as its deletion significantly affects fungal development in both organisms [100–102]. The RpdA homolog in *A. oryzae* (HdaB/*AoRpd3*) is also essential for cell integrity and is involved in stress tolerance [103, 104]. HosA is another KDAC class I member in *A. nidulans* that plays a minor role as an active enzyme [86, 101, 105], although its homolog in *A. oryzae* (HdaD) regulates growth, asexual development, secondary metabolite production, and stress response [103, 104].

HdaA (class 2) is the main contributor to the overall KDAC activity, and knockout cell lines were obtained in *A. nidulans*, *A. oryzae*, and *A. fumigatus* [86, 103, 106]. The *A. fumigatus* Δ*hdaA* strain showed a reduction in growth and in the production of secondary metabolites, but the reduction in virulence was not observed for the murine IA model [107]. The removal

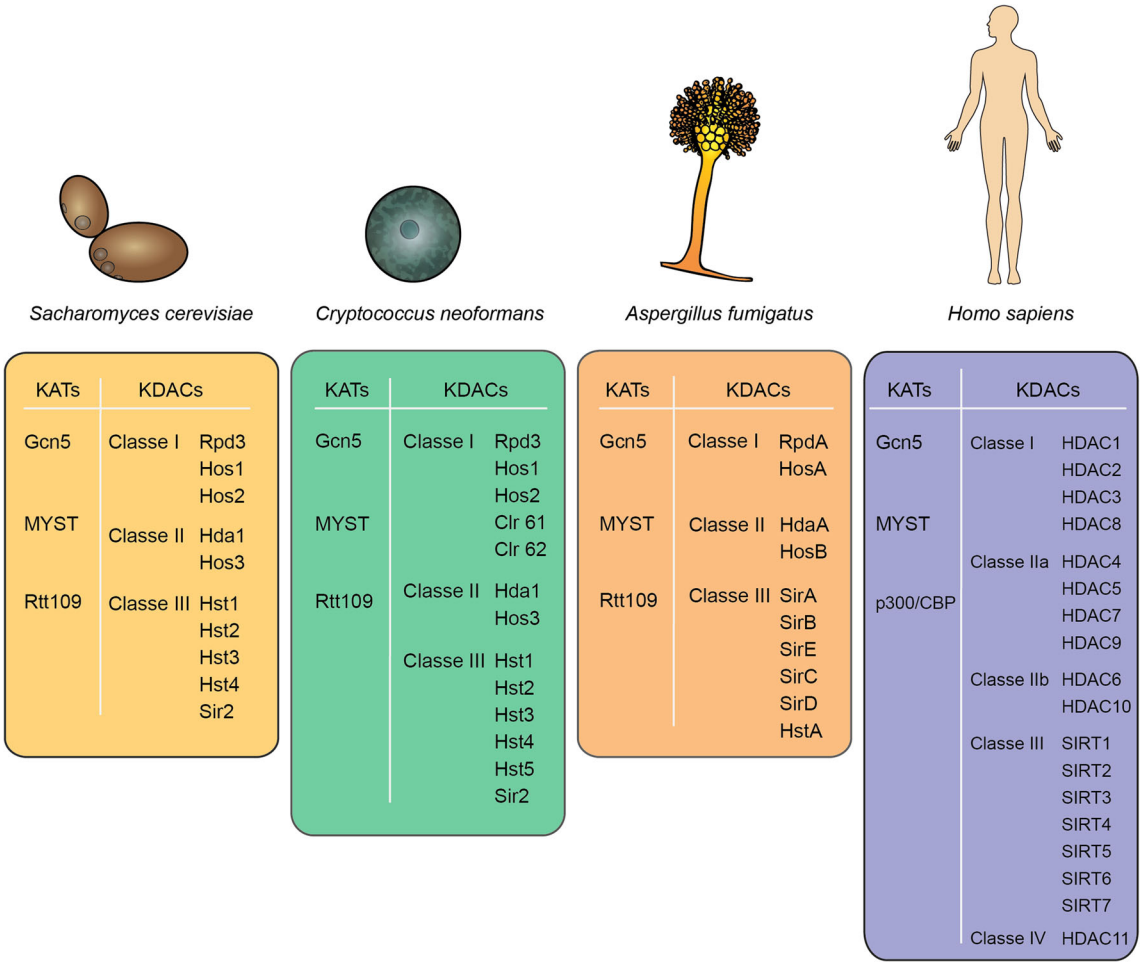


**Fig. 1** Lysine acetyltransferases and deacetylases in *S. cerevisiae*, *C. neoformans*, *A. fumigatus*, and *H. sapiens*

of *hdaA* from *A. oryzae* showed that this gene may play a role in stress response in liquid culture [103].

Sirtuins are involved in multiple cellular events, including transcriptional silencing, chromatin remodeling, mitosis, and lifespan duration [108]. Class I to III sirtuins are predicted in ascomycete filamentous fungi, whereas *S. cerevisiae* has only class I sirtuins (Sir2p, Hst1p, Hst2p, Hst3p, and Hst4p). In *A. nidulans*, HstA (class II sirtuin) has a predicted KDAC activity [109, 110]. Class I sirtuin HstD/*AoHst4* was removed from *A. oryzae* and associated with a significant role in fungal growth, sporulation, stress responses, and secondary metabolite production [104, 111]. The knockout of the *AoHst4* orthologue in *A. nidulans* (AN1226) resulted in decreased mycelial autolysis, conidiophore development, sterigmatocystin biosynthesis, and extracellular hydrolases production [87].

## KAT and KDAC inhibitors as potential antifungal enhancers

Epigenetics and PTMs have been reported to constitute an important regulatory mechanism in the transcription of genes and a link between genotype, phenotype, and environment in most eukaryotes, including fungi [112]. Changes in protein acetylation are relevant to many diseases such as obesity, diabetes mellitus, cancer, neurodegenerative, and inflammatory diseases, and several KATs and KATs inhibitors have been developed for treatment of these illnesses [113]. Some of these KATs and KDACs inhibitors could be repurposed for treatment of fungal infections, alone or combined with the classical compounds. In the next topics, we will summarize some inhibitors that have been tested in fungi.

Trichostatin A (TSA), an organic antibiotic produced by *Streptomyces hygroscopicus*, is known for its antifungal activity, with a broad spectrum of inhibition of class I and II KDACs, but its precise mode of action remains poorly understood [114, 115]. TSA showed a promising antifungal strategy for the treatment of *A. fumigatus* in combination with azole [116, 117]. Sodium butyrate (SB), another KDAC inhibitor, showed antifungal activity against the *Candida* species and *C. neoformans* [118]. Moreover, studies have indicated that SB affects some morphological and enzyme activity–related factors essential to the virulence of *C. neoformans* [119]. The study of the synergistic effect of MGCD290, a Hos2 fungal

KDAC inhibitor with different azoles in opportunistic fungal isolates, is one of the main studies that supports the use of KDAC inhibitors as antifungal drugs [120]. Nicotinamide is widely used as an overall sirtuin inhibitor [89, 121]. This inhibitor strongly inhibited the growth of *C. albicans*, *C. krusei*, *A. fumigatus*, and *A. nidulans* [75] and decreased the activity of some enzymes produced by *C. albicans*, *T. rubrum*, and *Trichophyton mentagrophytes* [122]. Other sirtuin inhibitors have been reported, such as sirtinol, splitomycin, salermide, cambinol, and 5-methylmellein. *A. nidulans* cultivated in 5-methylmellein showed an increase in the production of secondary metabolites, which could be used as a potential drug discovery tool [123].

Garcinol, a polyisoprenylated benzophenone derivative and a KAT inhibitor [124], caused a significant growth defect in *C. neoformans*, but the data showed the existence of off-target effects in addition to Gcn5 inhibition [125]. Anacardic acid (6-pentadecylsalicylic acid), a KAT inhibitor that inhibits p300, PCAF, and Tip60 in vitro, affects mycelial cell growth and conidial germination, also inducing apoptosis-like cell death in *Magnaporthe oryzae* [126]. Indeed, fungal-specific KAT Rtt109 was reported to be required for the treatment of pathogenesis caused by *C. albicans*, reinforcing the potential of KAT inhibitors as a therapeutic strategy [99].

## Conclusion and perspectives

In the past few years, the increase in fungal strains resistant to the main antifungal drugs used in clinical settings and in agriculture has been widely reported [127–130]. Thus, to avoid a global concern regarding the control of fungal infections and to prevent critical failures in medicine and food safety, more controlled use of triazoles by patients and in agriculture is necessary. In addition, the development of new antifungal classes and/or combinations of drugs with higher selectivity and low toxicity, which could contribute to overcoming the resistance in pathogenic fungi, are urgent [116].

Several KAT and KDAC inhibitors are currently under development as drugs for various human diseases, from tumors to fungal infections [131]. The Food and Drug Administration (FDA) has already approved some KDAC inhibitors for the treatment of cancer, such as vorinostat, romidepsin, belinostat, and panobinostat [132–137]. In addition, chidamide, another KDAC inhibitor, was recently approved in China for treatment of peripheral T cell lymphoma [138]. Several other KDAC inhibitors combined with classical chemotherapeutic compounds present promising results in preclinical and clinical trials.

Although not widely used for the treatment of fungal infections, there is great potential for use of available KAT and KDAC inhibitors or for the exploration of these proteins as drug targets for the development of new antifungal compounds. Despite the possible side effects, toxicity, and pleiotropic effects that these inhibitors could have, it is still valid not only to apply "drug repurposing" of the available approved inhibitors but also to perform large screening approaches to identify new compounds for further application in fungal infection treatments. Moreover, studies that not only identify new molecules but also understand their action mechanism—including off-target effects, structure-activity relations, pharmacokinetic/pharmacodynamic properties, and biomarkers design—are necessary to reduce toxicity, which will contribute enormously to the KAT and KDAC efficacy.

Thus, any efforts to learn the role of protein acetylation in the biology of *Aspergillus* and other fungi species will contribute not only to advance the understanding as to how these pathogens interact with their hosts and cause diseases but also to provide the opportunity of using KATs and KDACs as drug targets to develop new inhibitors that could be used to treat these diseases that affect millions of persons worldwide.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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