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(54) Title: INHIBITORS OF RTT109 AS ANTI-FUNGAL AGENTS

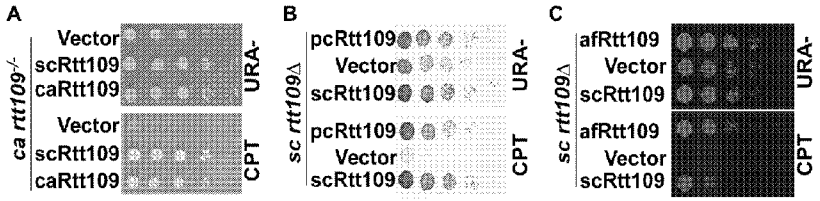

FIG. 1

(57) Abstract: This document relates to materials and methods for identifying chemical inhibitors of fungal targets useful for treating organisms infected with opportunistic fungal pathogens. For example, this document provides the materials and methods for identifying and optimizing chemical inhibitors of Rtt109, a fungal histone acetyltransferase. This document also provides the materials and methods for using high throughput screening technologies to identify candidate chemical inhibitors of Rtt109 as well as the materials and methods for evaluating candidate chemical inhibitors of Rtt109 in secondary screens for their binding efficacy, molecular specificity, and cellular toxicity.

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Inhibitors of Rtt109 as Anti-Fungal Agents

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of U.S. Provisional Appl. No. 61/499,940, filed June 22, 2011, which is incorporated by reference in its entirety.

TECHNICAL FIELD

This document relates to the materials and methods involved in identifying chemical inhibitors of fungal targets for treating organisms infected with opportunistic fungal pathogens.

BACKGROUND

Fungal infections in humans have increased significantly in the last century, mainly due to the increased number of immunocompromised patients, including individuals infected with HIV, transplant patients, and cancer patients treated with chemotherapeutic agents. Despite significant advances, the mortality rate from opportunistic fungal infections exceeds 50% in most human studies, and can be as high as 90%, as reported for bone marrow transplant recipients infected with *Aspergillus* species.

At least four major factors have impeded progress towards treating fungal infection. First, fungal pathogens are eukaryotes, and many genes involved in critical biological processes in fungi are conserved in humans. Therefore, many antifungal drugs can be toxic to humans. Second, the kingdom of fungi consists of many species and each has sophisticated strategies to survive the host and evade its immune system. No standardized vaccines have been identified for preventing fungal infections in humans. Third, it is often difficult to diagnose and confirm invasive fungal infections. Antifungal drugs are often prescribed prophylactically because of the high cost and mortality rate associated with fungal infection. Finally, fungal species can develop resistance to the most frequently used anti-fungal agents.

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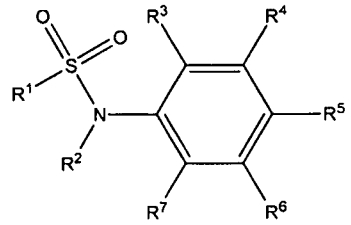
SUMMARY

Provided herein is a method of treating a fungal infection in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (1) or formula (2).

A compound of formula (1):



or a pharmaceutically acceptable salt form thereof,

wherein:

R^1 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl;

R^2 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl;

R^3, R^4 and R^5 are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, $(C_3\text{-}C_{14})$aryl, $-(CH_2)(C_3\text{-}C_{14})$aryl halogen, $-CN$, $-NO_2$, $-NR^8_2$, and $-OR^8$;

R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, $-CN$, $-NO_2$, $-NR^8_2$, and $-OR^8$, or R^6 and R^7 can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure; and

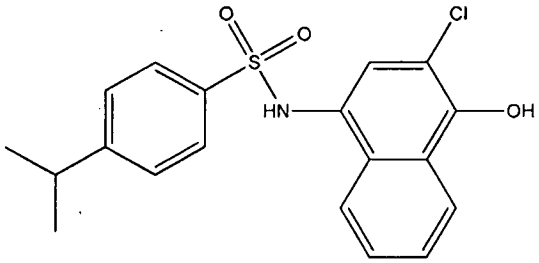
each R^8 is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

In some embodiments, R^1 is selected from the group consisting of hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl. For example, R^1 can be a substituted $(C_3\text{-}C_{14})$aryl, such as a a para-cumenyl moiety.

In some embodiments, R^2 is selected from hydrogen or substituted or unsubstituted $(C_1\text{-}C_6)$alkyl. For example, R^2 can be hydrogen, methyl, or isopropyl.

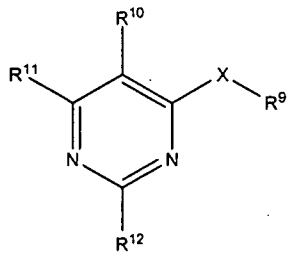
In some embodiments, R^3 is hydrogen. In some embodiments, R^4 is selected from hydrogen and halogen. In some embodiments, R^5 is hydrogen or $-OR^8$. For example, R^5 can be hydrogen, –OH, or $-OCH_3$. In some embodiments, R^6 and R^7 come together to form an unsubstituted fused $(C_4\text{-}C_6)$ ring structure. For example, the fused $(C_4\text{-}C_6)$ ring structure can be an aryl ring.

A non-limiting example of a compound of formula (1) includes:



or a pharmaceutically acceptable salt form thereof.

A compound of formula (2):



or a pharmaceutically acceptable salt form thereof,

wherein:

X is selected from O, S, and NR^{13};

R^9 is selected from the group consisting of: substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, substituted or

unsubstituted (C_3-C_7)heterocycloalkyl, substituted or unsubstituted (C_3-C_{14})aryl, and substituted or unsubstituted (C_3-C_{14})heteroaryl;

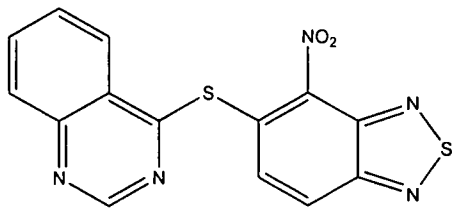
R^{10} and R^{11} are independently selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, –NO_2, –NR^{13}_2, and –OR^{13}, or R^{10} and R^{11} can come together to form a substituted or unsubstituted fused (C_4-C_6) ring structure;

R^{12} is selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, –NO_2, –NR^{13}_2, and –OR^{13}; and

each R^{13} is independently selected from the group consisting of hydrogen and (C_1-C_6)alkyl.

In some embodiments, X is S. In some embodiments, R^9 is a substituted or unsubstituted (C_3-C_7)heterocycloalkyl or substituted or unsubstituted (C_3-C_{14})heteroaryl. In some embodiments, R^{10} and R^{11} come together to form an unsubstituted fused (C_4-C_6) ring structure. For example, the fused (C_4-C_6) ring structure can be an aryl ring. In some embodiments, R^{12} is hydrogen.

A non-limiting example of a compound of formula (2) includes:



or a pharmaceutically acceptable salt form thereof.

The compounds and compositions provided herein can also be useful to inhibit inhibiting a fungal infection in a patient, fungal growth in a patient, and Rtt109 activity in a cell.

In some embodiments, the patient is immunocompromised.

In some embodiments, the fungal infection is caused by a fungal organism selected from *C. albicans*, *P. carinii*, and *A. fumigatus*.

Further provided herein is a method for screening a test compound for antifungal activity, the method comprising determining if the test compound inhibits the histone acetyltransferase activity of a fungal Rtt109 polypeptide, wherein the fungal Rtt109

polypeptide has at least 80% identity to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6.

In some embodiments, the fungal Rtt109 polypeptide has at least about 85% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, the fungal Rtt109 polypeptide has at least about 90% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, the fungal Rtt109 polypeptide has at least about 95% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, the fungal Rtt109 polypeptide has at least about 98% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, the fungal Rtt109 polypeptide is selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, the fungal Rtt109 polypeptide is complexed with Vps75.

In some embodiments, the determining comprises contacting the test compound with a composition comprising a fungal Rtt109, a substrate for a fungal Rtt109 polypeptide, and acetyl coenzyme A; and determining the level of a de-acetylated coenzyme A.

A suitable substrate for the screening assay includes a fungal histone. For example, a lysine containing histone. In some embodiments, the lysine containing histone is an Asf-1-H3-H4 complex or H3K56.

De-acetylated coenzyme A can be detected by labeling either the coenzyme A itself or the de-acetylated coenzyme A. For example, the assay solution can further include a label (e.g., a fluorophore) that reacts with de-acetylated coenzyme A to form a detectable complex.

In some embodiments, the method further comprises determining whether the test compound inhibits a human p300/CBP polypeptide. For example, the test compound can exhibit preferential inhibition of the fungal Rtt109 activity compared to human p300/CBP activity. In some embodiments, the method further includes determining whether the test compound inhibits a yeast Gcn5 polypeptide.

In some embodiments, the test compound inhibits growth of a fungal species. For example, the test compound inhibits growth of a fungal species selected from *C. albicans*, *P. carinii*, and *A. fumigatus*.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figures 1A-C depict the results of spot test experiments. Expression of Rtt109 from different fungal species suppressed the growth inhibitory effects of camptothecin (CPT). Expression of scRtt109 suppressed CPT sensitivity of *C. albicans* cells lacking Rtt109 (A). Expression of pcRtt109 (B) and afRtt109 (C) suppressed the CPT sensitivity of *S. cerevisiae* cells lacking Rtt109. Ten-fold serial dilutions of *C. albicans* (A) or *S. cerevisiae* cells (B and C) expressing Rtt109 were spotted onto media with or without CPT. Cell growth was recorded after a two-day incubation period.

Figure 2 depicts the reduced toxicity against human cells by *C. albicans* cells lacking both copies of Rtt109 (rtt109-/-). Three different wild type *C. albicans* strains, a strain containing one copy of the Rtt109 gene (rtt109+/-), and one lacking both copies of the Rtt109 gene (rtt109-/-) were used to infect H4 enterocytes. The infected enterocytes' lactate dehydrogenease (LDH) was quantified and used to measure the extent of tissue damage as a result of infection.

Figure 3 depicts how the Rtt109-Vps75 complex utilizes the Asf1-H3-H4 complex as the substrate for acetylation.

Figures 4 depicts an inhibitor against Rtt109 preferentially inhibits the activity of Rtt109 over p300/CBP (A) and Gcn5 (B) . Using Asf-H3-H4 as the substrate, candidate Rtt109 inhibitors were assessed for their ability to inhibit p300, scRtt109-Vps75 and Gcn5.

Figures 5A-D depict the results of experiments assessing three identified Rtt109 inhibitors against the growth of three fungal species. Each compound (100 μM) was

added to exponentially growing *S. cerevisiae* (A) and *C. albicans* (B). After the indicated incubation time (hours), the cell density was determined (A-B). Growth effects of these inhibitors against *P. Carinii* (C) was measured using real-time PCR by detecting fungal specific heat-shock protein 70 mRNA, against *A. fumigatus* (D) using spot tests.

Figure 6 is a bar graph depicting the results of experiments testing the toxicity of the Rtt109 inhibitors on human cells. After overnight incubation, the indicated concentration of each Rtt109 inhibitor was added to cultures of human lung epithelial cells (line A549). Cell proliferation and viability was then measured and quantified.

Figure 7 is a graphical representation outlining the screening strategy for identifying Rtt109 inhibitors, including secondary screens and SAR compound optimization.

Figure 8 shows the sequence homology of Rtt109 polypeptides from different fungal species.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this disclosure belongs. All patents, applications, published applications, and other publications cited herein are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms cited herein, those in this section prevail unless otherwise stated.

For the terms "for example" and "such as," and grammatical equivalences thereof, the phrase "and without limitation" is understood to follow unless explicitly stated otherwise. As used herein, the term "about" is meant to account for variations due to experimental error. All measurements reported herein are understood to be modified by the term "about", whether or not the term is explicitly used, unless explicitly stated otherwise. As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise:

A "patient," as used herein, includes both humans and other animals, particularly mammals. Thus the methods are applicable to both human therapy and veterinary applications. In some embodiments, the patient is a mammal, for example, a primate. In some embodiments, the patient is a human.

The terms "treating" and "treatment" mean causing a therapeutically beneficial effect, such as ameliorating existing symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, postponing or preventing the further development of a disorder and/or reducing the severity of symptoms that will or are expected to develop.

A "therapeutically effective" amount of the compounds described herein is typically one which is sufficient to achieve the desired effect and may vary according to the nature and severity of the disease condition, and the potency of the compound. It will be appreciated that different concentrations may be employed for prophylaxis than for treatment of an active disease.

The term "contacting" means bringing at least two moieties together, whether in an *in vitro* system or an *in vivo* system.

As used herein, "antifungal activity" refers to inhibition of fungal growth and infection. In some embodiments, the term antifungal activity can include prevention of antifungal infection in a patient.

In general, reference to a certain element such as hydrogen or H is meant to include all isotopes of that element. For example if a R group is defined to represent hydrogen or H, it also includes deuterium and tritium.

The term "alkyl" includes straight-chain alkyl groups (e.g., methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, *etc.*) and branched-chain alkyl groups (isopropyl, tert-butyl, isobutyl, *etc.*), cycloalkyl (alicyclic) groups (cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl), alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has 6 or fewer carbon atoms in its backbone (e.g., C_{1-6} for straight chain, C_{3-10} for branched chain). The term C_{1-6} includes alkyl groups containing 1 to 6 carbon atoms.

The term "alkenyl" includes aliphatic groups containing at least one double bond and at least two carbon atoms. For example, the term "alkenyl" includes straight-chain alkenyl groups (*e.g.*, ethylenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, *etc.*) and branched-chain alkenyl groups. In certain embodiments, a straight chain or branched chain alkenyl group has 6 or fewer carbon atoms in its backbone (*e.g.*, C_{2-6} for straight chain, C_{3-6} for branched chain). The term C_{2-6} includes alkenyl groups containing 2 to 6 carbon atoms.

The term "alkynyl" includes unsaturated aliphatic groups analogous in length to the alkyls described above, but which contain at least one triple bond and two carbon atoms. For example, the term "alkynyl" includes straight-chain alkynyl groups (*e.g.*, ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl, *etc.*) and branched-chain alkynyl groups. In certain embodiments, a straight chain or branched chain alkynyl group has 6 or fewer carbon atoms in its backbone (*e.g.*, C_{2-6} for straight chain, C_{3-6} for branched chain). The term C_{2-6} includes alkynyl groups containing 2 to 6 carbon atoms.

The term "cycloalkyl" includes a cyclic aliphatic group which may be saturated or unsaturated. For example, cycloalkyl groups include cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. In some embodiments, cycloalkyls have from 3-8 carbon atoms in their ring structure, for example, they can have 3, 4, 5 or 6 carbons in the ring structure.

In general, the term "aryl" includes groups, including 5- and 6-membered single-ring aromatic groups, such as benzene and phenyl. Furthermore, the term "aryl" includes multicyclic aryl groups, e.g., tricyclic, bicyclic, such as naphthalene and anthracene.

The term "heteroaryl" includes groups, including 5- and 6- membered single-ring aromatic groups, that have from one to four heteroatoms, for example, pyrrole, furan, thiophene, thiazole, isothiaozole, imidazole, triazole, tetrazole, pyrazole, oxazole, isooxazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like. Furthermore, the term "heteroaryl" includes multicyclic heteroaryl groups, e.g., tricyclic, bicyclic, such as benzoxazole, benzodioxazole, benzothiazole, benzoimidazole, benzothiophene,

methylenedioxyphenyl, quinoline, isoquinoline, napthyridine, indole, benzofuran, purine, benzofuran, quinazoline, deazapurine, indazole, or indolizine.

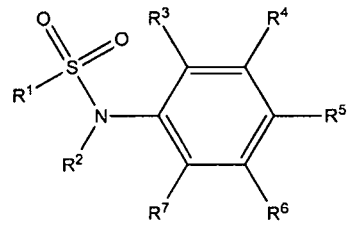
The term "heterocycloalkyl" includes groups, including but not limited to, 3- to 10-membered single or multiple rings having one to five heteroatoms, for example, piperazine, pyrrolidine, piperidine, or homopiperazine.

The term "substituted" means that an atom or group of atoms formally replaces hydrogen as a "substituent" attached to another group. For aryl and heteroaryl groups, the term "substituted", unless otherwise indicated, refers to any level of substitution, namely mono, di, tri, tetra, or penta substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position. In some cases two sites of substitution may come together to form a 3-10 membered cycloalkyl or heterocycloalkyl ring.

As used herein, "administration" refers to delivery of a compound or composition as described herein by any external route, including, without limitation, IV, intramuscular, SC, intranasal, inhalation, transdermal, oral, buccal, rectal, sublingual, and parenteral administration.

Compounds

Provided herein are compounds of formula (1):



or a pharmaceutically acceptable salt form thereof,

wherein:

R^1 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl;

R^2 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl;

R^3, R^4 and R^5 are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, $(C_3\text{-}C_{14})$aryl, $-(CH_2)(C_3\text{-}C_{14})$aryl halogen, $-CN$, $-NO_2$, $-NR^8_2$, and $-OR^8$;

R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, $-CN$, $-NO_2$, $-NR^8_2$, and $-OR^8$, or R^6 and R^7 can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure; and

each R^8 is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

In some embodiments, R^1 is selected from the group consisting of hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl. In some embodiments, R^1 is a substituted $(C_3\text{-}C_{14})$aryl. For example, R^1 can be a para-cumenyl moiety.

In some embodiments, R^2 is selected from hydrogen or substituted or unsubstituted $(C_1\text{-}C_6)$alkyl. For example, R^2 can be selected from the group consisting of hydrogen, methyl, and isopropyl. In some embodiments, R^2 is hydrogen.

In some embodiments, R^3 is hydrogen. In some embodiments, R^4 is selected from hydrogen and halogen. For example, R^4 can be Cl.

In some embodiments, R^5 is hydrogen or $-OR^8$. For example, R^5 can be selected from the group consisting of hydrogen, $-OH$, and $-OCH_3$.

In some embodiments, R^6 and R^7 come together to form an unsubstituted fused $(C_4\text{-}C_6)$ ring structure. The ring structure can include cycloalkyl, heterocycloalkyl (e.g., nitrogen containing heterocycloalkyls), aryl, and heteroaryl (e.g., nitrogen containing heteroaryls). For example, the fused $(C_4\text{-}C_6)$ ring structure can be an aryl ring.

A non-limiting example of a compound of formula (1) includes:

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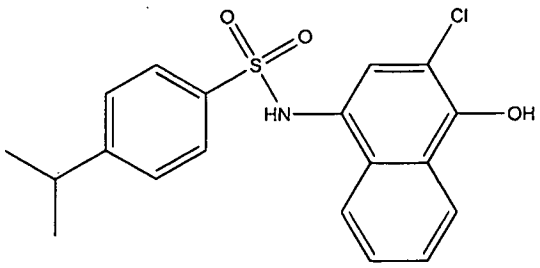
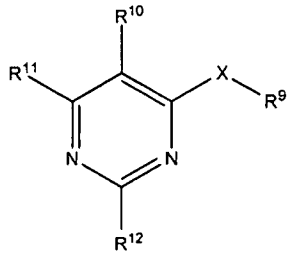


1-1

or a pharmaceutically acceptable salt form thereof.

Also provided herein are compounds according to formula (2):



or a pharmaceutically acceptable salt form thereof,

wherein:

X is selected from O, S, and NR^{13};

R^9 is selected from the group consisting of: substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, substituted or unsubstituted $(C_3\text{-}C_7)$heterocycloalkyl, substituted or unsubstituted $(C_3\text{-}C_{14})$aryl, and substituted or unsubstituted $(C_3\text{-}C_{14})$heteroaryl;

R^{10} and R^{11} are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$, or R^{10} and R^{11} can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure;

R^{12} is selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$; and

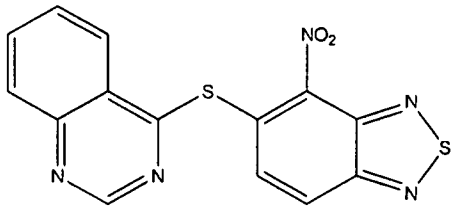
each R^{13} is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

In some embodiments, X is S. In some embodiments, R^9 is a substituted or unsubstituted (C_3-C_7)heterocycloalkyl or substituted or unsubstituted (C_3-C_{14})heteroaryl.

In some embodiments, R^{10} and R^{11} come together to form an unsubstituted fused (C_4-C_6) ring structure. The ring structure can include cycloalkyl, heterocycloalkyl (e.g., nitrogen containing heterocycloalkyls), aryl, and heteroaryl (e.g., nitrogen containing heteroaryls). For example, the fused (C_4-C_6) ring structure can be an aryl ring.

In some embodiments, R^{12} is hydrogen.

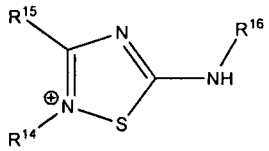
A non-limiting example of a compound of formula (2) includes:



2-1

or a pharmaceutically acceptable salt form thereof.

Further provided herein is a compound of formula (3):



or a pharmaceutically acceptable salt form thereof,

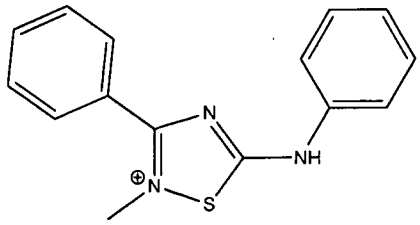
wherein:

R^{14} is selected from the group consisting of: hydrogen, substituted or unsubstituted (C_1-C_6)alkyl, substituted or unsubstituted (C_2-C_6)alkenyl, substituted or unsubstituted (C_2-C_6)alkynyl, substituted or unsubstituted (C_3-C_7)cycloalkyl; and

R^{15} and R^{16} are independently a substituted or unsubstituted (C_3-C_{14})aryl.

In some embodiments, R^{14} is a (C_1-C_6)alkyl. For example, R^{14} is CH_3. In some embodiments, R^{15} and R^{16} are substituted with electron-withdrawing or electron-donating substitutents. In some embodiments, R^{15} and R^{16} are an unsubstituted (C_3-C_{14})aryl. For example, R^{15} and R^{16} are phenyl.

A non-limiting example of a compound of formula (3) is:



3-1

or a pharmaceutically acceptable salt form thereof.

Compounds described herein, including pharmaceutically acceptable salts thereof, can be prepared using known organic synthesis techniques and can be synthesized according to any of numerous possible synthetic routes.

The reactions for preparing the compounds described herein can be carried out in suitable solvents which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially non-reactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, e.g., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected by the skilled artisan.

Preparation of compounds can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in *Protecting Group Chemistry*, 1st Ed., Oxford University Press, 2000; and *March's Advanced Organic chemistry: Reactions, Mechanisms, and Structure*, 5th Ed., Wiley-Interscience Publication, 2001 (each of which is incorporated herein by reference in their entirety).

Pharmaceutically Acceptable Salts and Compositions

Pharmaceutically acceptable salts of the compounds described herein include the acid addition and base salts thereof.

Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the.acetate, adipate, aspartate, benzoate, besylate,

bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, hydrogen phosphate, isethionate, D- and L-lactate, malate, maleate, malonate, mesylate, methylsulphate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen, phosphate/phosphate dihydrogen, pyroglutamate, saccharate, stearate, succinate, tannate, D- and L-tartrate, 1-hydroxy-2-naphthoate tosylate and xinafoate salts.

Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts.

Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

Compounds described herein intended for pharmaceutical use may be administered as crystalline or amorphous products. They may be obtained, for example, as solid plugs, powders, or films by methods such as precipitation, crystallization, freeze drying, spray drying, or evaporative drying. Microwave or radio frequency drying may be used for this purpose.

The compounds may be administered alone or in combination with one or more other compounds described herein or in combination with one or more other drugs (or as any combination thereof). Generally, they will be administered as a formulation in association with one or more pharmaceutically acceptable excipients. The term "excipient" is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

Non-limiting examples of pharmaceutical excipients suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. Pharmaceutically acceptable

excipients include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol polyethylene glycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium-chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethyl cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, and wool fat. Cyclodextrins such as α-, β, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-b-cyclodextrins, or other solubilized derivatives can also be advantageously used to enhance delivery of compounds of the formulae described herein. In some embodiments, the excipient is a physiologically acceptable saline solution.

The compositions can be, in one embodiment, formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration or in sterile solutions or suspensions for parenteral administration, as well as transdermal patch preparation and dry powder inhalers (see, e.g., *Ansel Introduction to Pharmaceutical Dosage Forms, Fourth Edition* 1985, 126).

The concentration of a compound in a pharmaceutical composition will depend on absorption, inactivation and excretion rates of the compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

The pharmaceutical composition may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values

may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular patient, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

The pharmaceutical compositions are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are, in one embodiment, formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal patients and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, solubilizing agents, pH buffering agents and the like, for example,

acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

Dosage forms or compositions containing a compound as described herein in the range of 0.005% to 100% with the balance made up from non-toxic carrier may be prepared. Methods for preparation of these compositions are known to those skilled in the art. The contemplated compositions may contain 0.001%-100% active ingredient, in one embodiment 0.1-95%, in another embodiment 75-85%.

Pharmaceutical compositions suitable for the delivery of compounds described herein and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company, 1995).

Methods of Use

The compounds and compositions provided herein can be used in a method of treating and/or inhibiting a fungal infection in a patient. For example, the method can include administering to the patient a therapeutically effective amount of a compound as provided herein. In some embodiments, inhibition of a fungal infection can include inhibiting fungal growth in the patient.

A patient as provided herein can include a patient that is immunocompromised. For example, a patient infected with HIV/AIDS, transplant patients (e.g., patients undergoing introduction of allogenic and/or autologous stem cells, patients undergoing bone marrow transplantation, and patients undergoing organ transplatation), and cancer patients treated with chemotherapeutic agents.

A fungal infection can be caused by any fungal organism that causes infection in a patient. For example, the fungal infection can be caused by a fungal organism selected from *C. albicans*, non-albicans Candida species, *P. carinii*, and *A. fumigatus*.

In some embodiments, a compound or composition provided herein inhibits the transferase activity of a fungal Rtt109 polypeptide.

Also provided herein is a method of inhibiting a fungal Rtt109 polypeptide in a cell. The method comprising contacting the cell with an effective amount of a compound

as provided herein. Uses of such *in vitro* methods include, but are not limited to, use in a screening assay (for example, wherein the compound is used as a positive control or standard compared to compounds of unknown activity or potency in any of the methods provided herein).

Screening target compounds for antifungal activity

Also provided herein are materials and methods involved in identifying chemical inhibitors of fungal targets for treating organisms infected with opportunistic fungal pathogens. For example, this document provides the materials and methods for identifying and optimizing chemical inhibitors of Rtt109 polypeptide, a fungal histone acetyltransferase (see Fig. 7). This document also provides the materials and methods for using high throughput screening (HTS) technologies to identify candidate chemical inhibitors of fungal Rtt109 as well as the materials and methods for evaluating candidate chemical inhibitors of Rtt109 in secondary screens for their binding efficacy, molecular specificity, and cellular toxicity.

As described herein, the histone acetyltransferase, Rtt109, is a lysine acetyltransferase (KAT) highly conserved in fungal species that exhibits no obvious sequence homology to other mammalian KATs. Compared to other histone acetyltransferases (HATs) discovered in yeast or other organisms, Rtt109 is unique in several ways. First, Rtt109 does not appear to have a classic acetyl-CoA binding motif found in other known HATs despite the fact that it utilizes acetyl-CoA as a co-factor. Second, in contrast to most HATs that utilize free histones or nucleosomal histones as substrates, Rtt109 can use a heterotrimeric complex Asf1-H3-H4. Rtt109 can form a complex with Vps75 that utilizes the Asf1-H3-H4 complex as the substrate during acetylation. Third, most HATs are conserved from yeast to humans, and yet the sequence homologs of Rtt109 appear not be present in humans. Finally, Rtt109 is critical for yeast cells to survive following treatment with DNA damaging agents. Taken together, Rtt109 may be a fungal-specific target that can be used to identify chemical inhibitors for treating organisms infected with opportunistic fungal pathogens.

Using Rtt109, chemical agents can be screened for their ability to inhibit or attenuate the function of the fungal target, which may compromise the health or survival of the fungal pathogen. Chemical agents can also disrupt the target's interactions with other proteins or protein complexes, which may compromise the health or survival of the fungal pathogen. As described herein, Rtt109 forms a complex with Vps75 and uses another complex consisting of Asf1-H3-H4 as a substrate during acetylation in *Saccharomyces cerevisiae*, which is required for DNA replication and cellular proliferation. In some cases, chemical agents can be screened for their ability to inhibit directly the function of Rtt109 or for their ability to disrupt the interactions between Rtt109-Vps75 and its substrate Asf1-H3-H4.

In some embodiments, a HTS screening assay can be developed based on the activity of the fungal target. For example, the fungal target can be an enzyme that acts post-translationally in biochemical processes, including acetylation, phosphorylation, methylation, and ubiquitination. These modifications regulate a variety of essential cellular processes including gene transcription, cell proliferation and differentiation. The HTS screening assay can be designed to detect the presence or absence of the products of the enzymatic reaction catalyzed by the fungal target. The HTS screening assay can also be designed to detect the presence or absence of the by-products of the enzymatic reaction catalyzed by the fungal target.

For example, a screening assay can be used to identify a test compound having antifungal activity, the assay can include determining if the test compound inhibits the histone acetyltransferase activity of a fungal Rtt109 polypeptide. A fungal Rtt109 polypeptide can include any polypeptide that has at least 80% identity (e.g., at least about 85%, at least about 90%, at least about 95%, at least about 98%, and at least about 99%) to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6 (see Fig. 9). In some embodiments, a fungal Rtt109 polypeptide is selected from the group consisting of SEQ ID NOs: 1-6. In some embodiments, a fungal Rtt109 is complexed with Vps75.

In some embodiments, if the test compound inhibits the histone acetyltransferase activity of a fungal Rtt109 polypeptide can be determined by contacting the test

compound with a composition comprising a fungal Rtt109 polypeptide, a substrate for a fungal Rtt109 polypeptide, and acetyl coenzyme A; and determining the level of a de-acetylated coenzyme A.

A substrate for a fungal Rtt109 polypeptide can be any substrate that facilitates the activity of the polypeptide. For example, the substrate is a fungal histone, such as a lysine containing histone. In some embodiments, the lysine containing histone is selected from an Asf-1-H3-H4 complex or H3K56.

Determining the level of a de-acetylated coenzyme A can be done by any method known to those of skill in the art. In some embodiments, a second reaction can be used in the HTS screening assay to detect the presence or absence of the products or by-products of the enzymatic reaction catalyzed by the fungal target (e.g., deacetylated coenzyme A). For example, the presence or absence of the products or by-products of the enzymatic reaction catalyzed by the fungal target can be monitored or detected using fluorescence, chemiluminescence, radioactivity, colorimetrics, and photometrics. In some embodiments, the coenzyme A is labeled. For example, the label can be a radioisotope or a fluorophore.

Suitable radionuclides include but are not limited to ^{2}H (deuterium), ^{3}H (tritium), ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{18}F, ^{35}S, ^{36}Cl, ^{82}Br, ^{75}Br, ^{7}Br, ^{77}Br, ^{123}I, ^{124}I, ^{125}I and ^{131}I. In some embodiments, the label is a radioisotope, such as ^{3}H, ^{14}C, ^{35}S, and ^{125}I. In some embodiments, the acetyl coenzyme A is ^{3}H-acetyl coenzyme A.

Suitable fluorophores include, for example, xanthene derivatives (e.g., fluorescein, rhodamine, Oregon green, eosin, Texas red, and Cal Fluor dyes), cyanine derivatives (e.g., cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, merocyanine, and Quasar dyes), naphthalene derivatives (e.g., dansyl and prodan derivatives), coumarin derivatives, oxadiazole derivatives (e.g., pyridyloxazole, nitrobenzoxadiazole and benzoxadiazole), pyrene derivatives (e.g., cascade blue), oxazine derivatives (e.g., Nile red, Nile blue, cresyl violet, oxazine 170), acridine derivatives (e.g., proflavin, acridine orange, acridine yellow), arylmethine derivatives (e.g., auramine, crystal violet, malachite green), and tetrapyrrole derivatives (e.g., porphin, phtalocyanine, bilirubin). In some embodiments, the assay solution can further

include a label that reacts with de-acetylated coenzyme A to form a detectable complex. For example, the assay solution can include 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin).

In some cases, a HTS screening assay can be developed in which candidate chemical inhibitors of the fungal target are identified from among hundreds of thousands of chemical agents contained in chemical libraries. The candidate chemical inhibitors of fungal targets contained in chemical libraries can have known or unknown biological effects or activities both *in vitro* and *in vivo*. In some cases, the HTS screening assay can be performed on an automated or semi-automated platform capable of screening thousands of candidate chemical inhibitors daily. For example, automated or semi-automated screening platforms can detect or monitor the readout of a HTS screening assay within individual wells of a 96 or 384-well screening plate. The readout of the HTS can be captured and processed electronically using computers and computerized robotics.

In some embodiments, once a candidate chemical inhibitor of a fungal target has been identified, the candidate chemical inhibitor can be screened in one or more secondary assays that assess the compounds binding efficacy, molecular specificity, and cellular toxicity. In some embodiments, the secondary assay is a repeat of the primary screen. In some embodiments, the secondary screen is designed to evaluate a separate feature of the candidate chemical inhibitors. For example, secondary screens can be designed to evaluate the binding kinetics of candidate chemical inhibitors, including the rates at which substrates are bound and released, or whether the candidate chemical inhibitors are reversible or non-reversible inhibitors. Secondary screens can also be designed to evaluate the effects of candidate chemical inhibitors on similar enzymes from similar or different species. Secondary screens can also be designed to evaluate candidate chemical inhibitors for their toxic effects on cells of similar or different species.

Suitable secondary screens can include, for example, determining whether the test compound inhibits a human p300/CBP polypeptide. In some embodiments, a desirable test compound exhibits preferential inhibition of the fungal Rtt109 activity compared to

human p300/CBP activity. Preferential inhibition of fungal Rtt109 over p300/CBP activity can indicate low toxicity of the compound to human patients. Another example of a secondary screen can include determining whether the test compound inhibits a yeast Gcn5 polypeptide.

The test compounds can also be examined to determine how each compound affects growth of one or more specific fungal species. For example, various doses of a target compound can be tested to determine how a particular does of the compound affects the growth of a fungal species. For example, the fungal species can be selected from *C. albicans*, *P. carinii*, and *A. fumigatus*.

In general, once primary and secondary screens for chemical inhibitors of a fungal target have been performed, the candidate chemical inhibitors can be used as the basis for further optimization. For example, candidate chemical inhibitors can be organized into groups based on structural and binding properties. This information can be used to synthesize other candidate chemical inhibitors that incorporate or enhance the features necessary for efficient binding, potency, specificity, and reduced toxicity. The optimized candidate chemical inhibitors can then be evaluated in the same primary and secondary screening assays for their ability to modify fungal targets. In some cases, candidate or optimized candidate chemical inhibitors can be used on mammals as a therapeutic agent for treating opportunistic fungal pathogens.

EXAMPLES

Example 1 – Identifying and optimizing inhibitors for the histone acetyltransferase Rtt109 complex

In vitro enzymatic assays for screening compounds that inhibit the activity of Rtt109 were developed based on the biochemical function of Rtt109, as schematized in Fig. 3. Using these assays, about 90,248 compounds were screened and 213 compounds were identified as having reasonable dose response. Eighteen compounds were counter-screened for inhibition of human p300 lysine acetyltransferase, and three lead compounds exhibited selective activity against scRtt109.

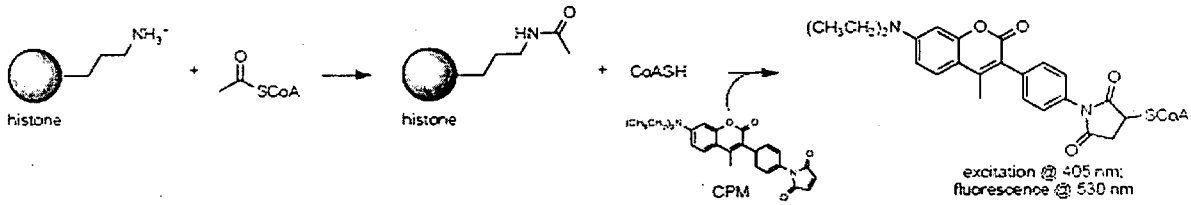
Example 2 – Complementation experiments using Rtt109 from different fungal species

The sequence homologs of scRtt109 have been identified from many fungal species (see Fig. 8). In *C. albicans,* cells lacking both copies of Rtt109 are less virulent than wild type controls (Fig. 2). It was determined that expressing scRtt109 in rtt109-/- *C. albicans* cells rendered the mutant cells resistant to CPT (Fig. 1A) to a similar degree as expressing caRtt109. It was also determined that expressing pcRtt109 or afRtt109 in *S. cerevisiae* cells lacking scRtt109 led to resistance to CPT and restoration of H3K56Ac (Fig. 1B). These results indicate that Rtt109 from these fungal species have similar functions and suggest that small molecule inhibitors against scRtt109 will also inhibit Rtt109 in other species.

Example 3 – Enzymatic assay to identify small molecule inhibitors of Rtt109

In *S. cerevisiae*, Rtt109 is the catalytic subunit of the Rtt109-Vps75 that utilizes Asf1-H3-H4 complexes as substrates. Rtt109 from other fungal species likely functions similarly. Therefore, to identify inhibitors against scRtt109, the Rtt109-Vps75 and Asf-1-H3-H4 complexes were purified and used as enzyme and substrate, respectively, for histone acetyltransferase assays (See Scheme 1).

Scheme 1



Assays conditions described in Trievel *et al.* were used with slight modifications (Trievel, R.C. *et al.* 2000. *Anal Biochem*, 287, 319-29). Briefly, 15 nL DMSO or 10 μM test compound were added to wells of Corning 384-well plate using an ECHO 550 contactless liquid handler. Controls were added to the appropriate wells using the same dispensing method. Then 5 μL 1X HAT buffer (50 mM Tris-HCl, 0.1 mM EDTA, 50 mM KCl, pH 8.0) was added using a Multidrop 384 bulk reagent liquid dispenser. Following this, Rtt109-Vsp75 (200 ng/well) and then Asf1-H3-H4 (800 ng/well), was added using the Multidrop. To initiate the reaction, 10 μL 15 μM Acetyl-Coenzyme A

was added to the entire plate. Following incubation at 30°C for 1-2 hours, 5 μL of 80 μM CPM (final concentration 20μM) was added. The fluorescence intensity was measured using a M2E plate reader (Molecular Devices) with excitation at 405 nm and emission at 530 nm. The assay was validated for top-to-bottom and edge-to-edge variability using a control plate where either Rtt109-Vps75, Acetyl-CoA, or Asf1-H3-H4 were not added and using duplicated assay runs of the LOPAC compound collection (Sigma-Aldrich, St. Louis, MO) on two separate days. Using this method, 90,248 compounds were screened and a 1.8% hit rate was achieved when 35% inhibition was set as the cutoff. Following the primary screen, 314 compounds were cherry-picked from the primary screening and 135 compounds were exhibited dose response. Dose-response confirmed hits were filtered to remove compounds that had low solubility (high molecular weight and/or high cLogP values), contained synthetically inaccessible functionalities, possessed known toxicophores, or were likely to be pan-assay interference compounds (PAINS). After this analysis, 50 compounds were purchased, 18 of which were selected and tested for inhibition of p300.

Example 4 – Testing candidate compounds for inhibition of p300/CBP

While scRtt109 does not share sequence homology with human p300/CBP, the structure of the catalytic domain of scRtt109 is similar to that of p300/CBP despite the fact that scRtt109 and p300 likely utilize distinct mechanisms for catalysis. In addition, p300/CBP can acetylate H3K56 in human cells. Therefore, commercially available human p300 was obtained and a counter screening assay was developed to determine whether the compounds identified in the primary assay inhibit the activity of p300/CBP. Eighteen compounds with lower IC_{50s} were chosen for counter-screening against human p300 using the same assay methodology for scRtt109-Vps75 as described in Example 3. Six compounds exhibited selective inhibition of scRtt109 over p300, one of which is shown in Fig. 4A.

Example 5 – Testing candidate compounds for inhibition of Gcn5

In addition to p300/CBP, human Gcn5 is another HAT that targets human H3K56.

In *S. cerevisiae*, it was determined that cells lacking both Gcn5 and Rtt109 exhibited more severe growth defects than cells lacking scRtt109 alone. Six compounds that inhibited the activity of scRtt109-Vps75 preferentially over human p300 were examined for their ability to inhibit yeast Gcn5 using an independent assay in which radioactive ^{3}H-labled-acetyl-CoA was used. This assay detected reaction products of acetylated H3 directly using a scintillation counter. It was found that two compounds preferentially inhibited the activity of scRtt109-Vps75 over Gcn5, one of which is shown in Fig. 4B.

Example 6 – Three compounds inhibit fungal pathogen growth but are not toxic to human cells

The effects on the growth of four fungal species, *S. cerevisiae*, *C. albicans*, *P. carinii*, and *A. fumigatus* were examined. Dose response experiments were performed with each compound using liquid cultures. Concentrations ranging from 10-100 μM of each compound inhibited growth. All three compounds inhibited the growth of *S. cerevisiae* to a similar extent. Two compounds (Compound 3-1 and Compound 1-1) inhibited growth of *C. albicans*, whereas one compound (Compound 2-1) exhibited partial activities (Fig. 5A-B).

To determine the effect of each of the three compounds on the growth of *P. carinii*, about 1×107 *P. carinii* cells were incubated in Ham's F12 media in one well of a 6-well plate. After incubation at 37°C for 24 hours, 1 μg/mL Pentamidine (a known inhibitor of *P. carinii* growth) or each compound at different concentrations (10, 50, and 100 μM) was added. After 24 hours, total RNA was isolated with Trizol reagents and cDNA was produced. The viability of organisms after treatment was monitored by measuring the mRNA of heat shock 70 protein (Hsp70) by quantitative real-time PCR. Compound 3-1 inhibited the growth of *P. carinii* at all three concentrations tested, and Compound 1-1 exhibited limited activity, whereas Compound 2-1 had no apparent effect on the growth. It was also observed that Compound 3-1 inhibited growth of *A. fumigatus* at 100 μM concentrations *in vitro* (Fig. 5C-D). Finally, none of these compounds exhibited significant toxicity against human cells at concentrations that can inhibit growth of fungal species (Fig. 6).

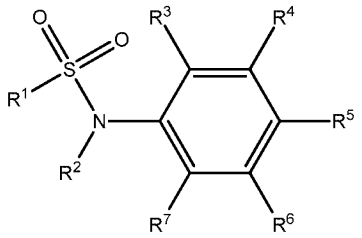
Example 7 – Deletion of Rtt109 from *C. albicans* resulted in reduced virulence

C. albicans lacking Rtt109 was tested for reduced virulence in a mammalian tissue culture model. This assay mimics the tissue injuries caused by *C. albicans* infection and takes advantage of the fact that epithelial cells damaged by *C. albicans* infection release lactate dehydrogenease (LDH) into the supernatant. Briefly, H4 enterocytes were cultured at a concentration of 2×104 cells per well and grown to approximately 80% confluence in a 96-well flat-bottomed tissue culture plate. The enterocytes were infected with wild type *C. albicans* and a *C. albicans* mutant lacking one or both copies of Rtt109 genes. The released LDH was quantified spectrophotometrically using the Cyto-Tox-96® assay (Promega). *C. albicans* lacking both copies of the Rtt109 gene caused significantly less damage to enterocytes compared to *C. albicans* lacking just one copy of the Rtt109 gene or compared different wild type yeast strains. This reduction was not due to slow growth of the rtt109 -/- cells.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating a fungal infection in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (1):



or a pharmaceutically acceptable salt form thereof,

wherein:

R^1 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl;

R^2 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, and substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl;

R^3, R^4 and R^5 are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, $(C_3\text{-}C_{14})$aryl, $-(CH_2)(C_3\text{-}C_{14})$aryl halogen, –CN, $–NO_2$, $–N(R^8)_2$, and $–OR^8$;

R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–N(R^8)_2$, and $–OR^8$, or R^6 and R^7 can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure; and

each R^8 is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

2. The method of claim 1, wherein R^1 is selected from the group consisting of hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl.

3. The method of claim 2, wherein R^1 is a substituted (C_3-C_{14})aryl.

4. The method of claim 3, wherein R^1 is a para-cumenyl moiety.

5. The method of claim 1, wherein R^2 is selected from hydrogen or substituted or unsubstituted (C_1-C_6)alkyl.

6. The method of claim 5, wherein R^2 is selected from the group consisting of hydrogen, methyl, and isopropyl.

7. The method of claim 6, wherein R^2 is hydrogen.

8. The method of claim 1, wherein R^3 is hydrogen.

9. The method of claim 1, wherein R^4 is selected from hydrogen and halogen.

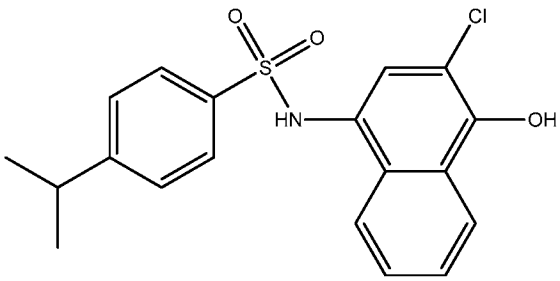
10. The method of claim 1, wherein R^5 is hydrogen or $-OR^8$.

11. The method of claim 10, wherein R^5 is selected from the group consisting of hydrogen, –OH, and $-OCH_3$.

12. The method of claim 1, wherein R^6 and R^7 come together to form an unsubstituted fused (C_4-C_6) ring structure.

13. The method of claim 12, wherein the fused (C_4-C_6) ring structure is an aryl ring.

14. The method of claim 1, wherein the compound of formula (1) is:

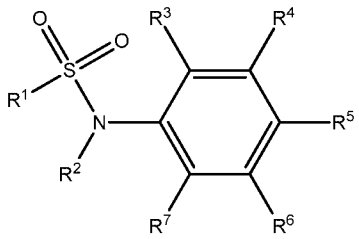


or a pharmaceutically acceptable salt form thereof.

15. The method of claim 1, wherein the patient is immunocompromised.

16. The method of claim 1, wherein the fungal infection is caused by a fungal organism selected from *C. albicans*, *P. carinii*, and *A. fumigatus*.

17. A method of inhibiting a fungal infection in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (1):



or a pharmaceutically acceptable salt form thereof,

wherein:

R^1 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl;

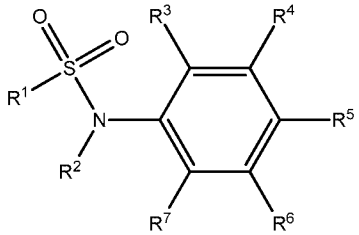
R^2 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, and substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl;

R^3, R^4 and R^5 are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, $(C_3\text{-}C_{14})$aryl, $\text{-}(CH_2)(C_3\text{-}C_{14})$aryl halogen, –CN, –NO_2, $\text{–N}(R^8)_2$, and –OR^8;

R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, –NO_2, $\text{–N}(R^8)_2$, and –OR^8, or R^6 and R^7 can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure; and

each R^8 is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

18. A method of inhibiting fungal growth in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (1):



or a pharmaceutically acceptable salt form thereof,

R^1 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, and substituted or unsubstituted $(C_3\text{-}C_{14})$aryl;

R^2 is selected from the group consisting of: hydrogen, substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, and substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl;

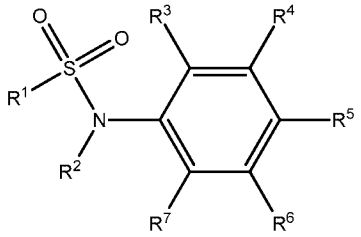
R^3, R^4 and R^5 are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, $(C_3\text{-}C_{14})$aryl, $\text{-}(CH_2)(C_3\text{-}C_{14})$aryl halogen, –CN, –NO_2, $\text{–N}(R^8)_2$, and –OR^8;

R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, –NO_2, $\text{–N}(R^8)_2$, and

–OR^8, or R^6 and R^7 can come together to form a substituted or unsubstituted fused (C_4-C_6) ring structure; and

each R^8 is independently selected from the group consisting of hydrogen and (C_1-C_6)alkyl.

19. A method of inhibiting Rtt109 in a cell, the method comprising contacting the cell with an effective amount of a compound of formula (1):



or a pharmaceutically acceptable salt form thereof,

wherein:

R^1 is selected from the group consisting of: hydrogen, substituted or unsubstituted (C_1-C_6)alkyl, substituted or unsubstituted (C_2-C_6)alkenyl, substituted or unsubstituted (C_2-C_6)alkynyl, substituted or unsubstituted (C_3-C_7)cycloalkyl, and substituted or unsubstituted (C_3-C_{14})aryl;

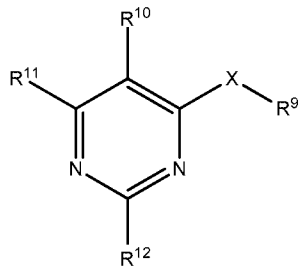
R^2 is selected from the group consisting of: hydrogen, substituted or unsubstituted (C_1-C_6)alkyl, substituted or unsubstituted (C_2-C_6)alkenyl, substituted or unsubstituted (C_2-C_6)alkynyl, and substituted or unsubstituted (C_3-C_7)cycloalkyl;

R^3, R^4 and R^5 are independently selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, (C_3-C_{14})aryl, -(CH_2)(C_3-C_{14})aryl halogen, –CN, –NO_2, –$N(R^8)_2$, and –OR^8;

R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, –NO_2, –$N(R^8)_2$, and –OR^8, or R^6 and R^7 can come together to form a substituted or unsubstituted fused (C_4-C_6) ring structure; and

each R^8 is independently selected from the group consisting of hydrogen and (C_1-C_6)alkyl.

20. A method of treating a fungal infection in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (2):



or a pharmaceutically acceptable salt form thereof,

wherein:

X is selected from O, S, and NR^{13};

R^9 is selected from the group consisting of: substituted or unsubstituted (C_1-C_6)alkyl, substituted or unsubstituted (C_2-C_6)alkenyl, substituted or unsubstituted (C_2-C_6)alkynyl, substituted or unsubstituted (C_3-C_7)cycloalkyl, substituted or unsubstituted (C_3-C_7)heterocycloalkyl, substituted or unsubstituted (C_3-C_{14})aryl, and substituted or unsubstituted (C_3-C_{14})heteroaryl;

R^{10} and R^{11} are independently selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$, or R^{10} and R^{11} can come together to form a substituted or unsubstituted fused (C_4-C_6) ring structure;

R^{12} is selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$; and

each R^{13} is independently selected from the group consisting of hydrogen and (C_1-C_6)alkyl.

21. The method of claim 20, wherein X is S.

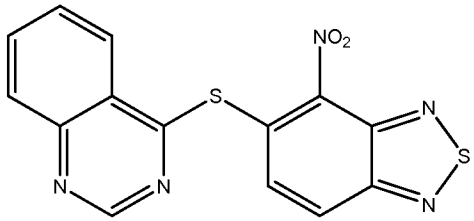
22. The method of claim 20, wherein R^9 is a substituted or unsubstituted (C_3-C_7)heterocycloalkyl or substituted or unsubstituted (C_3-C_{14})heteroaryl.

23. The method of claim 20, wherein R^{10} and R^{11} come together to form an unsubstituted fused (C_4-C_6) ring structure.

24. The method of claim 23, wherein the fused (C_4-C_6) ring structure is an aryl ring.

25. The method of claim 20, wherein R^{12} is hydrogen.

26. The method of claim 20, wherein the compound of formula (2) is:

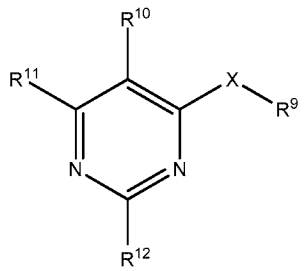


or a pharmaceutically acceptable salt form thereof.

27. The method of claim 20, wherein the patient is immunocompromised.

28. The method of claim 20, wherein the fungal infection is caused by a fungal organism selected from *C. albicans*, *P. carinii*, and *A. fumigatus*.

29. A method of inhibiting a fungal infection in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (2):



or a pharmaceutically acceptable salt form thereof,

wherein:

X is selected from O, S, and NR^{13};

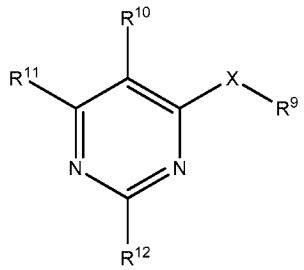
R^9 is selected from the group consisting of: substituted or unsubstituted (C_1-C_6)alkyl, substituted or unsubstituted (C_2-C_6)alkenyl, substituted or unsubstituted (C_2-C_6)alkynyl, substituted or unsubstituted (C_3-C_7)cycloalkyl, substituted or unsubstituted (C_3-C_7)heterocycloalkyl, substituted or unsubstituted (C_3-C_{14})aryl, and substituted or unsubstituted (C_3-C_{14})heteroaryl;

R^{10} and R^{11} are independently selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$, or R^{10} and R^{11} can come together to form a substituted or unsubstituted fused (C_4-C_6) ring structure;

R^{12} is selected from the group consisting of: hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$; and

each R^{13} is independently selected from the group consisting of hydrogen and (C_1-C_6)alkyl.

30. A method of inhibiting fungal growth in a patient, the method comprising administering to the patient a therapeutically effective compound of formula (2):



or a pharmaceutically acceptable salt form thereof,

wherein:

X is selected from O, S, and NR^{13};

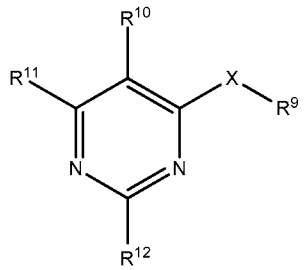
R^9 is selected from the group consisting of: substituted or unsubstituted (C_1-C_6)alkyl, substituted or unsubstituted (C_2-C_6)alkenyl, substituted or unsubstituted (C_2-C_6)alkynyl, substituted or unsubstituted (C_3-C_7)cycloalkyl, substituted or unsubstituted (C_3-C_7)heterocycloalkyl, substituted or unsubstituted (C_3-C_{14})aryl, and substituted or unsubstituted (C_3-C_{14})heteroaryl;

R^{10} and R^{11} are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$, or R^{10} and R^{11} can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure;

R^{12} is selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$; and

each R^{13} is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

31. A method of inhibiting Rtt109 in a cell, the method comprising contacting the cell with an effective amount of a compound of formula (2):



or a pharmaceutically acceptable salt form thereof,

wherein:

X is selected from O, S, and NR^{13};

R^9 is selected from the group consisting of: substituted or unsubstituted $(C_1\text{-}C_6)$alkyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkenyl, substituted or unsubstituted $(C_2\text{-}C_6)$alkynyl, substituted or unsubstituted $(C_3\text{-}C_7)$cycloalkyl, substituted or unsubstituted $(C_3\text{-}C_7)$heterocycloalkyl, substituted or unsubstituted $(C_3\text{-}C_{14})$aryl, and substituted or unsubstituted $(C_3\text{-}C_{14})$heteroaryl;

R^{10} and R^{11} are independently selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$, or R^{10} and R^{11} can come together to form a substituted or unsubstituted fused $(C_4\text{-}C_6)$ ring structure;

R^{12} is selected from the group consisting of: hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_6)$alkenyl, $(C_2\text{-}C_6)$alkynyl, halogen, –CN, $–NO_2$, $–NR^{13}{}_2$, and $–OR^{13}$; and

each R^{13} is independently selected from the group consisting of hydrogen and $(C_1\text{-}C_6)$alkyl.

32. A method for screening a test compound for antifungal activity, the method comprising determining if the test compound inhibits the histone acetyltransferase activity of a fungal Rtt109 polypeptide, wherein the fungal Rtt109 polypeptide has at least 80% identity to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6.

33. The method of claim 32, wherein the fungal Rtt109 polypeptide has at least about 85% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6.

34. The method of claim 33, wherein the fungal Rtt109 polypeptide has at least about 90% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6.

35. The method of claim 34, wherein the fungal Rtt109 polypeptide has at least about 95% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6.

36. The method of claim 35, wherein the fungal Rtt109 polypeptide has at least about 98% sequence identity to a polypeptide comprising an amino acid selected from the group consisting of SEQ ID NOs: 1-6.

37. The method of claim 32, wherein the fungal Rtt109 polypeptide is selected from the group consisting of SEQ ID NOs: 1-6.

38. The method of claim 32, wherein the determining comprises contacting the test compound with a composition comprising a fungal Rtt109, a substrate for a fungal

Rtt109 polypeptide, and acetyl coenzyme A; and determining the level of a de-acetylated coenzyme A.

39. The method of claim 38, wherein the substrate is a fungal histone.

40. The method of claim 39, wherein the fungal histone is a lysine containing histone.

41. The method of claim 40, wherein the lysine containing histone is an Asf-1-H3-H4 complex.

42. The method of claim 40, wherein the lysine containing histone is H3K56.

43. The method of claim 38, wherein the coenzyme A is labeled.

44. The method of claim 38, wherein the de-acetylated coenzyme A is labeled.

45. The method of claim 32, wherein the fungal Rtt109 polypeptide is complexed with Vps75.

46. The method of claim 38, wherein the assay solution further comprises a label that reacts with de-acetylated coenzyme A to form a detectable complex.

47. The method of claim 46, wherein the label is a fluorophore.

48. The method of claim 32, wherein the method further comprises determining whether the test compound inhibits a human p300/CBP polypeptide.

49. The method of claim 48, wherein the test compound exhibits preferential inhibition of the fungal Rtt109 activity compared to human p300/CBP activity.

50. The method of claim 32, wherein the method further comprises determining whether the test compound inhibits a yeast Gcn5 polypeptide.

51. The method of claim 32, wherein the test compound inhibits growth of a fungal species.

52. The method of claim 51, wherein the fungal species is selected from *C. albicans*, *P. carinii*, and *A. fumigatus*.

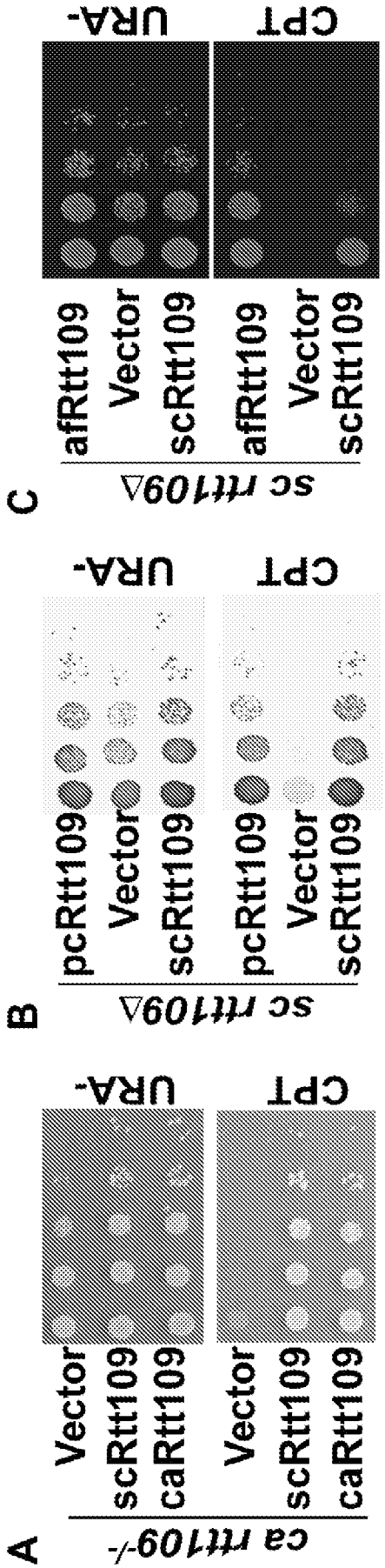

A
ca rtt109-/-
Vector
scRtt109
caRtt109
Vector
scRtt109
caRtt109
CPT
URA-
B
sc rtt109Δ
pcRtt109
Vector
scRtt109
pcRtt109
Vector
scRtt109
CPT
URA-
C
sc rtt109Δ
afRtt109
Vector
scRtt109
afRtt109
Vector
scRtt109
CPT
URA-

FIG. 1

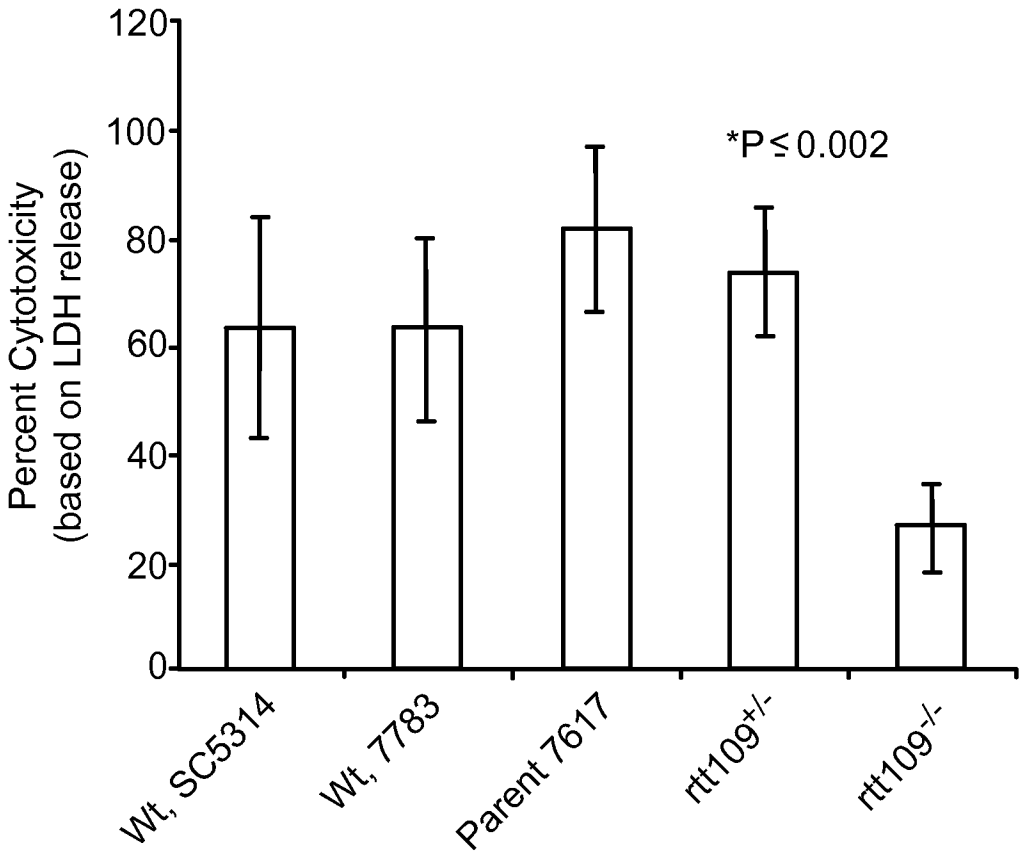

120
100
80
60
40
20
0
Percent Cytotoxicity
(based on LDH release)
*P ≤ 0.002
Wt, SC5314
Wt, 7783
Parent 7617
rtt109+/-
rtt109-/-

FIG. 2

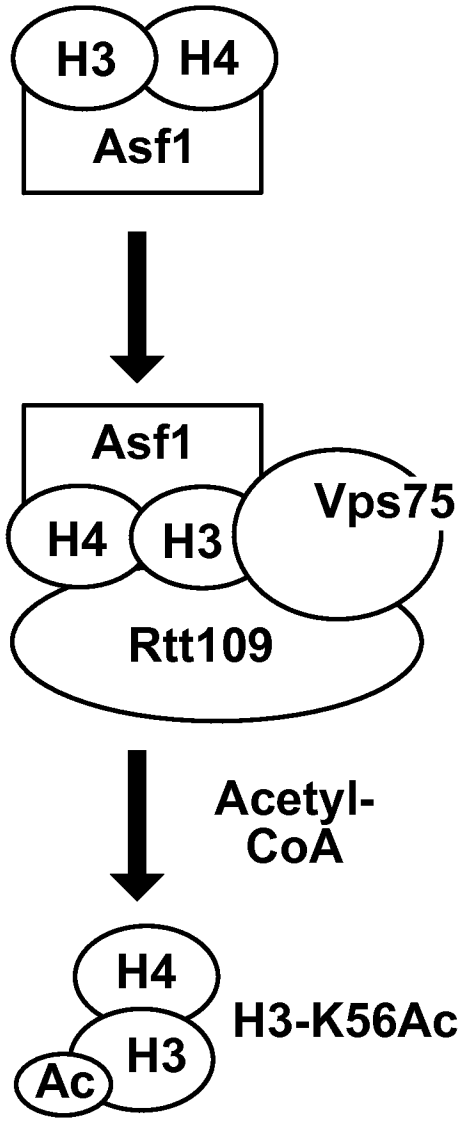


FIG. 3

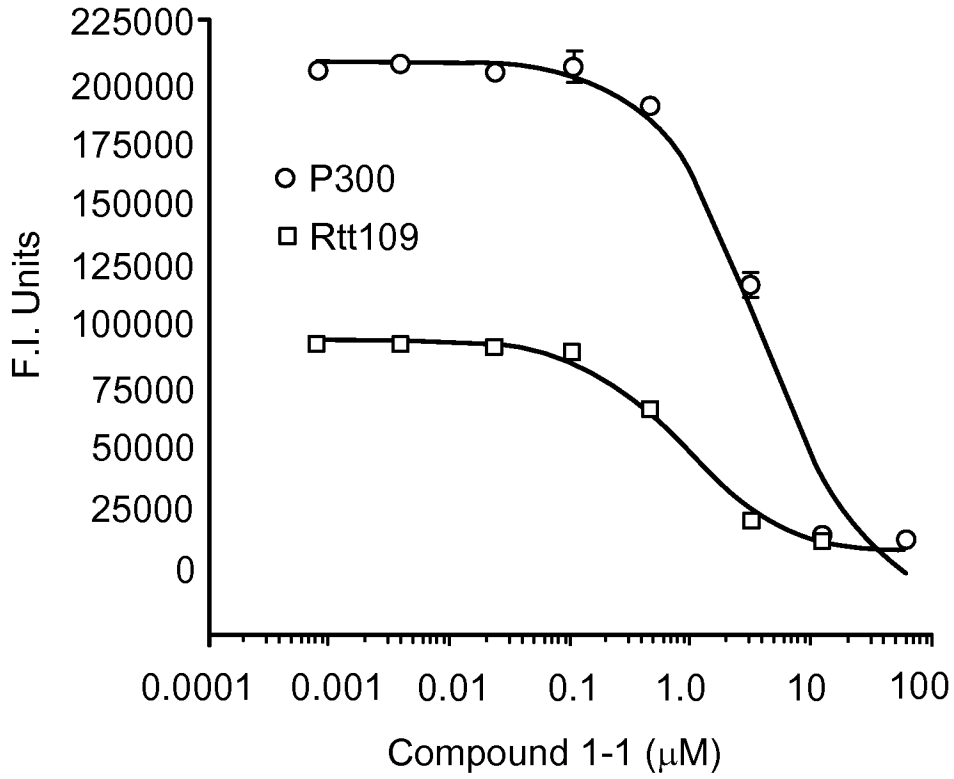

225000
200000
175000
150000
125000
100000
75000
50000
25000
0
F.I. Units
P300
Rtt109
0.0001
0.001
0.01
0.1
1.0
10
100
Compound 1-1 (μM)

FIG. 4A

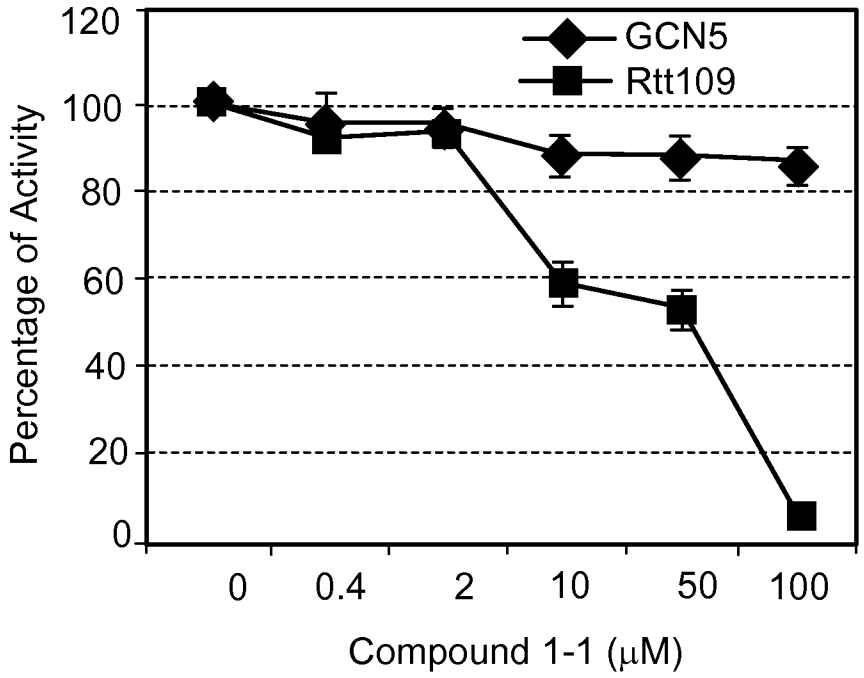

120
100
80
60
40
20
0
Percentage of Activity
GCN5
Rtt109
0
0.4
2
10
50
100
Compound 1-1 (μM)

FIG. 4B

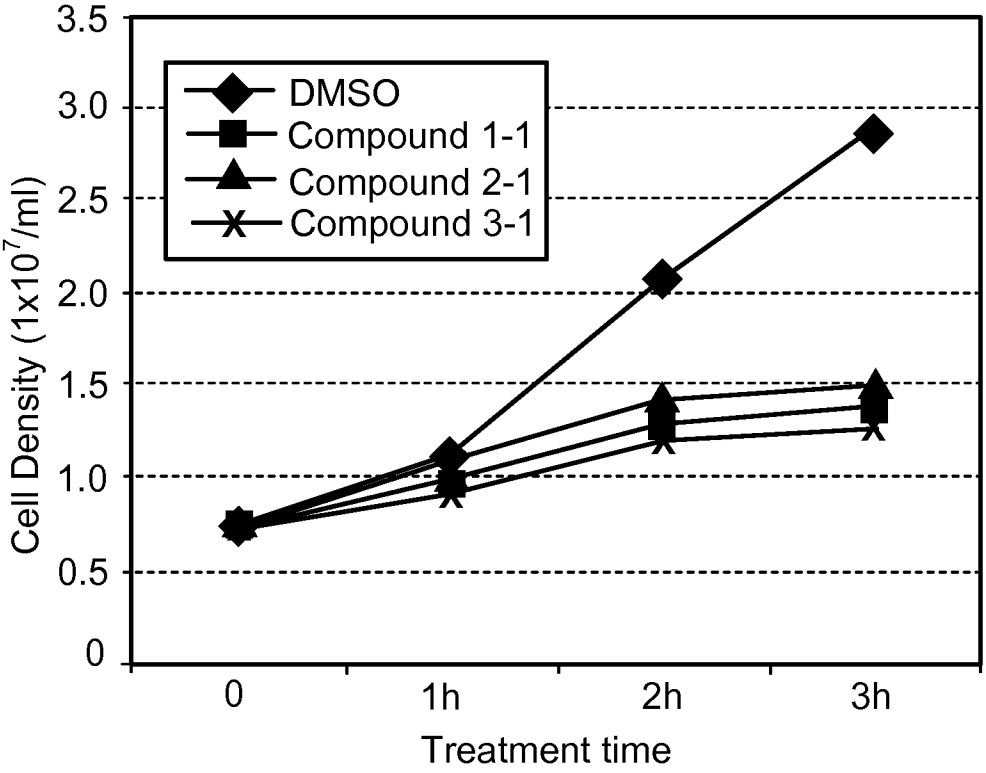

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Cell Density (1x10^7/ml)
DMSO
Compound 1-1
Compound 2-1
Compound 3-1
0
1h
2h
3h
Treatment time

FIG. 5A

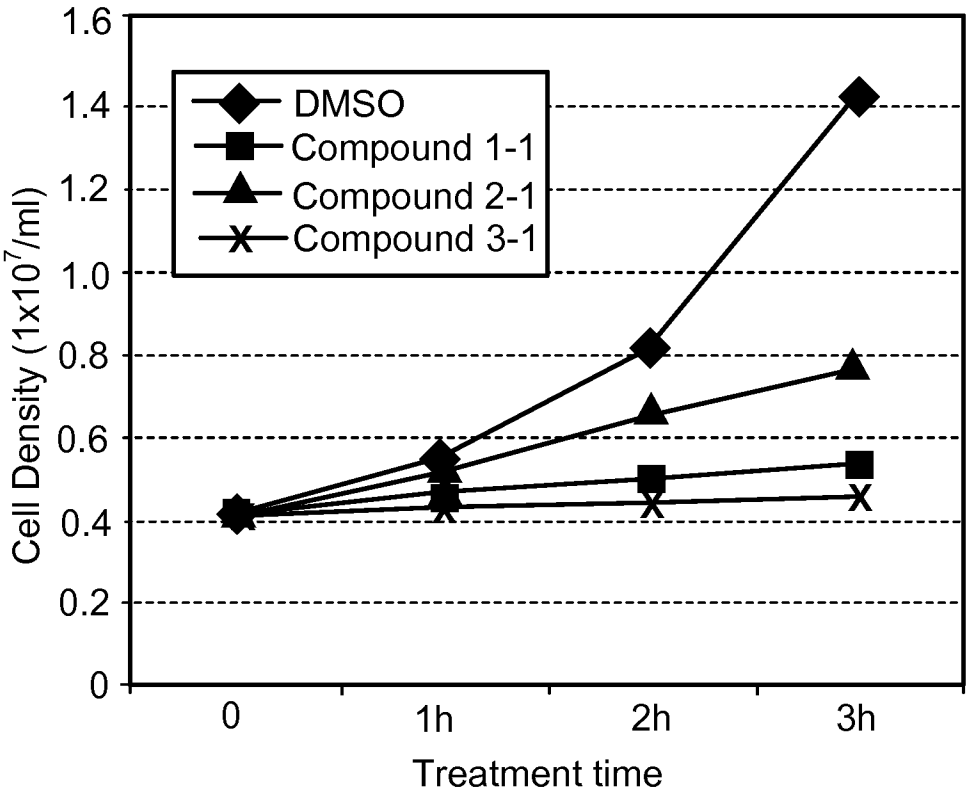

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Cell Density (1x10^7/ml)
DMSO
Compound 1-1
Compound 2-1
Compound 3-1
0
1h
2h
3h
Treatment time

FIG. 5B

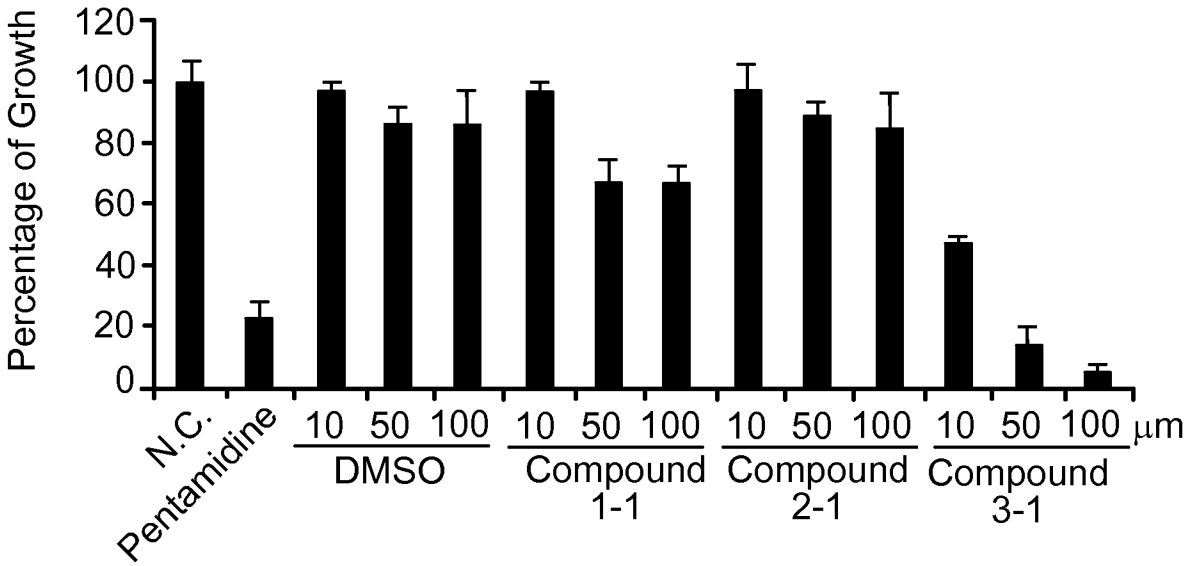

Percentage of Growth
120
100
80
60
40
20
0
N.C.
Pentamidine
10 50 100
DMSO
10 50 100
Compound 1-1
10 50 100
Compound 2-1
10 50 100
Compound 3-1
μm

FIG. 5C

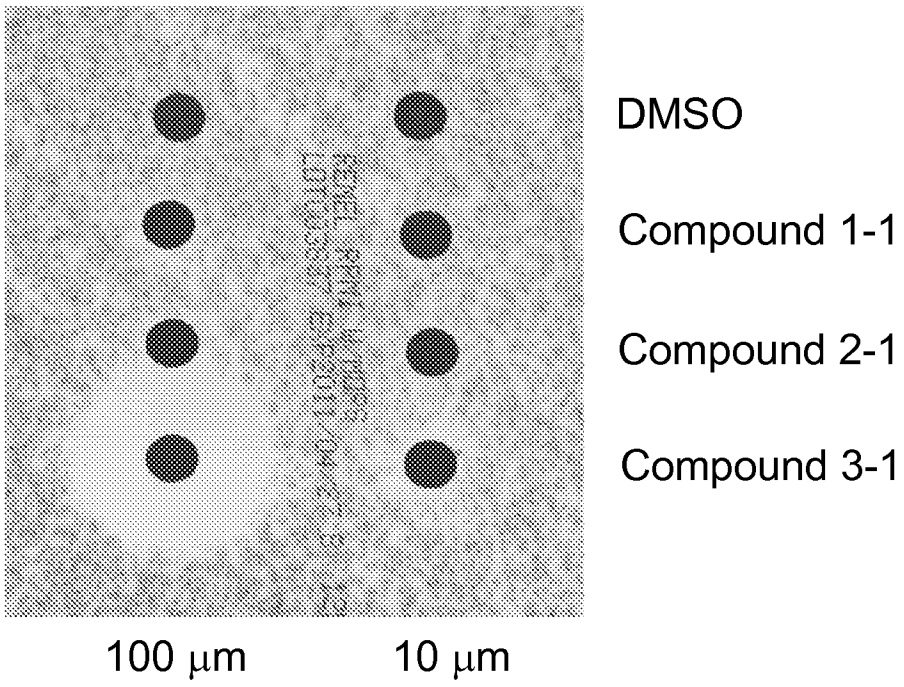

DMSO
Compound 1-1
Compound 2-1
Compound 3-1
100 μm
10 μm

FIG. 5D

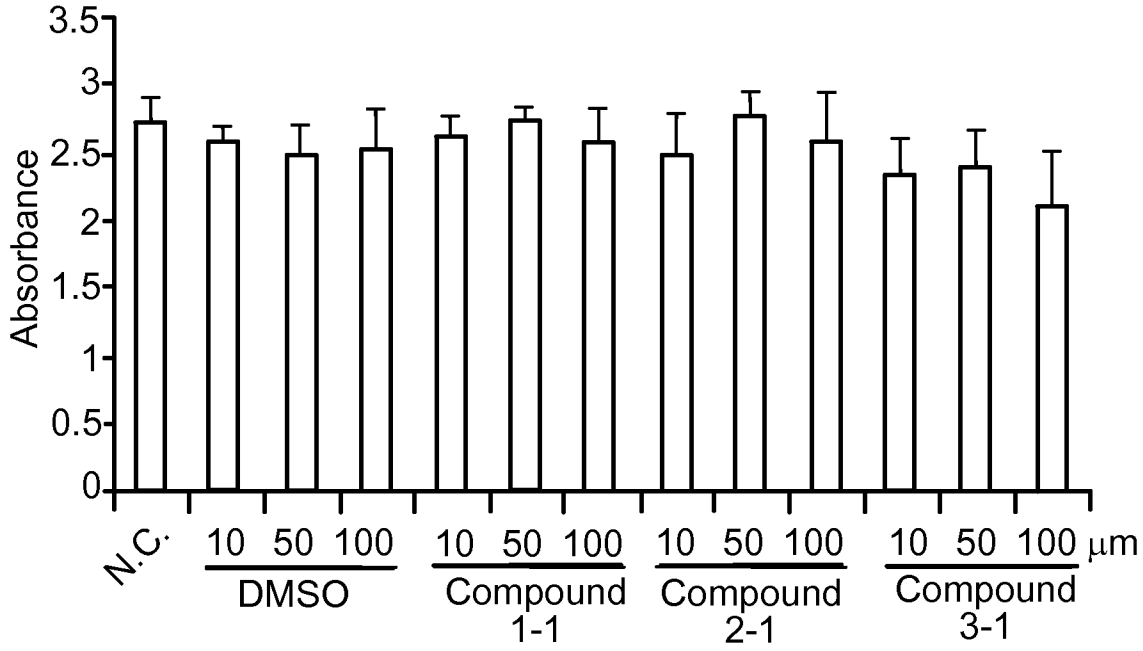

3.5
3
2.5
2
1.5
1
0.5
0
Absorbance
N.C.
10 50 100
DMSO
10 50 100
Compound 1-1
10 50 100
Compound 2-1
10 50 100 μm
Compound 3-1

FIG. 6

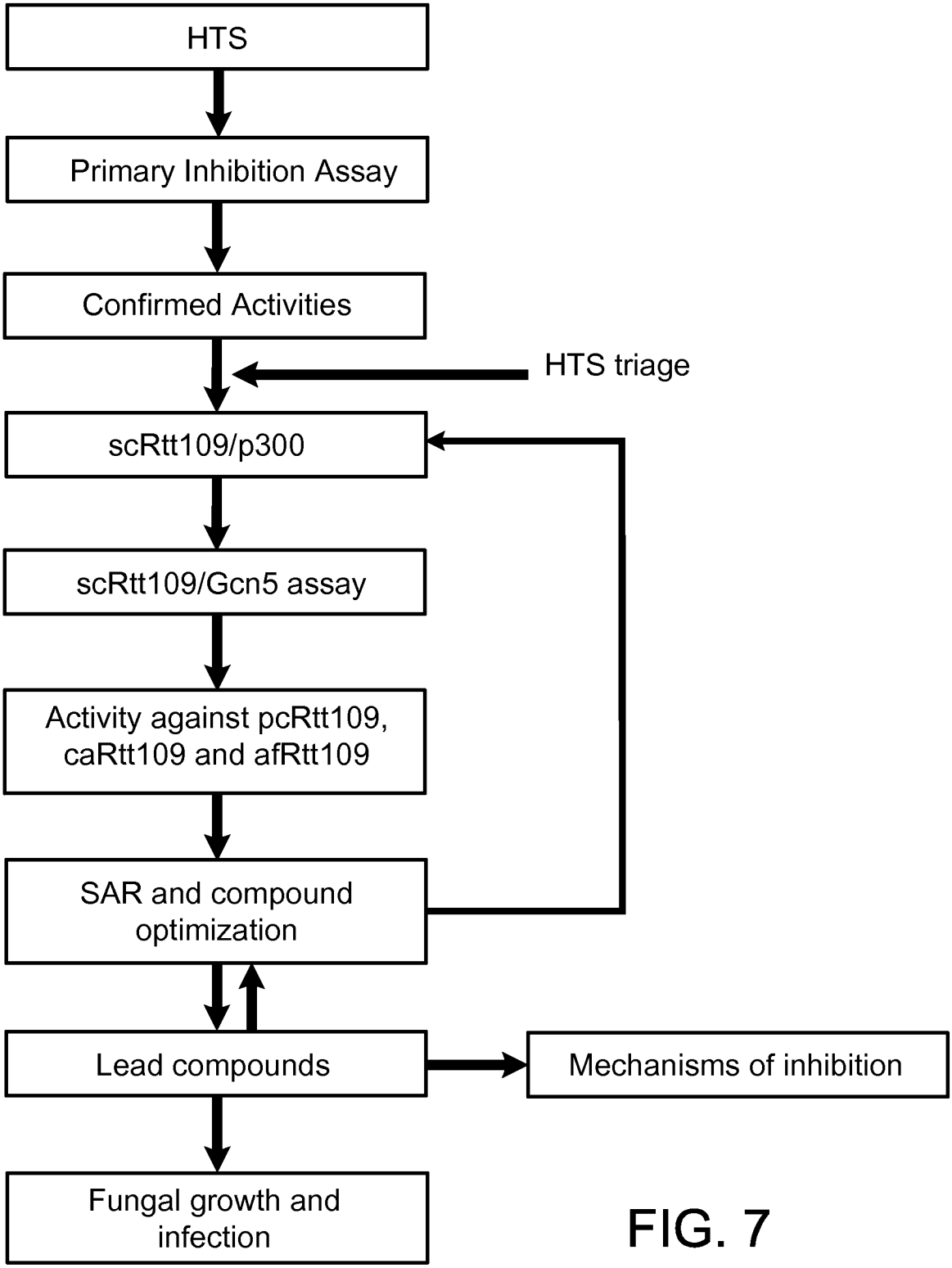

HTS
Primary Inhibition Assay
Confirmed Activities
HTS triage
scRtt109/p300
scRtt109/Gcn5 assay
Activity against pcRtt109, caRtt109 and afRtt109
SAR and compound optimization
Lead compounds
Mechanisms of inhibition
Fungal growth and infection

FIG. 7

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SEQ ID NO: 1  YEAST ----------MSLNDFLSSVLPVSEQFEYDSLQSIPLETHAVVTPNKDDKRVP---KSTIKTQHFFSLFHQGKVFFSLEVYVYVTLWDEAD--AER  81
SEQ ID NO: 2  KLULA ----------MSLECYIKDALPFGHEFEIINLQSIPRETSPITFPKREAEDVNGKTVKTIKTQHFVALCFEEKVVFALEIVVYFSLNSANNGPVER  86
SEQ ID NO: 3  ASHGO ----------MGLKELLAEALPAGHDFDVLHLQSPATERPPLTEASGCS---------PTTIAAQHFFTLSNNSKVFYALQVVVYICIEGTSG--DR  76
SEQ ID NO: 4  CANAL ----------MLPPDLLQNGEFETLYFQTNPTYIKSPIHIPKSTIGKP-----DTVKIRHFFALLHQDLVVLGLEVFVYLQIYSDFV---EK  74
SEQ ID NO: 5  SCHPO ----------MPDLWSESILEGRKLSIYHLKSTLEKCPFLFGQSKKSK---------DFQFGSHLFLVEEQNVFTFGMECIVYEKNK-----EF  70
SEQ ID NO: 6  ASPOR MTIAVLESLFSHENYGDVADPMSKVDVDLGDLLAKVLPTGVKVTIRHISSAPTPCTALTPPPGEESES----TFCENHFLTVSVNADEHDGPELIVFGIEVLVYSTAHLT 107

SEQ ID NO: 1  YEAST LIFVSKADTNGYCNTRVSVRDITKILEFTLSIDPNYYLQKVKPAIRSYKKIS-PELISAASTPARTLRILARRLKQSGSTVLKELESPRFQQDLYLSFTCPREILTKICLF 192
SEQ ID NO: 2  KLULA LLFVSKADTNGYCDLRLDIGQITKSIIAYILAISPGHYLRAVKPLIR--KALSGAKIIKRSTSVQRALEILSNRPLDS-KGITSRVKIP--DQDLVVSFPVPTEIVTKVSLF 193
SEQ ID NO: 3  ASHGO MLFVSKADTNGYCDVRVDVRRATRALLQFLLSIDPSHYLQRVR-RMR-IDPAACKGLITPATNAVDALRILAQRYRTGNLDVRAAADRAR----YTSFPCPPQYTTRLALF 181
SEQ ID NO: 4  CANAL YVYVSKCDTVGLEKSTIKIGKVIGPVLQYIINYN-GYKIKMKNLDEKSKDLSDPSTLVRLQRLRDKLPDIYPNLPYYNDIPPKEECIEYR----TLPKTQNLRICVF 176
SEQ ID NO: 5  SCHPO IVFVSKADSTGFGSKGVSCNSLAFCCLVTLIDGLRKQGAENVTI---------------------------------------------LTTLF 117
SEQ ID NO: 6  ASPOR TVFVSKADSTGFLHLIKNAPKVS--LIRRISNGFLSFLVQTHQ---------------------------------------------RPGVRLVVSLF 159

SEQ ID NO: 1  YEAST TRPASQYLFPDSSKNSKKHILNGEELMKWWGFILDRILIECFQND----------TQAKLRIPGELDPARVRSYLRGM----KYPLWQVG--DLFTSKENSL 277
SEQ ID NO: 2  KLULA TRAEPQYLFSNSSKNPRKHVLSGDKLLKWWLKIVDQIVVEDFDNK----------TKATLQIPGEEKRVISNYILRNT----AYQNWTVG--DIFSESPQDI 278
SEQ ID NO: 3  ASHGO TRAEPHYLFSESACNPAKHVLPGDRLLRWWLASVDDLLVTLFDPA----------TDAALLIPGEDSAVTAHYLRPV----RFPRWRVG--HVFGGSAEDP 266
SEQ ID NO: 4  CANAL TKPAKEYLFPNSAKNPYKNILNGQSLLRWWLSIIDSITKGWNN----------HKLMIPGADKYATRKFIE----KYSDWSEG--HIFK--KDGL 253
SEQ ID NO: 5  SCHPO AIAQGQYLFPESVDNGQKHVLNDSGLLRWWVNQLEKLRKYYTDSE---APNDSEKQKNSTLIPKAYLFVPGLENIRSYLPNR----HWIE---SNAITTGK--- 208
SEQ ID NO: 6  ASPOR ARAQNQYLFPGSIENSGKHVLDDRGLIKWWCRVVDPLLREYEPESGSHEKGLLDRAMESAKSSATAFLIVPGCDKFETRGTFPGTAKSDDKERRPWLNSYPLHQLCDNTNAP 271
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FIG. 8

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YEAST AVYNILFPDDPKARFTHQLAEDRL----------LKVSLSSFWIELQERQEFKLSVTSSVMG----------ISGYSLATPSLFPSSADVIVPKSRKQFRAIKYTL 365
KLULA AIVRIPLFPDDPKGRFLDHLISDGRI----------SKVNVLTFWTELQARQEFRLGSTVSVIG----------VSGKYIGR-CNEPERTDIIIPTSKKNFKNLKNYIL 365
ASHGO ALTRIPRFPDDPKGRFLDDLAHEGR----------NPSLRAFWTELVARQEFRLGVVVGVVG----------AAGLYTGP-CARPAPEDLICPASPADVWHVKHYV 351
CANAL AVQAIPLFPDDPKGRFLEIVIVECRY----------GKMTVSREYQELAYRQEFLIGDCVSLIG----------CCKENLEV----TYHDDSVSTVTISEYKEFMNSL 337
SCHPO AVEELPRFPDDPKCRYICELQDEKS----------DMSVEEFWDTLTYRQECSSGKLVGFETLQLQ---FYQTREFIAKDNFGDSGVMIPAKLYRVTVDTLLKHP 300
ASPOR PRCLVPRFPDDPKTRFLNLDDELPQKLEAAGSKEGAGQWRSVKSLDQFWEMMSFRQECSAGRLVGFLWLVINPPGLVNSVQMTSSRPVFKEKAEGSLTTTVPVLDKVDSKP 383

YEAST TGEEYDTEEG----------AIEAFTNLRDFLLLRMATNLQSLTG 400
KLULA TGEEYDTEEG----------AEEAYMNIRDIFDMHYSTKMIKVIG 400
ASHGO TAEEYATAAG----------ASEAHRNLWDCLLLRYGVAMPRVRG 386
CANAL KSVDFSDRVE----------VSNFVSNVRKSK---------- 359
SCHPO FGSLSDAQSS----------TEKFLSNTLSAVQNLKDFHYKRYKL 335
ASPOR TGTLAVSVSTDSQSSDTAKDSTAEATSGGPVQDPSTQTGSLSSETHPKVQPNTDQNAFYWPEAGRGHAVNSEEDYKTAINFLLEQDFYNEKVSIASTKAWSEKVASIVDQLWV 495

YEAST KREHRERNQPVPASNINTLAITMLKP---RKKAKALPKT---------- 436
KLULA NHHKIATTKRPEINTIN--VINTLSV---HRKPKS---------- 430
ASHGO AHSTTPRPAPTQDATKRP-AVNDLTARCVRKKPRC---------- 420
CANAL ---------- 359
SCHPO DICGLAKRDDRKNHNHSKPATQANILQPRKKVKK---------- 369
ASPOR GQQVTGRNTSGESADKHAPTTNIINTGLVRKRKKDEPSQATTATSAQKEGCEGNGTVSTAVTAEASTIGTNESPGVNVLQANLIRKKKKA 585
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FIG. 8 (Cont.)