Title: COMPOSITIONS AND METHODS FOR INHIBITING PRENYLTRANSFERASES

Abstract: The present invention relates in part to compositions and methods for inhibiting prenyltransferases.
Compositions and Methods for Inhibiting Prenyltransferases

Background of the Invention

The Ras proteins, including Ha-Ras, Ki4a-Ras, Ki4b-Ras and N-Ras, are part of a signalling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of action indicate that Ras functions like a regulatory G-protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation, Ras is induced to exchange GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulation signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D. R. Lowy et al. (1993) Ann. Rev. Biochem. 62:851-891; Barbacid (1987) Ann. Rev. Biochem. 56:779). Ras must be localized to the plasma membrane to achieve its normal function.

Covalent modification by lipids (prenylation) contributes to membrane interactions and biological activities of a rapidly expanding group of proteins, including Ras (see, for example, Maltese (1990) FASEB J 4:3319; and Glomset et al. (1990) Trends Biochem Sci 15:139). For example, either farnesyl (15-carbon) or geranylgeranyl (20-carbon) isoprenoids can be attached to specific proteins, with geranylgeranyl being the predominant isoprenoid found on proteins (Farnsworth et al. (1990) Science 247:320).

protein substrate to the α/β catalytic subunits. Each of these enzymes selectively uses farnesyl diphosphate or geranylgeranyl diphosphate as the isoprenoid donor and selectively recognizes the protein substrate. FPTase farnesylates CaaX-containing proteins that end with Ser, Met, Cys, Gln or Ala. GGPTase-I geranylgeranylates CaaX-containing proteins that end with Leu or Phe. For FPTase and GGPTase-I, CaaX tetrapeptides comprise the minimum region required for interaction of the protein substrate with the enzyme. GGPTase-II modifies XXCC and XCXC proteins, while the interaction between GGPTase-II and its protein substrates is more complex, requiring protein sequences in addition to the C-terminal amino acids for recognition. The enzymological characterization of these three enzymes has demonstrated that it is possible to selectively inhibit one with little inhibitory effect on the others (Moores et al. (1991) J. Biol. Chem. 266:17438). Some prenyltransferases have been recombinantly prepared (Omer et al. (1993) Biochem. 32).

In part, FPTase covalently modifies the Cys thiol group of the Ras CAAX box (Reiss et al. (1990) Cell 62:81-88; Schaber et al. (1990) J. Biol. Chem. 265:14701-14704; Schafer et al., (1990) Science 249:1133-1139; Manne et al. (1990) Proc. Natl. Acad. Sci USA 87:7541-7545). Other farnesylated proteins include the Ras-related GTP-binding proteins such as Rho, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James et al. (1994) J. Biol. Chem. 269:14182, have identified a peroxisome associated protein Pxf which is also farnesylated. James et al. have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed here. In part, GGPTase-I transfers the prenyl group from geranylgeranyl diphosphate to the sulphur atom in the Cys residue within the CAAX sequence. Proteins of the Ras superfamily, including Rho1, Rho2, Rsr1/Bu1, and Cdc42 appear to be GGPTase substrates (Madaule et al. (1987) PNAS 84:779-783; Bender et al. (1989) PNAS 86:9976-9980; and Johnson et al. (1990) J Cell Biol 111:143-152), as do Rap1A and Rac1. Other proteins may be both farnesylated and geranylgeranylated, including RhoB and Ki4b-Ras.

**Fungal infections**

Fungal infections of humans range from superficial conditions, usually caused by dermatophytes or Candida species, that affect the skin (such as dermatophytoses) to deeply invasive and often lethal infections (such as candidiasis and cryptococcosis). Pathogenic fungi occur worldwide, although particular species may predominate in certain geographic areas.
In the past 20 years, the incidence of fungal infections has increased dramatically, as have the numbers of potentially invasive species. Indeed, fungal infections, once dismissed as a nuisance, have begun to spread so widely that they are becoming a major concern in hospitals and health departments. Fungal infections occur more frequently in people whose immune system is compromised or suppressed (e.g., because of organ transplantation, cancer chemotherapy, or the human immunodeficiency virus), who have been treated with broad-spectrum antibacterial agents, or who have been subject to invasive procedures (catheters and prosthetic devices, for example). Fungal infections are now important causes of morbidity and mortality of hospitalized patients: the frequency of invasive candidiasis has increased tenfold to become the fourth most common blood culture isolate (Pannuti et al. (1992) Cancer 69:2653). Invasive pulmonary aspergillosis is a leading cause of mortality in bone-marrow transplant recipients (Pannuti et al., supra), while *Pneumocystis carinii* pneumonia is the cause of death in many patients with acquired immunodeficiency syndrome (AIDS) in North America and Europe (Hughes (1991) Pediatr Infect. Dis J. 10:391). Many opportunistic fungal infections cannot be diagnosed by usual blood culture and must be treated empirically in severely immuno-compromised patients (Walsh et al. (1991) Rev. Infect. Dis. 13:496).

The fungi responsible for life-threatening infections include *Candida* species (mainly *Candida albicans*, followed by *Candida tropicalis*), *Aspergillus* species, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Pneumocystis carinii* and some zygomycetes. Treatment of deeply invasive fungal infections has lagged behind bacterial chemotherapy.

There are numerous commentators who have speculated on this apparent neglect. See, for example, Georgopapadakou et al. (1994) Science 264:371. First, like mammalian cells, fungi are eukaryotes, and thus agents that inhibit fungal protein, RNA, or DNA biosynthesis may have the same activity in the patient's own cells, producing toxic side effects. Second, life-threatening fungal infections were thought, until recently, to be too infrequent to warrant aggressive research by the pharmaceutical industry. Other factors have included:

(i) Lack of drugs. A drug known as Amphotericin B has become the mainstay of therapy for fungal infection despite side effects so severe that the drug is known as "amphoterrible" by patients. Only a few second-tier drugs exist.
(ii) Increasing resistance. Long-term treatment of oral candidiasis in AIDS patients has begun to breed species resistant to older antifungal drugs. Several other species of fungi have also begun to exhibit resistance.

(iii) A growing list of pathogens. Species of fungi that once posed no threat to humans are now being detected as a cause of disease in immune-deficient people. Even low-virulence baker's yeast, found in the human mouth, has been found to cause infection in susceptible burn patients.

(iv) Lagging research. Because pathogenic fungi are difficult to culture, and because many of them do not reproduce sexually, microbiological and genetic research into the disease-causing organisms has lagged far behind research into other organisms.

In the past decade, however, more antifungal drugs have become available. Nevertheless, there are still major weaknesses in their spectra, potency, safety, and pharmacokinetick properties, and accordingly it is desirable to improve the panel of antifungal agents available to the practitioner.

The fungal cell wall is a structure that is both essential for the fungus and absent from mammalian cells, and consequently may be an ideal target for antifungal agents. Inhibitors of the biosynthesis of two important cell wall components, glucan and chitin, already exist. Polyoxins and the structurally related nikkomycins (both consist of a pyrimidine nucleoside linked to a peptide moiety) inhibit chitin synthase competitively, presumably acting as analogs of the substrate uridine diphosphate (UDP)-N-acetylglucosamine (chitin is an N-acetylglucosamine homopolymer), causing inhibition of septation and osmotic lysis. Unfortunately, the target of polyoxins and nikkomycins is in the inner leaflet of the plasma membrane; they are taken up by a dipeptide permease, and thus peptides in body fluids antagonize their transport.

In most fungi, glucans are the major components that strengthen the cell wall. The glucosyl units within these glucans are arranged as long coiling chains of \( \beta-(1,3) \)-linked residues, with occasional sidechains that involve \( \beta-(1,6) \) linkages. Three \( \beta-(1,3) \) chains running in parallel can associate to form a triple helix, and the aggregation of helices produces a network of water-insoluble fibrils. Even in the chitin-rich filamentous aspergilli, \( \beta-(1,3) \)-glucan is required to maintain the integrity and form of the cell wall (Kurtz et al. (1994) *Antimicrob Agents Chemother* 38:1408-1489), and, in *P. carinii*, it is important during the life cycle as a constituent of the cyst (ascus) wall (Nollstadt et al. (1994) *Antimicrob Agents Chemother* 38:2258-2265).
In a wide variety of fungi, β-(1,3)-glucan is produced by a synthase composed of at least two subunits (Tkacz, J.S. (1992), in Emerging Targets in Antibacterial and Antifungal Chemotherapy 495-523 (Sutcliffe and Georgopapadakou, Eds., Chapman & Hall); and Kang et al. (1986) PNAS 83:5808-5812). One subunit is localized to the plasma membrane and is thought to be the catalytic subunit, while the second subunit binds GTP and associates with and activates the catalytic subunit (Mol et al. (1994) J Biol Chem 269:31267-31274).

Two groups of anti-candidal antibiotics known in the art interfere with the formation of β-(1,3)-glucan: the papulacandins and the echinocandins (Hector et al. (1993) Clin Microbiol Rev 6:1-21). However, many of the papulacandins are not active against a variety of Candida species, or other pathogenic fungi including Aspergillus. The echinocandins, in addition to suffering from narrow activity spectrum, are not in wide use because of lack of bioavailability and toxicity.

The cell wall of many fungi, as set out above, is required to maintain cell shape and integrity. The main structural component responsible for the rigidity of the yeast cell wall is 1,3-β-linked glucan polymers with some branches through 1,6-β-linkages. The biochemistry of the yeast enzyme catalyzing the synthesis of 1,3-β-glucan chains has been studied extensively. A pair of closely related proteins (Gsc1/Fks1 and Gsc2/Fks2) had previously been described as subunits of the 1,3-β-glucan synthase (GS) (Inoue et al. (1995) supra; Douglas et al. (1994) PNAS 91:12907; Drgonova et al. (1996) Science 272:277). GS activity in many fungal species, including S. cerevisiae, requires GTP or a non-hydrolyzable analog (e.g., GTPγS) as a cofactor, suggesting that a GTP-binding protein is a regulatory subunit that stimulates this enzyme (Mol et al. (1994) J. Biol. Chem. 269:31267; Qadota et al. (1996) Science 272:279).

Rho-like GTPase activities are critically involved in cell wall integrity, hyphae formation, and other cellular functions critical to pathogenesis. Fungal Rho1 GTPase is required for glucan synthase activity, copurifies with 1,3-β-glucan synthase, and is found to associate with the Gsc1/Fks1 subunit of this complex in vivo. Rho1 is a regulatory subunit of 1,3-β-glucan synthase, and accordingly Rho1, and the resulting enzyme complex, are potential therapeutic targets for development of antifungal agents. Moreover, Rho1 is required for protein kinase C (PKC1) mediated MAPK activation, and confers upon PKC1 the ability to be stimulated by phosphatidylserine (PS), indicating that Rho1 controls signal transmission through PKC1. Loss of PKC1 activity results in cell lysis. Prenylation of Rho1 by GGPTase is a critical step to maintenance of cell wall integrity in yeast, and loss of Rho1 prenylation results in cell lysis.
Cancer

Mutated Ras genes are found in many human cancers. Transforming Ras genes are the oncogenes most frequently identified in human cancers. Clinical investigations have identified activated Ras genes in a wide variety of human neoplasms, including carcinomas, sarcomas, leukemias, and lymphomas. It is estimated that 40% of all human colon cancers and 95% of human pancreatic cancers contain activated Ras oncogenes (Kuzumaki (1991) Anticancer Res. 11:313-320).

Mammalian cells express at least four types of Ras proteins, Ha-Ras, Ki4a-Ras, Ki4b-Ras and N-Ras, among which Ki4b-Ras is the most frequently mutated form of Ras in human cancers. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulation signal. Mutations to the Ras proto-oncogene translate into amino acid substitutions in the GTP binding domain, activating the Ras protein and biasing this molecular switch in the "on" position. Thus, the Ras transformed cell behaves like a cell with a faulty switch, signaling extracellular hormone binding when none is present. Cells transformed in this way grow and differentiate in an abnormal manner.

Ras must be localized to the plasma membrane by prenylation to produce any oncogenic effect. Prenyltransferases, including FPTase, GGPTase I, and GGPTase II, are therefore potential targets for anticancer, antitumor, and other like agents. It was therefore one object of this invention to identify compounds that antagonize prenylation of low molecular weight G-proteins such as Ras.

Summary of the Invention

One aspect of the present invention relates to methods for treating or preventing fungal infections and infections involving other eukaryotic parasites of plants or animals, using compounds that inhibit the biological activity of a prenyltransferase. In certain embodiments, the prenyltransferase that is inhibited is GGPTase. The present invention also relates to the novel compositions of matter used in such methods. In certain embodiments, the subject inhibitors can be used for the treatment of mycotic infections in animals; as additives in feed for livestock to promote weight gain; as disinfectant formulations; and as in agricultural applications to prevent or treat fungal infection of plants. In preferred embodiments, the practice of the subject method utilizes inhibitors which are selective inhibitors of the fungal or parasitic prenyltransferase relative to any human prenyltransferases. In certain
preferred embodiments, the method can be used for treating a nosocomial fungal and skin/wound infection involving fungal organisms, including, among others, the species *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Epidermophyton*, *Hendersonula*, *Histoplasma*, *Microsporum*, *Paecilomyces*, *Paracoccidioides*, *Pneumocystis*, *Trichophyton*, and *Trichosporium*. In other preferred embodiments, the method can be used for treating an animal or plant parasites, such as infections involving liver flukes, nematodes or the like.

In other embodiments, the inhibitors of prenyltransferases may be used to treat cancer, neoplasms, or other forms or types of aberrant hyperproliferation or unwanted proliferation. In preferred embodiments, the practice of the subject method utilizes prenyltransferase inhibitors which are selective inhibitors of human GGPTase I, GGPTase II, or FPTase. The present invention also relates to the novel compositions of matter used in such methods. In certain embodiments, the practice of the subject method utilizes prenyltransferase inhibitors which are selective inhibitors of a specific prenyltransferase, such as GGPTase I, GGPTase II, or FPTase. Such specific inhibition may allow for inhibition of only those prenyltransferases that are active for a particular protein, such as those from the Ras family, encoded by a mutated gene, while inhibiting to some lesser degree any protein product of a nonmutated gene.

According to the present invention, treatment using the inhibitors of the present invention comprises the administration of a pharmaceutical composition of the invention in a therapeutically effective amount to an individual in need of such treatment. The compositions may be administered parenterally by intramuscular, intravenous, intraocular, intraperitoneal, or subcutaneous routes; inhalation; orally, topically and intranasally.

**Brief Description of the Drawings**

Figures 1-31 present various illustrative reaction schemes for preparing prenyltransferase inhibitors useful in the methods and compositions of the present invention.

Figure 32 is a demonstration of the effect of a prenyltransferase inhibitor on prenylation state of CaRHO1.
**Detailed Description of the Invention**

In one aspect, the present invention relates to methods for treating and/or preventing fungal infections using compounds that specifically inhibit the biological activity of fungal enzymes involved in cell wall integrity, hyphael formation, and other cellular functions critical to pathogenesis. In particular, it has been observed by us that prenylation of Rh01-like phosphatases by a geranylgeranylproteintransferase (GGPTase) activity can be critical to maintenance of cell wall integrity in yeast. As described in WO 97/38293, prenylation of, *inter alia*, Rh01-like GTPase(s) is required for sufficient glucan synthase activity. It was demonstrated that the prenylation of Rh01 by GGPTase I is not only critical to cell growth, but inhibition of the prenylation reaction is a potential target for developing a cytotoxic agent for killing various fungi. Moreover, the relatively high divergence between fungal and human GGPTase sequences suggests that selectivity for the fungal GGPTase activity can be obtained to provide antifungal agents having desirable therapeutic indices.

In another aspect of the present invention, the present invention relates to methods of treating and preventing cancer, neoplasms, and other aberrant hyperproliferation by using compounds that specifically inhibit the biological activity of prenyltransferases, including FTPase, GGPTase I, and GGPTase II. In general, proteins encoded by mutant Ras genes are able to transform cells to a malignant phenotype. Inhibition by compounds of the present invention may suppress the oncogenic potential of any protein encoded by a mutant Ras gene. The present invention also relates to the novel compositions of matter used in such methods.

Different substrate specificity among prenyltransferases allows for preparation of inhibitors of the present invention having improved therapeutic indexes. That is, certain inhibitors may inhibit some prenyltransferases and not others. As a result, inhibitors only for prenyltransferases encoded by oncogenes, and not wildtype enzymes, may be employed. Some of the reasons why different prenyltransferases may exhibit different specificity include the following. The β subunits for FTPase and GGPTase I are distinct, and such subunits contribute significantly to the activity of the enzyme. Also, there may be differences in effect for inhibition of GGPTase and FTPase, because geranylgeranyl protein in mammalian cells exceeds that of farnesylated proteins by a factor of about five. Numerous reports, which are detailed below, report that inhibitors of FTPase may not inhibit GGPTase and *vice versa*. Despite such differences, other reports indicate that
FTPase and GGTPase may, in certain circumstances, prenylate the same substrate (Caldwell et al. (1994) Proc. Natl. Acad. Sci. USA 91:1275-1279). It may be possible to inhibit one prenyltransferase selectively, which should allow for improved therapeutic indexes for any inhibitor when administered specifically for any cancer, neoplasm, or aberrant hyperproliferative disorder resulting from mutation of a particular gene encoding a prenyltransferase.

Thus, as described in greater detail below, the present invention provides methods and compositions for inhibiting prenyltransferases using small molecule (e.g., less than about 1000 amu) inhibitors. In the practice of the instant method, the preferred inhibitors inhibit a targeted prenyltransferase with a Kᵢ of 10 μM or less, more preferably 1 μM or less, and even more preferably with a Kᵢ less than 100 nM, 10 nM, or even 1 nM.

In one embodiment, for the treatment of humans or other animals, the subject method preferably employs prenyltransferase inhibitors, such as inhibitors of FPTase, GGPTase I, or GGPTase II to treat cancer, neoplasms and other aberrant hyperproliferative disorders. The chemotherapeutic properties of the compounds of the present invention may be determined from cell-based assays, as well as by other methods, including, inter alia, growth inhibition assays, flow cytometry analyses, and other standard assays known to those skilled in the art. Preferred anticancer agent pharmaceutical preparation, whether for topical, injection or oral delivery (or other route of administration), would provide a dose less than the ED₅₀ for modulation of prenyltransferase activity of nonmutated genes as compared to oncogenic ones, more preferably at least 1 order of magnitude less, more preferably at least 2, 3 or 4 orders of magnitude less.

Another parameter useful in identifying and measuring the effectiveness of the prenyltransferase inhibitor compounds of the invention as anticancer agents is the determination of the kinetics of the activity of such compounds. Such a determination can be made by determining the effect of an inhibitor, e.g., anticancer or antifungal activity, as a function of time. For treatment of fungal infections, in a preferred embodiment, the compounds display kinetics which result in efficient lysis of a fungal cell. In a preferred embodiment, the compounds are fungicidal. For treatment of cancer and other aberrant hyperproliferative disorders, the compounds display kinetics which result in at least slowing of cell proliferation, or more preferably, cell death for any oncogenic cell.
In another embodiment, for the treatment of humans or other animals, the subject method preferably employs prenyltransferase inhibitors which are selective for a fungal enzyme relative to the host animals’ prenyltransferase, e.g., the \( K_i \) for inhibition of the fungal enzyme is at least one order of magnitude less than the \( K_i \) for inhibition any prenyltransferase from human (or other animal), and even more preferably at least two, three, or even four orders of magnitude less. That is, in preferred embodiments, the practice of the subject method \textit{in vivo} in animals utilizes inhibitors with therapeutic indexes of at least 10, and more preferably at least 100 or 1000. Preferably, inhibitors for use as antifungal agents inhibit fungal GGPTase.

The antifungal properties of the compounds of the present invention may be determined from a fungal lysis assay, as well as by other methods, including, \textit{inter alia}, growth inhibition assays, fluorescence-based fungal viability assays, flow cytometry analyses, and other standard assays known to those skilled in the art. The assays for growth inhibition of a microbial target can be used to derive an \( ED_{50} \) value for the compound, that is, the concentration of compound required to kill 50% of the fungal sample being tested. Preferred antifungal agent pharmaceutical preparation, whether for topical, injection or oral delivery (or other route of administration), would provide a dose less than the \( ED_{50} \) for modulation of FPTase and/or GGPTase activity in the host (mammal), more preferably at least 1 order of magnitude less, more preferably at least 2, 3 or 4 orders of magnitude less.

Alternatively, growth inhibition by an antifungal compound of the invention may also be characterized in terms of the minimum inhibitory concentration (MIC), which is the concentration of compound required to achieve inhibition of fungal cell growth. Such values are well known to those in the art as representative of the effectiveness of a particular antifungal agent against a particular organism or group of organisms. For instance, cytolysis of a fungal population by an antifungal compound can also be characterized, as described above by the minimum inhibitory concentration, which is the concentration required to reduce the viable fungal population by 99.9%. The value of MIC\(_{50}\), defined as the concentration of a compound required to reduce the viable fungal population by 50%, can also be used. In preferred embodiments, the compounds of the present invention are selected for use based, \textit{inter alia}, on having MIC values of less than 25 \( \mu g/mL \), more preferably less than 7 \( \mu g/mL \), and even more preferably less than 1 \( \mu g/mL \) against a desired fungal target, e.g., \textit{Candida albicans}.

Furthermore, the preferred anticancer compounds of the invention display selective toxicity to target cells having mutated genes encoding for
prenyltransferases over the wildtype form. Determination of the toxic dose (or "LD_{50}") can be carried out using protocols well known in the field of pharmacology. Ascertaining the effect of a compound of the invention on mammalian cells is preferably performed using tissue culture assays, e.g., the present compounds can be evaluated according to standard methods known to those skilled in that art (see for example Gootz, T. D. (1990) Clin. Microbiol. Rev. 3:13-31). For mammalian cells, such assay methods include, *inter alia*, trypan blue exclusion and MTT assays (Moore et al. (1994) Compound Res. 7:265-269). Where a specific cell type may release a specific metabolite upon changes in membrane permeability, that specific metabolite may be assayed, e.g., the release of hemoglobin upon the lysis of red blood cells (Srinivas et al. (1992) J. Biol. Chem. 267:7121-7127). The compounds of the invention are preferably tested against primary cells, e.g., using human skin fibroblasts (HSF) or fetal equine kidney (FEK) cell cultures, or other primary cell cultures routinely used by those skilled in the art. Permanent cell lines may also be used, e.g., Jurkat cells. In preferred embodiments, the subject compounds are selected for use in animals, or animal cell/tissue culture based at least in part on having LD_{50}'s at least one order of magnitude greater than the MIC or ED_{50} as the case may be, and even more preferably at least two, three and even four orders of magnitude greater. That is, in preferred embodiments where the subject compounds are to be administered to an animal, a suitable therapeutic index is preferably greater than 10, and more preferably greater than 100, 1000 or even 10,000.

Furthermore, the preferred antifungal compounds of the invention display selective toxicity to target microorganisms and minimal toxicity to mammalian cells. Determination of the toxic dose (or "LD_{50}") can be carried out using protocols well known in the field of pharmacology. Ascertaining the effect of a compound of the invention on mammalian cells is preferably performed using tissue culture assays, e.g., the present compounds can be evaluated according to standard methods known to those skilled in that art (see for example Gootz, T. D. (1990) Clin. Microbiol. Rev. 3:13-31). For mammalian cells, such assay methods include, *inter alia*, trypan blue exclusion and MTT assays (Moore et al. (1994) Compound Research 7:265-269). Where a specific cell type may release a specific metabolite upon changes in membrane permeability, that specific metabolite may be assayed, e.g., the release of hemoglobin upon the lysis of red blood cells (Srinivas et al. (1992) J. Biol. Chem. 267:7121-7127). The compounds of the invention are preferably tested against primary cells, e.g., using human skin fibroblasts (HSF) or fetal equine kidney (FEK) cell cultures, or other primary cell cultures routinely used by those skilled in the art.
Permanent cell lines may also be used, e.g., Jurkat cells. In preferred embodiments, the subject compounds are selected for use in animals, or animal cell/tissue culture based at least in part on having LD<sub>50</sub>'s at least one order of magnitude greater than the MIC or ED<sub>50</sub> as the case may be, and even more preferably at least two, three, and even four orders of magnitude greater. That is, in preferred embodiments where the subject compounds are to be administered to an animal, a suitable therapeutic index is preferably greater than 10, and more preferably greater than 100, 1000 or even 10,000.

The invention is also directed to methods for treating a microbial infection in a host using the compositions of the invention. The compounds provided in the subject methods exhibit broad antifungal activity against various fungi and can be used as agents for treatment and prophylaxis of fungal infectious diseases. For instance, the subject method can be used to treat or prevent nosocomial fungal and skin/wound infection involving fungal organisms, including, among others, the species *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Epidermophyton*, *Hendersonula*, *Histoplasma*, *Microsporum*, *Paecilomyces*, *Paracoccidioides*, *Pneumocystis*, *Trichophyton*, and *Trichosporium*.

According to the present invention, treatment of such fungal infections comprises the administration of a pharmaceutical composition of the invention in a therapeutically effective amount to an individual in need of such treatment. The compositions may be administered parenterally by intramuscular, intravenous, intraocular, intraperitoneal, or subcutaneous routes; inhalation; orally, topically and intranasally.

The subject inhibitors of the present invention, and their method of use, may also be used to inhibit neoplastic growth or proliferative disorders in tissue culture. In addition, the subject inhibitors, and corresponding antifungal methods are also particularly useful in inhibiting unwanted fungal growth in tissue culture, especially those used for production of recombinant proteins or vectors for use in gene therapy.

The invention is also directed to pharmaceutical compositions containing one or more of the inhibitory compounds of the invention as the active ingredient which may be administered to a patient. In addition, the invention is also directed to pharmaceutical compositions containing one or more of the antimicrobial compounds of the invention as the active ingredient which may be administered to a host animal.

There have been a number of reports on methods for detecting inhibitors of
prenyltransferases and uses of such inhibitors. For example, inhibition of farnesyl-protein transferase has been shown to block the growth of Ras-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the Ras oncoprotein intracellularly (N. E. Kohl et al. (1993) Science 260:1934-1937; James et al. (1993) Science 260:1937-1942). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of Ras-dependent tumors in nude mice (N. E. Kohl et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91:9141-45) and induces regression of mammary and salivary carcinomas in Ras transgenic mice (N. E. Kohl et al. (1995) Nature Medicine 1:792-797).


Other uses of prenyltransferase inhibitors have been reported. For example, it has recently been reported that farnesyl-protein transferase inhibitors are inhibitors of proliferation of vascular smooth muscle cells and are therefore useful in the prevention and therapy of arteriosclerosis and diabetic disturbance of blood vessels.

5 **I. Definitions**

Before further description of the preferred embodiments of the subject invention, certain terms employed in the specification, examples, and appended claims are collected here for convenience.

The terms "aberrant proliferation" and "unwanted proliferation" are interchangeable and refer to proliferation of cells which is undesired, e.g., such as may arise it due to transformation and/or immortalization of the cells, e.g., neoplastic or hyperplastic.

The term "patient" refers to an animal, preferably a mammal, including humans as well as livestock and other veterinary subjects.

The terms "fungi" and "yeast" are used interchangeably herein and refer to the art recognized group of eukaryotic protists known as fungi. That is, unless clear from the context, "yeast" as used herein can encompass the two basic morphologic forms of yeast and mold and dimorphisms thereof.

As used herein, the term "antimicrobial" refers to the ability of the inhibitors of the invention to prevent, inhibit or destroy the growth of microbes such as bacteria, fungi, protozoa and viruses.

The term "prodrug" is intended to encompass compounds which, under physiological conditions, are converted into the inhibitor agents of the present invention. A common method for making a prodrug is to select moieties which are hydrolyzed under physiological conditions to provide the desired biologically active drug. In other embodiments, the prodrug is converted by an enzymatic activity of the patient or alternatively of a target fungi.

The term "ED$_{50}$" means the dose of a drug which produces 50% of its maximum response or effect. Alternatively, it may refer to the dose which produces a pre-determined response in 50% of test subjects or preparations.

The term "LD$_{50}$" means the dose of a drug which is lethal in 50% of test subjects.
The term "therapeutic index" refers to the therapeutic index of a drug defined as LD$_{50}$/ED$_{50}$.

The term "structure-activity relationship" or "SAR" refers to the way in which altering the molecular structure of drugs alters their interaction with a receptor, enzyme, etc.

The term "acylamino" is art-recognized and refers to a moiety that can be represented by the general formula:

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wherein R$_9$ is as defined above, and R$''$$_{11}$ represents a hydrogen, an alkyl, an alkenyl or -(CH$_2$)$_m$-R$_8$, where m and R$_8$ are as defined above.

Herein, the term "aliphatic group" refers to a straight-chain, branched-chain, or cyclic aliphatic hydrocarbon group and includes saturated and unsaturated aliphatic groups, such as an alkyl group, an alkenyl group, and an alkynyl group.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The terms "alkoxy" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxy groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxy, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, -O-(CH$_2$)$_m$-R$_8$, where m and R$_8$ are described above.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C$_1$-C$_{30}$ for straight chains, C$_3$-C$_{30}$ for branched chains), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.
Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxy carbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF₃, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF₃, -CN, and the like.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Throughout the application, preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and -S-(CH₂)ₘ-Rₘ, wherein m and Rₘ are defined above. Representative alkylthio groups include methylthio, ethylthio, and the like.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:
wherein R_9, R_{10} and R'_{10} each independently represent a hydrogen, an alkyl, an alkenyl, -(CH_2)_m-R_8, or R_9 and R_{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R_8 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In preferred embodiments, only one of R_9 or R_{10} can be a carbonyl, e.g., R_9, R_{10} and the nitrogen together do not form an imide. In even more preferred embodiments, R_9 and R_{10} (and optionally R'_{10}) each independently represent a hydrogen, an alkyl, an alkenyl, or -(CH_2)_m-R_8. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R_9 and R_{10} is an alkyl group.

The term "amido" is art-recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{R_9} \\
\text{R_{10}}
\end{array}
\]


wherein R_9, R_{10} are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The term "aryl" as used herein includes 5-, 6-, and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxy, amino, nitro, sulfdryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkythio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or...
heteroaromatic moieties, -CF₃, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

The term "carbonyl" is art-recognized and includes such moieties as can be represented by the general formula:

\[
\text{X} - \text{R}_{11}, \quad \text{X} - \text{R'}_{11}
\]

wherein X is a bond or represents an oxygen or a sulfur, and R₁₁ represents a hydrogen, an alkyl, an alkenyl, -(CH₂)m-R₈ or a pharmaceutically acceptable salt, R'₁₁ represents a hydrogen, an alkyl, an alkenyl or -(CH₂)m-R₈, where m and R₈ are as defined above. Where X is an oxygen and R₁₁ or R'₁₁ is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R₁₁ is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R₁₁ is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R'₁₁ is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiocarbonyl" group. Where X is a sulfur and R₁₁ or R'₁₁ is not hydrogen, the formula represents a "thioester." Where X is a sulfur and R₁₁ is hydrogen, the formula represents a "thiohydroxylic acid." Where X is a sulfur and R₁₁' is hydrogen, the formula represents a "thiolformate." On the other hand, where X is a bond, and R₁₁ is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R₁₁ is hydrogen, the above formula represents an "aldehyde" group.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran,
chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulphydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfanyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, -CN, or the like.

As used herein, the term "nitro" means -NO₂; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulphydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means -SO₂-.

A phenethylazaryl portion is a subunit having a structure according to the general formula:

\[
\begin{array}{c}
\text{K} \\
\text{U} \\
\text{A}
\end{array}
\]

wherein A represents a substituted or unsubstituted aryl or heteroaryl ring;

U represents a carbon or nitrogen atom, preferably an sp³-hybridized carbon atom, to which the linkage is attached; and

K represents a nitrogen-containing heteroaryl ring.

In certain embodiments, A represents a phenyl ring, preferably bearing from 1-3 substituents, even more preferably a disubstituted phenyl ring such as a 2,4-disubstituted phenyl ring. In certain embodiments, A is a phenyl ring substituted with at least one halogen atom. In certain embodiments, the phenyl ring is substituted with a halogen atom at an ortho and a para position.

In certain embodiments, K is a substituted or unsubstituted pyridine, imidazole, pyrrole, or triazole ring, preferably an imidazole or triazole ring. In preferred embodiments, K represents an unsubstituted imidazole or triazole ring linked through a nitrogen atom of the ring.

In certain embodiments, the phenethylazaryl portion has the formula:
wherein Y represents CH or N;
U represents a nitrogen or carbon atom, such as an sp\(^3\)-hybridized carbon atom, to
which the linkage is attached; and
R\(_7\) represents from 0 to 5 substituents on the ring to which it is attached, preferably
from 1 to 3 substituents, independently selected from fluoro, chloro, bromo,
iodo, nitro, and cyano.

In certain embodiments, R\(_7\) includes at least two halogen substituents, e.g.,
Cl and/or F, preferably located at an ortho and a para position on the phenyl ring. In
certain embodiments, R\(_7\) consists of two halogen substituents, e.g., Cl and/or F,
located at an ortho and a para position on the phenyl ring.

A “phosphonamidite” can be represented in the general formula:

\[
\begin{align*}
\text{OR}_{46} & \quad \text{OR}_{48} \\
\text{Q}_2 & \overset{\text{P}}{\text{O}} \quad \text{OR}_{46} \\
\text{N} & \left( \text{R}_9 \right) \text{R}_{10}
\end{align*}
\]

wherein R\(_9\) and R\(_{10}\) are as defined above, Q\(_2\) represents O, S or N, and R\(_{48}\)
represents a lower alkyl or an aryl, Q\(_2\) represents O, S or N.

A “phosphoramidite” can be represented in the general formula:

\[
\begin{align*}
\text{N} & \left( \text{R}_9 \right) \text{R}_{10} \\
\text{Q}_2 & \overset{\text{P}}{\text{O}} \quad \text{OR}_{46} \\
\text{OR}_{46} & \quad \text{OR}_{46}
\end{align*}
\]

wherein R\(_9\) and R\(_{10}\) are as defined above, and Q\(_2\) represents O, S or N.

A "phosphoryl" can in general be represented by the formula:

\[
\begin{align*}
\text{OR}_{46} & \\
\text{Q}_1 & \overset{\text{P}}{\text{O}}
\end{align*}
\]

wherein Q\(_1\) represented S or O, and R\(_{46}\) represents hydrogen, a lower alkyl or an
aryl. When used to substitute, for example, an alkyl, the phosphoryl group of the phosphorylalkyl can be represented by the general formula:

\[
\begin{align*}
\text{OR}_{46} \, \text{P} \equiv \text{O} & \quad \text{OR}_{46} \\
\end{align*}
\]

\[
\begin{align*}
\text{OR}_{46} \, \text{P} \equiv \text{O} & \quad \text{OR}_{46} \\
\end{align*}
\]

wherein \( Q_1 \) represented S or O, and each \( R_{46} \) independently represents hydrogen, a lower alkyl or an aryl, \( Q_2 \) represents O, S or N. When \( Q_1 \) is an S, the phosphoryl moiety is a “phosphorothioate”.

The terms "polycyclol" or "polycyclic group" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclols) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulphydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclol, an aromatic or heteroaromatic moiety, -CF<sub>3</sub>, -CN, or the like.

The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. Protective Groups in Organic Synthesis, 2nd ed.; Wiley: New York, 1991).

A "selenoalkyl" refers to an alkyl group having a substituted seleno group attached thereto. Exemplary "selenoethers" which may be substituted on the alkyl are selected from one of -Se-alkyl, -Se-alkenyl, -Se-alkynyl, and -Se-(CH<sub>2</sub>)<sub>2</sub>-R<sub>8</sub>, and R<sub>8</sub> being defined above.

As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate
organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

The term "sulfamoyl" is art-recognized and includes a moiety that can be represented by the general formula:

\[
\begin{array}{c}
\text{O} \\
\text{S} \quad \text{N} \\
\text{O} \\
\text{R}_{10} \\
\text{R}_{9}
\end{array}
\]

in which \(R_9\) and \(R_{10}\) are as defined above.

The term "sulfate" is art recognized and includes a moiety that can be represented by the general formula:

\[
\begin{array}{c}
\text{O} \\
\text{S} \\
\text{O} \\
\text{OR}_{41}
\end{array}
\]

in which \(R_{41}\) is as defined above.

The term "sulfonamido" is art recognized and includes a moiety that can be represented by the general formula:

\[
\begin{array}{c}
\text{O} \\
\text{N} \quad \text{S} \\
\text{O} \\
\text{R}_{9} \quad \text{R'_{11}}
\end{array}
\]

in which \(R_9\) and \(R'_{11}\) are as defined above.

The term "sulfonate" is art-recognized and includes a moiety that can be represented by the general formula:
in which $R_{41}$ is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

The terms "sulfoxido" or "sulfinyl", as used herein, refers to a moiety that can be represented by the general formula:

$$\text{S} - R_{44}$$

in which $R_{44}$ is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclic, aralkyl, or aryl.

Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminoaalkenyls, aminoaalkynyls, aminoaalkenyals, aminoaalkynyls, iminoaalkenyls, iminoaalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

As used herein, the definition of each expression, e.g., alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

The terms triflyl, tosyl, mesyl, and nonafllyl are art-recognized and refer to trifluoromethanesulfonyl, $p$-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, $p$-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

The abbreviations Me, Et, Ph, Tf, Nf, Ts, Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, $p$-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry*; this list is typically presented in a table entitled *Standard List of Abbreviations*. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-isomers, $R$- and $S$-enantiomers, diastereomers, (D)-isomers,
(1S)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts may be formed with an appropriate optically active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., the ability to inhibit hedgehog signaling), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover. Also for purposes of this invention, the term "hydrocarbon" is contemplated to include all permissible compounds having at least one hydrogen and one carbon atom. In a broad aspect, the permissible hydrocarbons include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic organic compounds which can be substituted or unsubstituted.

II. Compounds and Preparations Thereof

The present invention makes available a novel method for inhibiting cell growth by selectively inhibiting the activity of prenyltransferases. The compounds
presented below, useful in the subject methods, have been divided into three sections. Variables, such as \( W, X, \) and \( R_1 \), may have different definitions and preferred substituents in different sections.

1) In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula I:

\[
\text{R}_1^2N \bigg\{ \begin{array}{c} M \end{array} \bigg\}_{q} \text{R}_3 \quad \text{R}_1^2S \quad \text{R}_4 \quad \text{W} \quad \text{XR}_3 \\
\text{W} \quad \text{R}_4 \quad \text{R}_5 \quad \text{R}_6 \quad \text{R}_7 \quad \text{R}_8
\]

wherein

- \( W \) represents \(-\text{C}(=\text{Y})_{2}, -\text{S}(\text{O})_{2}, \text{or} -\text{S}(\text{O})_{2}, \) preferably \(-\text{C}(=\text{Y})_{2};\)
- \( Y \) is \( \text{O} \) or \( \text{S} \), preferably \( \text{O}; \)
- \( \text{R}_1 \) represents \( \text{H} \) or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
- \( \text{R}_2 \), independently for each occurrence, represents \( \text{H} \) or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
- \( X \) represents \( \text{O} \), \( \text{S} \), or \( \text{NR}_3 \), preferably \( \text{NR}_3; \)
- \( \text{R}_3 \), independently for each occurrence, represents \( \text{H} \), substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., \(-\text{(CH}_2\text{)}_{n}\text{cycloalkyl} \) (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., \(-\text{(CH}_2\text{)}_{n}\text{heterocyclyl} \) (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., \(-\text{(CH}_2\text{)}_{n}\text{aryl} \) (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., \(-\text{(CH}_2\text{)}_{n}\text{heteroaryl} \) (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two \( \text{R}_3 \) taken together may form a 4- to 8-membered ring, e.g., with \( \text{N} \), which ring may include one or more carbonyls and/or heteroatoms;
- \( \text{R}_4 \) represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from \( \text{H} \), or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, \( \text{N(R}_8\text{)}_{2}, \text{OR}_8, \text{SR}_8, \text{C}(=\text{O})\text{R}_8, \text{COOR}_8, \text{CON(R}_8\text{)}_{2}, \) or an amino acid residue;
- \( \text{R}_8 \), independently for each occurrence, represents \( \text{H} \) or substituted or
unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as \(-\text{CH}_2\)-, \(-\text{CHF}\)-, \(-\text{CHOH}\)-, \(-\text{CH(Me)}\)-, \(-\text{C(=O)}\)-, etc., a heteroatom selected from O, S, or NR', a subunit selected from \(-\text{C(=Y)}\)-, \(-\text{S(O)}\)-, or \(-\text{S(O)}_2\)-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R_3 nor R_4 includes a linkage to a phenethylazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula II:

\[
\begin{array}{c}
\text{Q} \quad \text{M} \\
\text{W} \quad \text{NR}_3 \\
\end{array}
\]

wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

Ar represents an aryl or heteroaryl ring, e.g., a substituted or unsubstituted phenyl ring;

W represents \(-\text{C(=Y)}\)-, \(-\text{S(O)}\)-, or \(-\text{S(O)}_2\)-, preferably \(-\text{C(=Y)}\)-;

Y is O or S, preferably O;

R_3, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., \(-\text{(CH}_2\)_n}\)cycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., \(-\text{(CH}_2\)_n}\)heterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., \(-\text{(CH}_2\)_n}\)aryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., \(-\text{(CH}_2\)_n}\)heteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R_3 taken
together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkyne, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)₂-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)₂-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents imidazolyl, Q may be attached to M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzyl, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments wherein Q is imidazolyl, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.

In certain embodiments, at least one occurrence of R₃ is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments of Formula I, both occurrences of R₃ are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, R₃ may represent a benzyl group substituted with one or more halogens. In certain embodiments of Formula I, both occurrences of R₃ are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula
II, R₃ represents a substituent other than H, and in certain embodiments, the two substituents of the nitrogen to which R₃ is attached are identical, e.g., both benzyl, m-chlorobenzyl, etc.

In certain embodiments, an occurrence of M directly bound to nitrogen represents –C(=Y)–, CH₂, –S(O)–, or –S(O)₂–, e.g., –C(=O)–, while in other embodiments, the occurrence of M bound to N represents CH₂, or an alkyl-substituted methylene group. In certain embodiments, occurrences of M not bound to nitrogen represent CH₂ or an alkyl-substituted methylene group.

In certain embodiments, R₄ is absent. In certain embodiments, R₄ represents one substituted or unsubstituted alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group, e.g., located adjacent to the nitrogen bound to M. In certain embodiments of Formula I where R₄ is present at this position, the composition is substantially pure or enriched in the diastereomer wherein the stereocenter where R₄ is attached has the S designation. In certain embodiments, R₁ and R₂ are absent for all occurrences.

In certain embodiments, a subject compound is substantially pure or enriched in one or more diastereomers of the above-described compounds. In certain embodiments of Formula I, the compound is substantially pure or enriched in a diastereomer wherein the stereocenter where M is attached to the nitrogen- and sulfur-bearing substituent is R.

In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula III:

```
\[ \text{III} \]
```

wherein

Y is O or S, preferably O;

R₁ represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

R₂, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y) -, -S(O) -, or -S(O)₂ -, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3, preferably from 1-2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula IV:

![Diagram](IV)
Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

Ar represents an aryl or heteroaryl ring, e.g., a substituted or unsubstituted phenyl ring;

Y is O or S, preferably O;

X represents O, S, or NR₃, preferably NR₃;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)₂-, or -SO₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3, preferably from 1-2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.
In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents imidazolyl, Q may be attached to M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzyl, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.

In certain embodiments, at least one occurrence of R₃ is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments of Formula III, both occurrences of R₃ are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, R₃ may represent a benzyl group substituted with one or more halogens. In certain embodiments of Formula III, both occurrences of R₃ are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula IV, R₃ represents a substituent other than H, and in certain embodiments, the two substituents of the nitrogen to which R₃ is attached are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula IV, Ar represents a substituted or unsubstituted phenyl ring, e.g., phenyl, m-chlorophenyl, etc.

In certain embodiments, an occurrence of M directly bound to nitrogen represents -C(=Y)-, CH₂, -S(O) -, or -S(O)₂-, e.g., -C(=O)-, while in other embodiments, the occurrence of M bound to N represents CH₂, or an alkyl-substituted methylene group. In certain embodiments, occurrences of M not bound to nitrogen represent CH₂ or an alkyl-substituted methylene group.

In certain embodiments, R₄ is absent. In certain embodiments, R₄ represents one substituted or unsubstituted alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group, e.g., located adjacent to the nitrogen bound to M. In certain embodiments of Formula III where R₄ is present at this position, the composition is substantially pure or enriched in the diastereomer wherein the stereocenter where R₄ is attached has the S designation. In certain embodiments, R₁ and R₂ are absent for all occurrences.

In certain embodiments, a subject compound is substantially pure or enriched in one or more diastereomers of the above-described compounds. In certain embodiments of Formula III, the compound is substantially pure or enriched in a diastereomer wherein the stereocenter where M is attached to the nitrogen- and sulfur-bearing substituent is R.
In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula \( V \):

\[
\begin{align*}
\text{Wherein:} \\
W & \text{ represents } -C(=Y)\, -S(O)\, -S(O)\, \text{ or } -S(O)\, \text{ preferably } -C(=Y); \\
X & \text{ represents } O, S, \text{ or } NR_3, \text{ preferably } NR_3; \\
Y & \text{ is } O \text{ or } S, \text{ preferably } O; \\
Z & \text{ is } H \text{ or } OH; \\
R_1 & \text{ represents } H \text{ or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl}; \\
R_2, \text{ independently for each occurrence, represents } H \text{ or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl}; \\
X & \text{ represents } O, S, \text{ or } NR_3, \text{ preferably } NR_3; \\
R_3, \text{ independently for each occurrence, represents } H, \text{ substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., } -\text{CH}_2\text{h-cycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., } -\text{CH}_2\text{h-heterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., } -\text{CH}_2\text{h-aryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., } -\text{CH}_2\text{h-heteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two } R_3 \text{ taken together may form a 4- to 8-membered ring, e.g., with } N, \text{ which ring may include one or more carbonyls and/or heteroatoms}; \\
R_4 & \text{ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from } H, \text{ or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, } N(R_8)\, OR_8, \text{ SR_8, } C(=O)R_8, \text{ COOR_8, CON(R_8)\, or an amino acid residue}; \\
R_8, \text{ independently for each occurrence, represents } H \text{ or substituted or }
\end{align*}
\]
unsubstituted alkyl, alkenyl, alkyne, cycloalkyl, heterocyclyl, heterocyclalkyl,
aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3;

x and y represent, independently, 0, 1, or 2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula VI:

\[
\begin{align*}
Q & \quad \left( \begin{array}{c}
M \\
q
\end{array} \right) \\
& \quad \left( \begin{array}{c}
Z \\
x \\
R₄
\end{array} \right) \\
& \quad \left( \begin{array}{c}
W \\
NR₃
\end{array} \right)
\end{align*}
\]

wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure, such as a pyridyl or imidazoyl ring;

Ar represents an aryl or heteroaryl ring, e.g., a substituted or unsubstituted phenyl ring;

W represents -C(=Y)-, -S(O)-, or -S(O)₂-, preferably -C(=Y)-;

Y is O or S, preferably O;

Z is H or OH;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl,
aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3;

x and y represent, independently, 0, 1, or 2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents imidazolyl, Q may be attached to M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzyl, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments wherein Q is imidazolyl, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.
In certain embodiments, at least one occurrence of \( R_3 \) is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments of Formula V wherein \( X \) is \( NR_3 \), both occurrences of \( R_3 \) are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, \( R_3 \) may represent a benzyl group substituted with one or more halogens. In certain embodiments of Formula V wherein \( X \) is \( NR_3 \), both occurrences of \( R_3 \) are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula VI, \( R_3 \) represents a substituent other than \( H \), and in certain embodiments, the two substituents of the nitrogen to which \( R_3 \) is attached are identical, e.g., both benzyl, m-chlorobenzyl, etc.

In certain embodiments, \( R_4 \) is absent. In certain embodiments, \( R_4 \) represents one substituted or unsubstituted alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group, e.g., located adjacent to the carbon bound to \( M \).

In certain embodiments, the sum of \( x \) and \( y \) is two, e.g., \( x \) is 2 and \( y \) is 0, or each of \( x \) and \( y \) is one. When \( Z \) is \( OH \), the occurrence of \( M \) bound to the carbon bearing \( Z \) preferably represents a substituted or unsubstituted methylene group.

In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula VII:

\[
\begin{align*}
R_2 & \quad NR_3 \\
R_4 & \quad X
\end{align*}
\]

(VII)

wherein:

- \( X \) represents \( O, S, \) or \( NR_3 \), preferably \( NR_3 \);
- \( Y \) is \( O \) or \( S \), preferably \( O \);
- \( Z \) represents \( H \) or \( OH \);
- \( R_1 \) represents \( H \) or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
- \( R_2 \), independently for each occurrence, represents \( H \) or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ is selected from H, substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me) -, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)₂-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3, preferably from 1-2;

x and y represent, independently, 0, 1, or 2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula IX:

![Diagram](attachment:image.png)
wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

Ar represents an aryl or heteroaryl ring, e.g., a substituted or unsubstituted phenyl ring;

Y is O or S, preferably O;

Z represents H or OH;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclylalkyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatom(s);

R₄ is selected from H, substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3, preferably from 1-2;

x and y represent, independently, 0, 1, or 2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.
In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents imidazolyl, Q may be attached to M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzyl, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.

In certain embodiments, at least one occurrence of R₃ is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments of Formula VII wherein X is NR₃, both occurrences of R₃ are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, R₃ may represent a benzyl group substituted with one or more halogens. In certain embodiments of Formula VII wherein X is NR₃, both occurrences of R₃ are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula IX, R₃ represents a substituent other than H, and in certain embodiments, the two substituents of the nitrogen to which R₃ is attached are identical, e.g., both benzyl, m-chlorobenzyl, etc.

In certain embodiments, R₄ is absent. In certain embodiments, R₄ is a substituted or unsubstituted alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group. In certain embodiments, R₁ and R₂ are absent for all occurrences.

In certain embodiments, the sum of x and y is two, e.g., x is 2 and y is 0, or each of x and y is one. When Z is OH, the occurrence of M bound to the carbon bearing Z preferably represents a substituted or unsubstituted methylene group.

In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula X:

\[
\begin{array}{c}
\text{Q} \\
\text{V} \\
\text{Ar} \\
\text{Z} \\
\text{M} \\
\text{x} \\
\text{y} \\
\text{W} \\
\text{XR₃}
\end{array}
\]

wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at
least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

Ar, independently for each occurrence, represents an aryl or heteroaryl ring, e.g., a substituted or unsubstituted phenyl ring;

V is H or OH;

W represents \(-\text{C}(=\text{Y})\), \(-\text{S(O)}\), or \(-\text{S(O)}_2\), preferably \(-\text{C}(=\text{Y})\);

X represents O, S, or NR₃, preferably NR₃;

Y is O or S, preferably O;

Z represents H or OH;

R₁ represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

R₂, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., \(\text{-(CH}_2\text{)}\)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., \(\text{-(CH}_2\text{)}\)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., \(\text{-(CH}_2\text{)}\)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., \(\text{-(CH}_2\text{)}\)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as \(-\text{CH}_2\), \(-\text{CHF}\), \(-\text{CHOH}\), \(-\text{CH(Me)}\), \(-\text{C}(=\text{O})\), etc., a heteroatom selected from O, S, or NR₈, a subunit selected from \(-\text{C}(=\text{Y})\), \(-\text{S(O)}\), or \(-\text{S(O)}_2\), or two M taken together represent substituted or unsubstituted
ethene or ethyne;

q represents an integer from 0 to 3, preferably from 1-2;

x and y represent, independently, 0, 1, or 2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula XI:

\[ \text{(XI)} \]

wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

Ar represents an aryl or heteroaryl ring, e.g., a substituted or unsubstituted phenyl ring;

V is H or OH;

Y is O or S, preferably O;

Z represents H or OH;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to
which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkylnyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteratom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3, preferably from 1-2;

x and y represent, independently, 0, 1, or 2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents imidazolyl, Q may be attached to the carbon bearing M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzy1, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.

In certain embodiments, Ar bound to the carbon bearing Q represents a substituted or unsubstituted aryl ring, such as a benzene ring. In certain embodiments, Ar bound to the carbon bearing Q includes at least two aryl rings, e.g., fused (such as naphthyl), linked (such as biphenyl), or tethered (such as a diphenyl ether or diphenyl amine, etc.).

In certain embodiments, at least one occurrence of R₃ is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments of Formula X
wherein X represents NR₃, both occurrences of R₃ are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, R₃ may represent a benzyl group substituted with one or more halogens. In certain embodiments of Formula X wherein X represents NR₃, both occurrences of R₃ are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula XI, R₃ represents a substituent other than H, and in certain embodiments, the two substituents of the nitrogen to which R₃ is attached are identical, e.g., both benzyl, m-chlorobenzyl, etc. In certain embodiments of Formula XI, Ar represents a substituted or unsubstituted phenyl ring, e.g., phenyl, m-chlorophenyl, etc.

In certain embodiments, the sum of x and y is two, e.g., x is 2 and y is 0, or each of x and y is one. When Z is OH, the occurrence of M bound to the carbon bearing Z preferably represents a substituted or unsubstituted methylene group. When V is OH, the occurrence of M bound to the carbon bearing V preferably represents a substituted or unsubstituted methylene group.

In certain embodiments, R₄ is absent. In certain embodiments, R₄ represents one substituted or unsubstituted alkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl group, e.g., located adjacent to the carbon bound to M.

3) In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula XII:

\[
\begin{align*}
&\text{wherein} \\
&W \text{ represents } -\text{C}(-\text{Y})-, -\text{S(O)}-, \text{ or } -\text{S(O)}_2-, \text{ preferably } -\text{C}(-\text{Y})--; \\
&Y \text{ is O or S, preferably O;} \\
&R_1 \text{ represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;} \\
&R_2, \text{ independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;} \\
&X \text{ represents O, S, or NR}_3, \text{ preferably } NR_3;
\end{align*}
\]
R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y)-, -S(O)-, or -S(O)₂-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethyiazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula XIII:

![Diagram](XIII)

wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at
least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

W represents –C(=Y)–, -S(O)–, or –S(O)₂–, preferably –C(=Y)–;

Y is O or S, preferably O;

X represents O, S, or NR₃, preferably NR₃;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroarylalkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroarylalkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroarylalkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂–, -CHF–, -CHOH–, -CH(Me)–, -C(=O)–, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from –C(=Y)–, -S(O)–, or –S(O)₂–, or two M taken together represent substituted or unsubstituted ethene or ethyne;

q represents an integer from 0 to 3; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably neither R₃ nor R₄ includes a linkage to a phenethylazaryl moiety.

In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents
imidazolyl, Q may be attached to M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzyl, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments wherein Q is imidazolyl, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.

In certain embodiments, at least one occurrence of R₃ is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments, both occurrences of R₃ are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, R₃ may represent a benzyl group substituted with one or more halogens. In certain embodiments, both occurrences of R₃ are identical, e.g., both benzyl, m-chlorobenzyl, etc.

In certain embodiments, an occurrence of M directly bound to the pyrrolidine ring represents NR₈, e.g., NMe, NBn, or NH. In certain embodiments, M₄ includes an amide, urea, carbamate, or amine linkage, e.g., -CH₂NR₈- or -C(=O)NR₈, where R₈ represents, for example, H or substituted or unsubstituted alkyl or aralkyl, e.g., H, Me, Bn, etc.

In certain embodiments, R₄ is absent. In certain embodiments, R₁ and R₂ are absent for all occurrences.

In certain embodiments, a subject compound is substantially pure or enriched in one or more diastereomers of the above-described compounds. In certain embodiments of Formula XII, the compound is substantially pure or enriched in a diastereomer wherein the stereocenter where M is attached to the ring has the designation R. In certain embodiments of Formula XII, the compound is substantially pure or enriched in a diastereomer wherein the stereocenter where M is attached to the nitrogen- and sulfur-bearing substituent is R. In certain embodiments, both of these stereocenters are of the R designation.

In certain embodiments of Formula XIII, the compound is substantially pure or enriched in an enantiomer or diastereomer wherein the stereocenter where M is attached to the ring has the designation S.

In certain embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula XIV:
wherein

Y is O or S, preferably O;

R₁ represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

R₂, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

X represents O, S, or NR₃, preferably NR₃;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH₂)ₙcycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH₂)ₙheterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH₂)ₙaryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH₂)ₙheteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two R₃ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH₂-, -CHF-, -CHOH-, -CH(Me) -, -C(=O) -, etc., a heteroatom selected from O, S, or NR₈, a subunit selected from -C(=Y) -, -S(O) -, or -S(O)₃ -, or two M taken together represent substituted or unsubstituted ethene or ethyne;

r represents an integer from 0 to 2, preferably from 1-2; and

n, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,
wherein preferably $R_3$ does not include a linkage to a phenethylazaryl moiety.

In certain other embodiments, the subject method can be practiced using an inhibitor of a prenyltransferase represented by the general formula XV:

$$
\begin{align*}
Q & \quad (M)_{r} \quad N \quad (Y) \quad \text{Ni}(R_3)_{b} \\
\end{align*}
$$

wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure, such as a pyridyl or imidazolyl ring;

Y is O or S, preferably O;

X represents O, S, or NR$_3$, preferably NR$_3$;

$R_3$, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkylnyl, cycloalkyl, cycloalkylalkyl, e.g., -(CH$_2$)$_n$-cycloalkyl (e.g., substituted or unsubstituted), heterocyclyl, heterocyclylalkyl, e.g., -(CH$_2$)$_n$-heterocyclyl (e.g., substituted or unsubstituted), aryl, aralkyl, e.g., -(CH$_2$)$_n$-aryl (e.g., substituted or unsubstituted), heteroaryl, heteroaralkyl, e.g., -(CH$_2$)$_n$-heteroaryl (e.g., substituted or unsubstituted), or a natural or unnatural amino acid residue (e.g., an alpha-amino acid residue), or two $R_3$ taken together may form a 4- to 8-membered ring, e.g., with N, which ring may include one or more carbonyls and/or heteroatoms;

$R_8$, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkylnyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, such as -CH$_2$-, -CHF-, -CHOH-, -CH(Me)-, -C(=O)-, etc., a heteroatom selected from O, S, or NR$_8$, a subunit selected from -C(=Y)-, -S(O)$_2$-, or -S(O)$_2$-, or two M taken together represent substituted or unsubstituted ethene or ethyne;

$r$ represents an integer from 0 to 2, preferably from 1-2; and

$n$, individually for each occurrence, represents an integer from 0 to 10, preferably from 0 to 5,

wherein preferably $R_3$ does not include a linkage to a phenethylazaryl moiety.
In certain embodiments, Q represents substituted or unsubstituted imidazolyl, oxazolyl, pyrrolyl, pyridyl, or thiazolyl. In embodiments wherein Q represents imidazolyl, Q may be attached to M at nitrogen, or may include an alkyl or aralkyl substituent on nitrogen, e.g., methyl, benzyl, etc. Preferably, Q represents pyridyl, imidazolyl, or N-methylimidazolyl, and may, in certain embodiments, be attached to the subject inhibitor at the 5-position of the imidazole ring. In embodiments wherein Q represents pyridyl, Q may be attached at the meta-position or the para-position, for example.

In certain embodiments, at least one occurrence of R₃ is an aralkyl group, e.g., a substituted or unsubstituted benzyl group. In certain embodiments, both occurrences of R₃ are aralkyl, e.g., substituted or unsubstituted benzyl, groups. In certain embodiments, R₃ may represent a benzyl group substituted with one or more halogens. In certain embodiments, both occurrences of R₃ are identical, e.g., both benzyl, both m-chlorobenzyl, etc.

In certain embodiments, an occurrence of M directly bound to nitrogen represents –C(=Y)–, -CH₂-, -S(O)-, or –S(O)₂-. In certain embodiments, M₉-NR₈ includes an amide, urea, carbamate, or amine linkage, e.g., –CH₂NR₈– or –C(=O)NR₈, where R₈ represents, for example, H or substituted or unsubstituted alkyl or aralkyl, e.g., H, Me, Bn, etc.

In certain embodiments, a subject compound is substantially pure or enriched in one or more diastereomers of the above-described compounds. In certain embodiments of Formula XIV, the compound is substantially pure or enriched in a diastereomer wherein the amine-bearing stereocenter of the pyrroline ring has the designation R. In certain embodiments of Formula XIV, the compound is substantially pure or enriched in a diastereomer wherein the stereocenter where M is attached to the nitrogen- and sulfur-bearing substituent is R. In certain embodiments, both of these stereocenters are of the R designation.

In certain embodiments of Formula XV, the compound is substantially pure or enriched in an enantiomer or diastereomer wherein the amine-bearing stereocenter of the pyrroline ring has the designation S.

In certain embodiments of all the structures above, substituents that include an aryl or heteroaryl moiety do not include a second aryl or heteroaryl moiety, e.g.,
such substituents are unsubstituted or substituted with one or more of a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxy carbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkyl, an alkenyl, an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, or a heterocyclyl.

**Permease Tags**

In certain embodiments, the ability of fungal cells to transport ectopically added compounds, particularly inhibitors of the present invention, can be enhanced by conjugation of the compound with an amino acid residue or oligopeptide (preferably a dipeptide or tripeptide) which is itself taken up by the cell in a permease-mediated transport mechanism. Thus, another aspect of the invention features prenyltransferase inhibitors which include a “permease tag”, e.g., which comprises an amino acid residue, dipeptide or tripeptide which facilitates permease-mediated transport of the inhibitor into the fungal pathogen. Such compounds can have desirable pharmacokinetic properties due to, for example, increased bioavailability and/or increased selectivity. With regard to the latter, in preferred embodiments, the permease tag does not increase the cellular uptake of the inhibitor by mammalian cells to any greater degree than it does for cellular uptake by the fungal pathogen, though in the most preferred embodiments, the permease tag increases the uptake by fungal cells to a greater degree than for uptake by mammalian cells.

In another embodiments, the permease tag is removed from the inhibitor as a result of its permease-mediated transport into the fungal pathogen.

In other embodiments the amino acid or oligopeptide of the permease tag includes a free N-terminal amine, or a group hydrolyzable thereto under the conditions that the pathogen is contacted with the inhibitor.

As demonstrated in the appended examples, in one embodiment the permease tag facilitates permease-mediated transport by an alanine transporter of the fungal pathogen. For example, the inhibitor is derivatized at a free amine with L-alanine, or a dipeptide or tripeptide including L-alanine. In preferred embodiments, the L-alanine moiety is attached to the prenyltransferase inhibitor through an amide linkage through either an amine or carboxyl group of the inhibitor, and provides the
complementary functionality in the permease tag. For instance, the L-alanine containing permease tag is provided by derivatization of a free amine on the inhibitor with a carboxyl group on an L-alanine containing oligopeptide, with the oligopeptide providing a free amine (or a group which is hydrolyzable thereto).

Other Candida permeases are known in the art, and appropriate permease tags can be generated for facilitating uptake of the subject inhibitors by other permease-mediated mechanisms. For instance, the permease tag can be selected to increase uptake of the inhibitor by any one of the following Candida permeases:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Permease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mukherjee et al. (1998) Yeast 14:335-45</td>
<td>Arginine permease</td>
</tr>
<tr>
<td>Matijekova et al. (1997) FEBS Lett 408: 89-93</td>
<td>Candida albicans CAN1 gene, encoding a high-affinity permease for arginine, lysine and histidine</td>
</tr>
<tr>
<td>Jethwaney et al. (1997) Microbiology 143:397</td>
<td>Proline permease</td>
</tr>
<tr>
<td>Grobler et al. (1995) Yeast 11:1485</td>
<td>mae1 gene, permease for malate and other C4 dicarboxylic acids</td>
</tr>
</tbody>
</table>

Moreover, many more permeases have been identified in S. cerevisiae through various genomic projects. Applicants contemplate that the subject permease tags can be selected to increase permease-mediated uptake by a mechanism relying on a Candida homolog of any one of the following S. cerevisiae permeases:

<table>
<thead>
<tr>
<th>Cerevisae gene</th>
<th>Transporter activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGP1</td>
<td>asparagine and glutamine permease</td>
</tr>
<tr>
<td>DIP5</td>
<td>dicarboxylic amino acid permease</td>
</tr>
<tr>
<td>MUP1</td>
<td>high affinity methionine permease</td>
</tr>
<tr>
<td>TAT2</td>
<td>high affinity tryptophan transport protein</td>
</tr>
<tr>
<td>GNP1</td>
<td>high-affinity glutamine permease</td>
</tr>
<tr>
<td>ALP1</td>
<td>high-affinity permease for basic amino acids</td>
</tr>
<tr>
<td>HIP1</td>
<td>histidine permease</td>
</tr>
<tr>
<td>STP4</td>
<td>involved in pre-tRNA splicing and in uptake of branched-chain amino acids</td>
</tr>
<tr>
<td>BAP2</td>
<td>leucine permease, high-affinity (S1)</td>
</tr>
<tr>
<td>LYP1</td>
<td>lysine-specific high-affinity permease</td>
</tr>
</tbody>
</table>
ARG11 | member of the mitochondrial carrier family (MCF)
---|---
PUT4 | proline and gamma-aminobutyrate permease
BAP3 | valine transporter

**Pharmaceutical Compositions**

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically effective amount of one or more compounds of the subject invention, such as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents for use in the treatment of fungal infections. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally or intravectally, for example, as a pessary, cream or foam. In certain embodiments, the pharmaceutical preparations may be non-pyrogenic, i.e., do not elevate the body temperature of a patient.

The phrase "therapeutically effective amount" as used herein means that amount of a compound, material, or composition comprising an inhibitor of the subject invention which is effective for producing some desired therapeutic effect. Such therapeutic effect may result from, for example, inhibition of aberrant hyperproliferation of a cell resulting from transformation of a Ras-related gene, or alternatively, by inhibiting fungal cell wall biosynthesis.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject compounds from one organ, or portion of the body, to
another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycérin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain embodiments of the present subject compounds may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of such inhibitors of prenyltransferases. These salts can be prepared in situ during the final isolation and purification of the compounds of the present invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19)

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of an inhibitor of prenyltransferases. These salts can likewise be prepared in situ during the final isolation and purification of the compounds of the present invention, or by separately reacting the purified compound
in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., supra)

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of inhibitor which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association an inhibitor of the
present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. An inhibitor of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered inhibitor moistened with an inert liquid diluent.
The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulations so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active inhibitor(s) of the present invention, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metaphosphate, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared
by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active inhibitor.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active prenyltransferase inhibitor, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the inhibitor of the present invention in the proper medium. Absorption enhancers can also be used to increase the flux of the drug across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound of the present invention in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more inhibitors of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous
solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and other antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the therapeutic effect of an inhibitor, it is desirable to slow the absorption of the inhibitor from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the inhibitor then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered inhibitor form is accomplished by dissolving or suspending the inhibitor in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject inhibitors in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.
When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

Regardless of the route of administration selected, the prenyltransferase inhibitors useful in the subject method may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response, e.g., antifungal or anticancer activity, for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular prenyltransferase inhibitor employed, or the ester, salt or
amid thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular inhibitor employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a potent prenyltransferase inhibitor, e.g., having an EC50 in the range of 1 mM to sub-nanomolar, will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated antifungal effects, will range from about 0.0001 to about 1000mg per kilogram of body weight per day, though preferably 0.5 to 300mg per kilogram.

If desired, the effective daily dose of the active inhibitor may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

In a preferred embodiment, the inhibitor agent is formulated for oral administration, as for example in the form of a solid tablet, pill, capsule, caplet or the like (collectively hereinafter "tablet") or an aqueous solution or suspension. The inhibitor agent of the present invention may be, for example, an anticancer agent or an antifungal agent. In a preferred embodiment of the tablet form of the inhibitor agent, the tablets are preferably formulated such that the amount of inhibitor agent (or inhibitor agents) provided in 20 tablets, if taken together, would provide a dose of at least the median effective dose (ED50), e.g., the dose at which at least 50% of individuals exhibited a therapeutic affect. For example, for an antifungal agent, the therapeutic effect would be a quantal effect of inhibition of fungal cell growth or protection (e.g., a statistically significant reduction in infection). More preferably, the tablets are formulated such that the total amount of inhibitor agent (or inhibitor agents) provided in 10, 5, 2 or 1 tablets would provide at least an ED50 dose to a
patient (human or non-human mammal). In other embodiments, the amount of inhibitor agent (or inhibitor agents) provided in 20, 10, 5 or 2 tablets taken in a 24 hour time period would provide a dosage regimen providing, on average, a mean plasma level of the inhibitor agent(s) of at least the ED50 concentration (the concentration for 50% of maximal effect of, e.g., inhibiting fungal cell growth), though preferably less than 100 times the ED50, and even more preferably less than 10 or 5 times the ED50. In preferred embodiments, a single dose of tablets (1-20 tablets) provides about .25 mg to 1250 mg of an inhibitor agent(s).

Likewise, the inhibitor agents can be formulated for parenteral administration, as for example, for subcutaneous, intramuscular or intravenous injection, e.g., the inhibitor agent can be provided in a sterile solution or suspension (collectively hereinafter "injectable solution"). The injectable solution is preferably formulated such that the amount of antifungal agent (or antifungal agents) provided in a 200 cc bolus injection would provide a dose of at least the median effective dose, though preferably less than 100 times the ED50, and even more preferably less than 10 or 5 times the ED50. More preferably, the injectable solution is formulated such that the total amount of antifungal agent (or antifungal agents) provided in 100, 50, 25, 10, 5, 2.5, or 1 cc injections would provide an ED50 dose to a patient, and preferably less than 100 times the ED50, and even more preferably less than 10 or 5 times the ED50. In other embodiments, the amount of inhibitor agent (or inhibitor agents) provided in a total volume of 100cc, 50, 25, 5 or 2cc to be injected at least twice in a 24 hour time period would provide a dosage regimen providing, on average, a mean plasma level of the inhibitor agent(s) of at least the ED50 concentration, though preferably less than 100 times the ED50, and even more preferably less than 10 or 5 times the ED50. In preferred embodiments, a single dose injection provides about .25 mg to 1250 mg of inhibitor agent.

For continuous intravenous infusion, e.g., drip or push, the inhibitor agent may be provided in a sterile dilute solution or suspension (collectively hereinafter "i.v. injectable solution"). The i.v. injectable solution is preferably formulated such that the amount of inhibitor agent (or inhibitor agents) provided in a 1L solution would provide a dose, if administered over 15 minutes or less, of at least the median effective dose, though preferably less than 100 times the ED50, and even more preferably less than 10 or 5 times the ED50. More preferably, the i.v. injectable solution is formulated such that the total amount of inhibitor agent (or inhibitor agents) provided in 1L solution administered over 60, 90, 120 or 240 minutes would provide an ED50 dose to a patient, though preferably less than 100 times the ED50,
and even more preferably less than 10 or 5 times the ED50. In preferred embodiments, a single i.v. "bag" provides about .25 mg to 5000 mg of inhibitor agent per liter i.v. solution, more preferably .25 mg to 2500 mg, and even more preferably .25 mg to 1250 mg. As already indicated, an inhibitor agent may be, for example, an antifungal agent or an anticancer agent.

As discussed above, the preferred antifungal agent pharmaceutical preparation, whether for injection or oral delivery (or other route of administration), would provide a dose less than the ED50 for modulation of FPTase, GGPTase, and/or other prenyltransferase activity in the host, more preferably at least 1 order of magnitude less, and more preferably at least 2, 3 or 4 orders magnitude less. In a similar fashion, the preferred anticancer agent pharmaceutical preparation, whether for injection or oral delivery (or other route of administration), would provide a dose less than the ED50 for modulation of any patient’s prenyltransferase other than the prenyltransferase corresponding to any oncogene that is responsible for any aberrant hyperproliferation, cancer or the like, more preferably at least 1 order of magnitude less, more preferably at least 2, 3 or 4 orders magnitude less.

An ED50 dose, for a human, is based on a body weight of from 10 lbs to 250 lbs, though more preferably for an adult in the range of 100 to 250 lbs.

Potential inhibitors may be assessed for ED50 values for any inhibition, including for example anticancer or antifungal activity, using any of a number of well known techniques in the art.

III. Identifying Candidate Inhibitor Agents

There are a variety of assay formats for testing compounds of the present invention for appropriate inhibition of prenyltransferase activity. For agents to be used for treating cancer and other aberrant hyperproliferative conditions or diseases, the inhibitors selected for use in the subject method will be orders of magnitude better inhibitors of a prenyltransferase that prenylates the protein product of an oncogene, e.g., a particular Ras protein, as compared to other prenyltransferases that prenylate protein products expressed from nonmutated genes of the patient. For use as antifungal agents, the inhibitors that may be selected for use in the subject method may be better inhibitors, on the order of magnitudes, of a fungal GGPTase or other prenyltransferase than a mammalian GGPTase or other prenyltransferase, and/or have greater membrane permeance through a fungal cell wall than a mammalian cell membrane.
In general, compositions of matter of the present invention that are candidate inhibitors of prenyltransferase will be screened for activity in appropriate assays. Compounds that display desired characteristics in a given assay may serve as lead compounds for the discovery of more potent inhibitors. Additionally, compounds active against fungal prenyltransferases, e.g., GGPTase I, will be screened independently against mammalian prenyltransferases. Additionally, compounds against any particular mammalian prenyltransferase will be screened against other mammalian prenyltransferases. The present invention is not limited in terms of the methods relied upon for pinpointing potent inhibitors. Compounds selected based on their activity in vitro will be screened subsequently in vivo.

In one embodiment, a candidate inhibitor can be tested in an assay comprising a prenylation reaction system that includes a prenyltransferase, such as FPTase, GGPTase I, and GGPTase II; a suitable protein for prenylation by the particular prenyltransferase of the assay, or a portion thereof, which serves as a prenylation target substrate; and an activated moiety to serve as the isoprenoid donor which can be covalently attached to the prenylation substrate by the prenyltransferase. The level of prenylation of the target substrate brought about by the system is measured in the presence and absence of a candidate agent, and a statistically significant decrease in the level prenylation is indicative of a potential activity for the candidate agent of interest. In a preferred embodiment, the prenyltransferase is a fungal GGPTase, the suitable protein for prenylation is a fungal GTPase protein or portion thereof, and the activated moiety is a geranylgeranyl moiety. In other preferred embodiments, the prenylation system is designed for use with mammalian GGPTase, mammalian FTPase, or fungal FTPase.

As described below, the level of prenylation of the target protein can be measured by determining the actual concentration of substrate:isoprenoid conjugates formed; or inferred by detecting some other quality of the target substrate affected by prenylation, including membrane localization of the target. In certain embodiments, the present assay comprises an in vivo prenylation system, such as a cell able to conduct the target substrate through at least a portion of a isoprenoid conjugation pathway. In other embodiments, the present assay comprises an in vitro prenylation system in which at least the ability to transfer isoprenoids to the target protein is constituted. Still other embodiments provide assay formats which detect protein-protein interaction between the prenyltransferase and a target protein, rather than enzymatic activity per se.
Cell-free Assay Formats

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins or cell-lysates, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with upstream or downstream elements. Accordingly, in an exemplary screening assay of the present invention, a reaction mixture is generated to include a polypeptide for prenylation, such as Ras or other protein having GTPase-activity, candidate inhibitor(s) of interest, and a polypeptide having prenylation activity, such as FPTase, GGPTase I, or GGPTase II or a potion thereof retaining enzymatic activity. Detection and quantification of the enzymatic conversion of the polypeptide for prenylation or the formation of complexes containing the polypeptide for prenylation and the polypeptide having prenylation activity provide a means for determining a compound's efficacy at inhibiting (or potentiating) the complex bioactivity of any prenyltransferase. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay may also be performed to provide a baseline for comparison.

In one embodiment, the subject drug screening assay comprises a prenylation system, e.g., a reaction mixture which enzymatically conjugates isoprenoids to a target protein, which is arranged to detect inhibitors of the prenylation of a Rho-like GTPase. For instance, in one embodiment of a cell-free prenylation system, one or more cell lysates including a prenyltransferase, a Rho-like GTPase (or substrate analog thereof), and an activated isoprenoid group are incubated with the test compound and the level of prenylation of the Rho-like GTPase substrate is detected. Lysates can be derived from cells expressing one or more of the relevant proteins, and mixed appropriately (or split) where no single lysate contains all the components necessary for generating the prenylation system. In preferred embodiments, one or more of the components, especially the substrate target, are recombinantly produced in a cell used to generate a lysate, or added by spiking a lysate mixture with a
purified or semi-purified preparation of the substrate. These embodiments have several advantages including: the ability to use a labeled substrate, e.g., a dansylated peptide, or fusion protein, e.g., a Rho1-GST fusion protein, for facilitating purification; the ability to carefully control reaction conditions with respect to concentrations of reactants; and where targets are derived from fungal pathogens, the ability to work in a non-pathogenic system by recombinantly or synthetically by producing components from the pathogen for constituting the prenylation system. In other preferred embodiments, the prenyltransferase is either fungal or mammalian FTPase, GGPTase I, or GGPTase II. In other preferred embodiments, the prenyltransferase is fungal GGPTase.

The prenylation systems can be derived from any number of cell types, ranging from bacterial cells to yeast cells to cells from metazoan organisms including insects and mammalian cells. To illustrate, a fungal prenylation system can be reconstituted by mixing cell lysates derived from insect cells expressing prenyltransferase subunits cloned into baculoviral expression vectors. For example, the exemplary GGPTase-I expression vectors described below can be recloned into baculoviral vectors (e.g., pVL vectors), and recombinant GGPTase-I produced in transfected Spodoptera frugiperda cells. The level of activity can be assessed by enzymatic activity, or by quantitating the level of expression by detecting, e.g., an exogenous tag added to the recombinant protein. Substrate and activated geranylgeranyl diphosphate can be added to the lysate mixtures. As appropriate, the transfected cells can be cells which lack an endogenous GGPTase activity, or the substrate can be chosen to be particularly sensitive to prenylation by the exogenous fungal GGPTase relative to any endogenous activity of the cells. In other embodiments, other prenyltransferases are employed.

In other cell-free embodiments of the present assay, the prenylation system comprises a reconstituted protein mixture of at least semi-purified proteins. By semi-purified, it is meant that the proteins utilized in the reconstituted mixture have been previously separated from other cellular proteins. For instance, in contrast to cell lysates, the proteins involved in conjugation of geranylgeranyl moieties to a target protein, together with the target protein, are present in the mixture to at least 50% purity relative to all other proteins in the mixture, and more preferably are present at 90-95% purity. In certain embodiments of the subject method, the reconstituted protein mixture is derived by mixing highly purified proteins such that the reconstituted mixture substantially lacks other proteins which might interfere with or
otherwise alter the ability to measure specific prenylation rates of the target GTPase substrate.

In the subject method, prenylation systems derived from purified proteins may have certain advantages over cell lysate based assays. Unlike the reconstituted protein system, the prenylation activity of a cell-lysate may not be readily controlled. Measuring kinetic parameters is made tedious by the fact that cell lysates may be inconsistent from batch to batch, with potentially significant variation between preparations. In vitro evidence indicates that prenyltransferases have the ability to cross-prenylate CAAAX-related sequences, so that prenyltransferase not of interest present in a lysate may provide an unwanted kinetic parameter. Moreover, cycling of prenylated proteins by guanine nucleotide dissociation inhibitor (GDI)-like proteins in the lysate could further complicate kinetics of the reaction mixture. Evaluation of a potential inhibitor using a lysate system is also complicated in those circumstances where the lysate is charged with mRNA encoding the a substrate polypeptide, e.g., GTPase, or prenyltransferase activity, as such lysates may continue to synthesize proteins active in the assay during the development period of the assay, and can do so at unpredictable rates. Knowledge of the concentration of each component of the prenylation system can be required for each lysate batch, along with the overall kinetic data, in order to determine the necessary time course and calculate the sensitivity of experiments performed from one lysate preparation to the next. The use of reconstituted protein mixtures can allow more careful control of the reaction conditions in the prenylation reaction.

The purified protein mixture includes a purified preparation of the substrate polypeptide and a isoprenoid moiety (or analog thereof) under conditions which drive the conjugation of the two molecules. For instance, the mixture can include a fungal GGPTase I complex including RAM2 and CDC43 subunits, a geranylgeranyl diphosphate, a divalent cation, and a substrate polypeptide, such as may be derived from Rho1.

Prenylation of the target regulatory protein via an in vitro prenylation system, in the presence and absence of a candidate inhibitor, can be accomplished in any vessel suitable for containing the reactants. Examples include microtitre plates, test tubes, and micro-centrifuge tubes. In such embodiments, a wide range of detection means can be practiced to score for the presence of the prenylated protein.

In one embodiment of the present assay, the products of a prenylation system are separated by gel electrophoresis, and the level of prenylated substrate
polypeptide assessed, using standard electrophoresis protocols, by measuring an increase in molecular weight of the target substrate that corresponds to the addition of one or more isoprenoid moieties. For example, one or both of the target substrate and isoprenoid group can be labeled with a radioisotope such as $^{35}$S, $^{14}$C, or $^{3}$H, and the isotopically labeled protein bands quantified by autoradiographic techniques. Standardization of the assay samples can be accomplished, for instance, by adding known quantities of labeled proteins which are not themselves subject to prenylation or degradation under the conditions which the assay is performed. Similarly, other means of detecting electrophoretically separated proteins can be employed to quantify the level of prenylation of the target substrate, including immunoblot analysis using antibodies specific for either the target substrate or isoprenoid epitopes.

As described below, the antibody can be replaced with another molecule able to bind one of either the target substrate or the isoprenoid. By way of illustration, one embodiment of the present assay comprises the use of a biotinylated target substrate in the conjugating system. For example, biotinylated GGPTase substrates have been described in the art (c.f. Yokoyama et al. (1995) *Biochemistry* 34:1344-1354). The biotin label is detected in a gel during a subsequent detection step by contacting the electrophoretic products (or a blot thereof) with a streptavidin-conjugated label, such as a streptavidin linked fluorochrome or enzyme, which can be readily detected by conventional techniques. Moreover, where a reconstituted protein mixture is used (rather than a lysate) as the conjugating system, it may be possible to simply detect the target substrate and isoprenoid conjugates in the gel by standard staining protocols, including coomassie blue and silver staining.

In a similar fashion, prenylated and unprenylated substrate can be separated by other chromatographic techniques, and the relative quantities of each determined. For example, HPLC can be used to quantitate prenylated and unprenylated substrate (Pickett et al. (1995) *Analytical Biochem* 225:60-63), and the effect of a test compound on that ratio determined.

In another embodiment, an immunoassay or similar binding assay, is used to detect and quantify the level of prenylated target substrate produced in the prenylation system. Many different immunoassay techniques are amenable for such use and can be employed to detect and quantitate the conjugates. For example, the wells of a microtitre plate (or other suitable solid phase) can be coated with an antibody which specifically binds one of either the target substrate or isoprenoid groups. After incubation of the prenylation system with and without the candidate
agent, the products are contacted with the matrix bound antibody, unbound material removed by washing, and prenylated conjugates of the target substrate specifically detected. To illustrate, if an antibody which binds the target substrate is used to sequester the protein on the matrix, then a detectable anti-isoprenoid antibody can be used to score for the presence of prenylated target substrate on the matrix.

Still a variety of other formats exist which are amenable to high throughput analysis on microtitre plates or the like. The prenylation substrate can be immobilized throughout the reaction, such as by cross-linking to activated polymer, or sequestered to the well walls after the development of the prenylation reaction. In one illustrative embodiment, a Rho-like GTPase, e.g., a fungal Rho1, Rho2, Cdc42 or Rsr1/Bud1, is cross-linked to the polymeric support of the well, the prenylation system set up in that well, and after completion, the well washed and the amount of geranylgeranyl sidechains attached to the immobilized GTPase detected. In another illustrative embodiment, wells of a microtitre plate are coated with streptavidin and contacted with a developed prenylation system under conditions wherein a biotinylated substrate binds to and is sequestered in the wells. Unbound material is washed from the wells, and the level of prenylated target substrate is detected in each well. There are, as evidenced by this specification, a variety of techniques for detecting the level of prenylation of the immobilized substrate. For example, by the use of dansylated (described infra) or radiolabelled isoprenoid moieties in the reaction mixture, addition of appropriate scintillant to the wells will permit detection of the label directly in the microtitre wells. Alternatively, the substrate can be released and detected, for example, by any of those means described above, e.g., by radiolabel, gel electrophoresis, etc. Reversibly bound substrate, such as the biotin-conjugated substrate set out above, is particularly amenable to the latter approach. In other embodiments, only the isoprenoid moiety is released for detection. For instance, the thioether linkage of the isoprenoid with the substrate peptide sequence can be cleaved by treatment with methyl iodide. The released isoprenoid products can be detected, e.g., by radioactivity, HPLC, or other convenient format.

Other isoprenoid derivatives include detectable labels which do not interfere greatly with the conjugation of that group to the target substrate. For example, in an illustrative embodiment, the assay format provides fluorescence assay which relies on a change in fluorescent activity of a group associated with a prenyltransferase substrate to assess test compounds against a prenyltransferase. To illustrate, prenylation activity of any prenyltransferase may be measured by a modified version of the continuous fluorescence assay described for farnesyl transferases (Cassidy et
al., (1985) Methods Enzymol. 250: 30-43; Pickett et al. (1995) Analytical Biochem 225:60-63; and Stirtan et al. (1995) Arch Biochem Biophys 321:182-190). In an illustrative embodiment, dansyl-Gly-Cys-Ile-Ile-Leu (d-GCIIIL) and geranylgeranyl diphosphate are added to assay buffer, along with the test agent or control. This mixture is preincubated at 30 °C for a few minutes before the reaction is initiated with the addition of GGPTase enzyme. The sample is vigorously mixed, and an aliquot of the reaction mixture immediately transferred to a prewarmed cuvette, and the fluorescence intensity measured for 5 minutes. Useful excitation and emission wavelengths are 340 and 486 nm, respectively, with a bandpass of 5.1 nm for both excitation and emission monochromators. Generally, fluorescence data are collected with a selected time increment, and the inhibitory activity of the test agent is determined by detecting a decrease in the initial velocity of the reaction relative to samples which lack a test agent.

In yet another embodiment, the prenyltransferase activity against a particular substrate can be detected in the subject assay by using a phosphocellulose paper absorption system (Roskoski et al. (1994) Analytical Biochem 222:275-280), or the like. To effect binding of a peptidyl substrate to phosphocellulose at low pH, several basic residues can be added, preferably to the amino-terminal side of the target sequence of the peptide, to produce a peptide with a minimal minimum charge of +2 or +3 at pH less than 2. This follows the strategy used for the phosphocellulose absorption assay for protein kinases. In one embodiment; the transfer of a [H³]-isoprenoid group from [H³]-isoprenoid pyrophosphate to acceptor peptides can be measured under conditions similar to the farnesyl transferase reactions described by Reiss et al. (Reiss et al., (1990) Cell 62: 81-88). In an illustrative embodiment, the transfer of the [H³] geranylgeranyl group from [H³]-geranylgeranyl pyrophosphate to KLKCAL can be measured. Reaction mixtures can be generated to contain 50 mM Tris-HCL (pH 7.5), 50 μM ZnCl₂, 20 mM KCl, 1 mM dithiothreitol, 250 μM KLKCAL, 0.4 μM [H³] geranylgeranyl pyrophosphate, and 10-1000 μg/ml of purified fungal GGPTase protein. After incubation, e.g., for 30 minutes at 37 °C, samples are applied to Whatman P81 phosphocellulose paper strips. After the liquid permeates the paper (a few seconds), the strips are washed in ethanol/phosphoric acid (prepared by mixing equal volumes of 95% ethanol and 75 mM phosphoric acid) to remove unbound isoprenoids. The samples are air dried, and radioactivity can be measured by liquid scintillation spectrometry. Background values are obtained by using reaction mixture with buffer in place of enzyme.
An added feature of this strategy is that it produces hydrophilic peptides that are more readily dissolved in water. Moreover, the procedure outlined above works equally well for protein substrates (most proteins bind to phosphocellulose at acidic pH), so should be useful where full length protein, e.g., Rho1 or Cdc42, are utilized as the prenylation substrate, e.g., substrate for GGPTase.

Cell-based Assay Formats

In other embodiments, compounds for use in the subject method can be detected using a screening assay derived to include a whole cell expressing a GTPase protein, e.g., Ras, along with a prenyltransferase, e.g., FTPase, GGPTase I, and GGPTase II. In preferred embodiments, the reagent cell is a mammalian cell that has been engineered to express one or more of these proteins from mammalian recombinant genes. In other preferred embodiments, the reagent cell is a fungal cell that has been engineered to express one or more of these proteins from fungal recombinant genes. The reagent cell may be manipulated so that the recombinant gene(s) complement a loss-of-function mutation to the homologous gene in the reagent cell.

In preferred embodiments, the reagent cell is a non-pathogenic cell which has been engineered to express one or more of these proteins from recombinant genes cloned from a pathogenic fungus. For example, non-pathogenic fungal cells, such as S. cerevisiae, can be derived to express a Rho-like GTPase from a fungal pathogen such as Candida albicans. In an exemplary embodiment, a non-pathogenic yeast cell is engineered to express a Rho-like GTPase, e.g., Rho1, and at least one of the subunits of a GGPTase, e.g., RAM2 and/or Cdc43, derived from a fungal protein. One salient feature to such reagent cells is the ability of the practitioner to work with a non-pathogenic strain rather than the pathogen itself. Another advantage derives from the level of knowledge, and available strains, when working with such reagent cells as S. cerevisiae.

In other embodiments, compounds for use in the subject method can be detected using a screening assay derived to include a whole cell expressing a substrate for a prenyltransferase, e.g., a GTPase protein, along with a prenyltransferase. In preferred embodiments, the reagent cell is a mammalian cell which has been engineered to express one or more of these proteins from recombinant mammalian genes. In other preferred embodiments, the reagent cell is a non-fungal cell which has been engineered to express one or more of these proteins.
from recombinant mammalian genes. In other preferred embodiments, the reagent cell is a non-pathogenic cell which has been engineered to express one or more of these proteins from recombinant genes cloned from a pathogenic fungus. For example, non-pathogenic fungal cells, such as S. cerevisiae, can be derived to express a Rho-like GTPase from a fungal pathogen such as Candida albicans. In an exemplary embodiment, a non-pathogenic yeast cell is engineered to express a Rho-like GTPase, e.g., Rho1, and at least one of the subunits of a GGPTase, e.g., RAM2 and/or Cdc43, derived from a fungal protein. One salient feature to such reagent cells is the ability of the practitioner to work with a non-pathogenic strain rather than the pathogen itself. Another advantage derives from the level of knowledge, and available strains, when working with such reagent cells as S. cerevisiae. For all such embodiments, the reagent cell may be manipulated such that the recombinant gene(s) complement a loss-of-function mutation to the homologous gene in the reagent cell.

The ability of a test agent to alter the activity of a prenyltransferase may be detected by analysis of the cell or products produced by the cell. For example, agonists and antagonists of the GTPase biological activity can be detected by scoring for alterations in growth or viability of the cell. Other embodiments will permit inference of the level of GTPase activity based on, for example, detecting expression of a reporter, the induction of which is directly or indirectly dependent on the activity of a Rho-like GTPase. General techniques for detecting each are well known, and will vary with respect to the source of the particular reagent cell utilized in any given assay.

For example, quantification of proliferation of cells in the presence and absence of a candidate agent can be measured with a number of techniques well known in the art, including simple measurement of population growth curves. For instance, where the assay involves proliferation in a liquid medium, turbidimetric techniques (i.e., absorption/transmission of light of a given wavelength through the sample) can be utilized. For example, in the instance where the reagent cell is a yeast cell, measurement of absorption of light at a wavelength between 540 and 600 nm can provide a conveniently fast measure of cell growth. Likewise, ability to form colonies in solid medium (e.g., agar) can be used to readily score for proliferation. In other embodiments, a GTPase substrate protein, such as a histone, can be provided as a fusion protein which permits the substrate to be isolated from cell lysates and the degree of acetylation detected. Each of these techniques are suitable for high through-put analysis necessary for rapid screening of large numbers of candidate agents.
Additionally, visual inspection of the morphology of the reagent cell can be used to determine whether the biological activity of the targeted protein, e.g., GTPase, has been affected by the added agent. To illustrate, the ability of an agent to create a lytic phenotype which is mediated in some way by a recombinant GTPase protein can be assessed by visual microscopy.

The nature of the effect of test agent on reagent cell can be assessed by measuring levels of expression of specific genes, e.g., by reverse transcription-PCR. Another method of scoring for effect on protein activity of interest, e.g., GTPase, is by detecting cell-type specific marker expression through immunofluorescent staining. Many such markers are known in the art, and antibodies are readily available.

In yet another embodiment, in order to enhance detection of cell lysis for fungal inhibitors, the target cell can be provided with a cytoplasmic reporter which is readily detectable, either because it has "leaked" outside the cell, or substrate has "leaked" into the cell, by perturbations in the cell wall. Preferred reporters are proteins which can be recombinantly expressed by the target cell, do not interfere with cell wall integrity, and which have an enzymatic activity for which chromogenic or fluorogenic substrates are available. In one example, a fungal cell can be constructed to recombinantly express the β-galactosidase gene from a construct (optionally) including an inducible promoter. At some time prior to contacting the cell with a test agent, expression of the reporter protein is induced. Agents which inhibit prenylation of a Rho-like GTPase in the cell, or the subsequent involvement of a Rho-like GTPase in cell wall integrity, can be detected by an increase in the reporter protein activity in the culture supernatant or from permeation of a substrate in the cell. Thus, for example, β-galactosidase activity can be scored using such colorimetric substrates as 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside or fluorescent substrates such as methylumbelliferyl-β-D-galactopyranoside. Permeation of the substrate into the cell, or leakage of the reporter into the culture media, is thus readily detectable.

In still another embodiment, the membrane localization resulting from prenylation of a GTPase can be exploited to generate the cell-based assay. For instance, the subject assay can be derived with a reagent cell having: (i) a reporter gene construct including a transcriptional regulatory element which can induce expression of the reporter upon interaction of the transcriptional regulatory protein portion of the above fusion protein. For example, a ga14 protein can be fused with a Rho1 polypeptide sequence which includes the CAAX prenylation target. In the
absence of inhibitors of GGPTase activity in the reagent cell, prenylation of the fusion protein will result in partitioning of the fusion protein at the cell surface membrane. This provides a basal level of expression of the reporter gene construct. When contacted with an agent that inhibits prenylation of the fusion protein, partitioning is lost and, with the concomitant increase in nuclear concentration of the protein, expression from the reporter construct is increased.

In a preferred embodiment, the cell is engineered such that inhibition by fungal inhibitors of the GGPTase activity does not result in cell lysis. For example, as described in Ohya et al. (1993) Mol Cell Biol 4:1017-1025, mutation of the C-terminus of Rho1 and cdc42 can provide proteins which are targets of farnesyl transferase rather than geranylgeranyl transferase. As Ohya et al. describe, such mutants can be used to render the GGPTase I activity dispensable. Accordingly, providing a reporter gene construct and an expression vector for the GGPTase substrate/transcription factor fusion protein in such cells as YOT35953 cells (Ohya et al., vide supra) generates a cell whose viability vis-à-vis the GGPTase activity is determined by the reporter construct, if at all, rather than by prenylation of an endogenous Rho-like GTPase by the GGPTase. Of course, the reporter gene product can be derived to have no effect on cell viability, providing for example another type of detectable marker (described, infra). Such cells can be engineered to express an exogenous GGPTase activity in place of an endogenous activity, or can rely on the endogenous activity. To further illustrate, the C41 mutant YOT35953 cell can be further manipulated to express a C41 homolog from, e.g., a fungal pathogen or a mammalian cell.

Alternatively, where fungal inhibition of a GGPTase activity causes cell lysis and reporter gene expression, the leakage assay provided above can be utilized to detect expression of the reporter protein. For instance, the reporter gene may encode β-galactosidase, and inhibition of the GGPTases activity scored for by the presence of cells which take up substrate due to loss of cell wall integrity, and convert substrate due to the expression of the reporter gene.

In other preferred embodiments, the reporter gene is a gene whose expression causes a phenotypic change which is screenable or selectable. If the change is selectable, the phenotypic change creates a difference in the growth or survival rate between cells which express the reporter gene and those which do not. If the change is screenable, the phenotype change creates a difference in some detectable characteristic of the cells, by which the cells which express the marker may be distinguished from those which do not.
The marker gene is coupled to GTPase-dependent activity, be it membrane association, or a downstream signaling pathway induced by a GTPase complex, so that expression of the marker gene is dependent on the activity of the GTPase. This coupling may be achieved by operably linking the marker gene to a promoter responsive to the therapeutically targeted event. The term "GTPase-responsive promoter" indicates a promoter which is regulated by some product or activity of the fungal GTPase. By this manner, the activity of a prenyltransferase may be detected by its effects on prenylation of GTPase and, accordingly, the downstream targets of the prenylated protein. Thus, in certain embodiments, transcriptional regulatory sequences responsive to signals generated by PKC/GTPase, GS/GTPase and/or other GTPase complexes, or to signals by other proteins in such complexes which are interrupted by GTPase binding, can be used to detect function of Rho-like GTPases such as Rho1 and cdc42.

In the case of nonfungal systems, suitable positively selectable (beneficial) genes include the following: For yeast, suitable positively selectable (beneficial) genes include the following: URA3, LYS2, HIS3, LEU2, TRP1; ADE1, 2, 3, 4, 5, 7, 8; ARG1, 3, 4, 5, 6, 8; HIS1, 4, 5; ILV1, 2, 5; THR1, 4; TRP2, 3, 4, 5; LEU1, 4; MET2, 3, 4, 8, 9, 14, 16, 19; URA1, 2, 4, 5, 10; HOM3, 6; ASP3; CHO1; ARO 2, 7; CY3; OLE1; IN01, 2, 4; PR01, 3. Countless other genes are potential selective markers. The above are involved in well-characterized biosynthetic pathways. The imidazoleglycerol phosphate dehydratase (IGP dehydratase) gene (HIS3) is preferred because it is both quite sensitive and can be selected over a broad range of expression levels. In the simplest case, the cell is auxotrophic for histidine (requires histidine for growth) in the absence of activation. Activation of the gene leads to synthesis of the enzyme and the cell becomes prototrophic for histidine (does not require histidine). Thus the selection is for growth in the absence of histidine. Since only a few molecules per cell of IGP dehydratase are required for histidine prototrophy, the assay is very sensitive.

The marker gene may also be a screenable gene. The screened characteristic may be a change in cell morphology, metabolism or other screenable features. Suitable markers include beta-galactosidase (Xgal, C12FDG, Salmon-gal, Magenta-Gal (latter two from Biosynth Ag)), alkaline phosphatase, horseradish peroxidase, exo-glucanase (product of yeast ebx1 gene; nonessential, secreted); luciferase; bacterial green fluorescent protein; (human placental) secreted alkaline phosphatase (SEAP); and chloramphenicol transferase (CAT). Some of the above can be engineered so that they are secreted (although not β-galactosidase). A
preferred screenable marker gene is β-galactosidase; for in yeast cells, for example, expression of the enzyme converts the colorless substrate Xgal into a blue pigment.

It has also been observed in the art that mutations to Gsc1 (Fks1) confer hypersensitivity to the immunosuppressants FK506 and cyclosporin A (Douglas et al. (1994) PNAS 91:12907). The mechanism of action of such agents is understood to involve inhibition of expression of the Fks2 gene (Mazur et al. (1995) Mol Cell Biol 15:5671). Similar to the echinocandin-sensitivity assay embodiments provided above, another assay format provides a cell in which Fks2 activity is compromised. Synergism of the Fks2 impairment with a test compound can be used to identify inhibitors of, for example, the glucan synthase subunit Gsc1. For instance, FK506 or cyclosporin A can be used to impair Fks2 activity, as can mutations to calcineurin or to the Fks2 gene.

These observations also suggest that, in certain embodiments, Cal1-1 cells or the like, e.g., impaired for certain prenyltransferase activities, are suitable for use in assays to detect GS inhibitors, as such cells are more sensitive to the effects of GS inhibitors. The benefits to enhanced sensitivity include speedier development of assay readouts, and the further prejudicing of the assay towards GS inhibitors rather than other targets which may not provide cytotoxicity. The latter can provide the ability to identify potential hits which may not themselves be potent GS inhibitors, but which can be manipulated, e.g., by combinatorial chemistry approaches, to provide potent and specific GS inhibitors.

Returning to the teachings of Ohya et al. (1993), vide supra, it is noted that there are only two essential targets of GGPTase in S. cerevisiae, the Rho-like GTPases Rho1 and cdc42. With such observations in mind, yet another embodiment of the subject assay utilizes a side-by-side comparison of the effect of a test agent on (i) a cell which prenylates a Rho-like GTPase by adding geranylgeranyl moieties, and (ii) a cell which prenylates an equivalent Rho-like GTPase by adding farnesyl moieties. In particular, the assay makes use of the ability to suppress GGPTase I defects in yeast by altering the C-terminal tail of Rho1 and cdc42 to become substrate targets of farnesyl transferase (see Ohya et al., supra). According to the present embodiment, the assay is arranged by providing a yeast cell in which the target Rho-like GTPases is prenylated by a GGPTase activity of the cell. Both the GGPTase and GTPase can be endogenous to the "test" cell, or one or both can be recombinantly expressed in the cell. The level of prenylation of the GTPase is detected, e.g., cell lysis or other means described above. The ability of the test compound to inhibit the addition of geranylgeranyl groups to the GTPase in the first
cell is compared against the ability of test compound to inhibit the farnesylation of the GTPase in a control cell. The "control" cell is preferably identical to the test cell, with the exception that the targeted GTPase(s) are mutated at their CAAX sequence to become substrates for FPTases rather than GGPTases. Agents which inhibit prenylation in the test cell but not the control cell are selected as potential antifungal agents. Such differential screens can be exquisitely sensitive to inhibitors of GGPTase I prenylation of Rho-like GTPases. In a preferred embodiment, the test cell is derived from the S. cerevisiae cell YOT35953 (Ohya et al., supra) or the like which is defective in GGPTase subunit cdc43. The cell is then engineered with a cdc43 subunit from a fungal pathogen such as Candida albicans to generate the test cell, and additionally with the mutated Rho-like GTPases to generate the control cell.

**Differential Screening Formats**

In a preferred embodiment, assays can be used to identify compounds that have favorable therapeutic indexes. For instance, anticancer agents can be identified by the present assays which inhibit particular prenyltransferases, and thereby may treating conditions resulting from mutations in the specific gene encoding that prenyltransferase. In another instance, antifungal agents can be identified by the present assays which inhibit proliferation of yeast cells or other lower eukaryotes, but which have a substantially reduced effect on mammalian cells, thereby improving therapeutic index of the drug as an anti-mycotic agent.

In one embodiment, differential screening assays can be used to exploit the difference in protein interactions and/or catalytic mechanism of different prenyltransferases in order to identify agents which display a statistically significant increase in specificity for inhibiting certain prenylation reactions relative to others. Thus, lead compounds which act specifically on the certain prenylation reactions can be developed.

In another embodiment, differential screening assays can be used to exploit the difference in protein interactions and/or catalytic mechanism of mammalian and fungal GGPTases in order to identify agents which display a statistically significant increase in specificity for inhibiting the fungal prenylation reaction relative to the mammalian prenylation reaction. Thus, lead compounds which act specifically on the prenylation reaction in pathogens, such as fungus involved in mycotic infections, can be developed. By way of illustration, the present assays can be used to screen for
agents which may ultimately be useful for inhibiting the growth of at least one fungus implicated in such mycosis as *candidiasis*, *aspergillosis*, *mucormycosis*, *blastomycosis*, *geotrichosis*, *cryptococcosis*, *chromoblastomycosis*, *coccidioidomycosis*, *conidiosporosis*, *histoplasmosis*, *maduromycosis*, *rhinosporidiosis*, *nocaidiosis*, *para-actinomycosis*, *penicilliosis*, *monoliasis*, or *sporotrichosis*. For example, if the mycotic infection to which treatment is desired is candidiasis, the present assay can comprise comparing the relative effectiveness of a test compound on inhibiting the prenylation of a mammalian GTPase protein with its effectiveness towards inhibiting the prenylation of a GTPase from a yeast selected from the group consisting of *Candida albicans*, *Candida stellatoidea*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida pseudotropicalis*, *Candida guilliermondii*, or *Candida rugosa*. Likewise, the present assay can be used to identify antifungal agents which may have therapeutic value in the treatment of *aspergillosis* by selectively targeting, relative to human cells, GTPase homologs from yeast such as *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, or *Aspergillus terreus*. Where the mycotic infection is mucormycosis, the GTPase system to be screened can be derived from yeast such as *Rhizopus arrhizus*, *Rhizopus oryzae*, *Absidia corymbifera*, *Absidia ramosa*, or *Mucor pusillus*. Sources of other assay reagents for includes the pathogen *Pneumocystis carinii*.

**IV. Exemplification**

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

*Preparation of compounds of the present invention*

**a. Illustrative synthetic schemes**

Exemplary synthesis schemes for generating prenyltransferase inhibitors useful in the methods and compositions of the present invention are shown in Figures 1-31.

The reaction conditions in the illustrated schemes of Figure 1-31 are as follows:
1) R₁CH₂CN, NaNH₂, toluene
   (Arzneim-Forsch, 1990, 40, 11, 1242)
2) H₂SO₄, H₂O, reflux
   (Arzneim-Forsch, 1990, 40, 11, 1242)
3) H₂SO₄, EtOH, reflux
   (Arzneim-Forsch, 1990, 40, 11, 1242)
4) NaOH, EtOH, reflux
5) (Boc)₂O, 2M NaOH, THF
6) LiHDMDS, R₁X, THF
   (Merck Patent Applic # WO 96/06609)
7) Pd-C, H₂, MeOH
8) t-BuONO, CuBr, HBr, H₂O
   (J. Org. Chem. 1977, 42, 2426)
9) ArBr(OH)₂, Pd(PPh₃)₄, Dioxane
10) R₁₂(H)=CR₁₃R₁₄, Pd(OAc)₂, Et₃N, DMF
   (Org. React. 1982, 27, 345)
11) Tf₂O, THF
    (J. Am. Chem. Soc. 1987, 109, 5478-5486)
12) ArSnBu₃, Pd(PPh₃)₄, Dioxane
    (J. Am. Chem. Soc. 1987, 109, 5478-5486)
13) KMnO₄, Py, H₂O
14) NaOR₁, THF
15) NaSR₁, THF
16) HNR₁R₁₃, THF
17) HONO, NaBF₄
18) Pd(OAc)₂, NaH, DPPF, PhCH₃, R₁OH
19) i. R₁X, Et₃N, CH₂Cl₂, ii. R₁₃X
20) SCl₂, cat DMF
21) CH₂N₂, Et₂O
22) Ag₂O, Na₂CO₃, Na₂S₂O₃, H₂O
    (Tetrahedron Lett. 1979, 2667)
23) AgO₂CPh, Et₃N, MeOH
24) LiOH, THF-MeOH
25) (EtO)₂P(O)CH₂CO₂R, BuLi, THF
26) MeO₂CCH(Br)=P(Ph)₃, benzene
27) KOH or KOTBu
28) Base, X(CH₂)nCO₂R
29) DPPA, Et₃N, toluene
    (Synthesis 1985, 220)
30) HONO, H₂O
31) SO₂, CuCl, HCl, H₂O
(Synthesis 1969, 1-10, 6)
32) Lawesson's reagent, toluene
   (Tetrahedron Asym. 1996, 7, 12, 3553)
33) R2M, solvent
34) 30% H2O2, glacial CH3CO2H
35) triphosgene, CH2Cl2
36) i. (EtO)2P(O)CHLiSO2Oi-Pr, THF, ii. NaI
37) Ph3PCH3I, NaCH2S(O)CH3, DMSO
   (Synthesis 1987, 498)
38) Br2, CHCl3 or other solvent
   (Synthesis 1987, 498)
39) BuLi, Bu3SnCl
40) ClSO2OTMS, CCl4
41) MeOH-HCl, reflux
42) LAH, Et2O or LiBH4, EtOH or BH3-THF
43) MsCl, Et3N, CH2Cl2
44) Na2SO3, H2O
45) R2R4NH, Et3N, CH2Cl2
46) R2M, solvent
47) CH3NH(OCH3), EDC, HOBT, DIEA, CH2Cl2 or DMF
   (Tetrahedron Lett, 1981, 22, 3815)
48) MeLi, THF
49) mCPBA, CH2Cl2
50) HONO, Cu2O, Cu(NO3)2, H2O
   (J. Org. Chem. 1977, 42, 2053)
51) R1M, solvent
52) HONO, NaS(S)COEt, H2O
   (Org. Synth. 1947, 27, 81)
53) HSR2 or HSR4, CH2Cl2
54) i-BuOC(O)Cl, Et3N, NH3, THF
55) R2R4NH, CH2Cl2, NaBH(OAc)3
56) R2R4NH, MeOH/CH3CO2H, NaBH3CN
57) R2OH, EDC, HOBT, DIEA, CH2Cl2 or DMF
58) R2OH, HBTU, HOBT, DIEA, CH2Cl2 or DMF
59) R2R4NH, EDC, HOBT, DIEA, CH2Cl2 or DMF
60) R2R4NH, HBTU, HOBT, DIEA, CH2Cl2 or DMF
61) POCl3, Py, CH2Cl2
62) R2R4NCO, solvent
63) R2OC(O)Cl, Et3N, solvent
64) R2CO2H, EDC or HBTU, HOBT, DIEA, CH2Cl2 or DMF
65) R2X, Et3N, solvent
66) (CH₃S)₂C=N(CN), DMF, EtOH
   (J. Med. Chem. 1994, 37, 57-66)
67) R₂SO₂Cl, Et₃N, CH₂Cl₂
68) R₂- or R₃- or R₄CHO, MeOH/CH₃CO₂H, NaBH₃CN
   (Synthesis 1975, 135-146)
69) Boc(Tr)₂-D or L-CysOH, HBTU, HOBt, DIEA, CH₂Cl₂ or DMF
70) Boc(Tr)₂-D or L-CysH, NaBH₃CN, MeOH/CH₃CO₂H
   (Synthesis 1975, 135-146)
71) S-Tr-N-Boc cysteinal, ClCH₂CH₂Cl or THF, NaBH(OAc)₃
72) TFA, CH₂Cl₂, Et₃SiH or (3:1:1) thioanisole/ethanedithioi/DMS
73) TFA, CH₂Cl₂
74) DPPA, Et₃N, toluene, HOCH₂CH₂SiCH₃
   (Tetrahedron Lett. 1984, 25, 3515)
75) TBAF, THF
76) Base, TrSH or BnSH
77) Base, R₂X or R₄X
78) R₃NH₂, MeOH/CH₃CO₂H, NaBH₃CN
79) N₂H₄, KOH
80) Pd₂(dbat)₃, (p-o-tol)₃, RNH₂, NaOtBu, Dioxane, R₁NH₂
81) Cyanamid.
82) Fmoc-Cl, sodium bicarbonate.
83) BnCOCl, sodium carbonate.
84) AllyloCOCl, pyridine.
85) Benzyl bromide, base.
86) Oxalyl chloride, DMSO.
87) RCONH₂.
88) Carboxyldiimidazole, neutral solvents (e.g., DCM, DMF, THF, toluene).
89) Thiocarboxyldiimidazole, neutral solvents (e.g., DCM, DMF, THF, toluene).
90) Cyanogen bromide, neutral solvents (e.g., DCM, DMF, THF, toluene).
91) ROCOCI, Triethylamine
92) RNHNH₂, EDC.
93) RO₂CCOCl, Et₃N, DCM.
94) MsOH, Pyridine (J. Het. Chem., 1980, 607.)
95) Base, neutral solvents (e.g., DCM, toluene, THF).
96) H₂NOR, EDC.
97) RCONH₂.
98) ROCOCHBrR, neutral solvents (e.g., DCM, DMF, THF, toluene), (Org. Proc.
99) CH₂N₂, HCl. (Synthesis, 1993, 197).
100) NH₂NHR, neutral solvents (e.g., DCM, DMF, THF, toluene).
103) NOCl or Cl₂ (J. Org. Chem., 1990, 55, 3916).
104) H₂NOH, neutral solvents (e.g., DCM, DMF, THF, toluene).
105) RCR₃, neutral solvents (DCM, THF, Toluene).
106) RCHCHR, neutral solvents (DCM, THF, Toluene).
107) H₂NOH, HCl.
110) HNO₂, HCl.
111) CICH₂CO₂Et (Org. Reactions, 1959, 10, 143).
113) RCOCHR′CN
114) RCOCHR′CO₂Et
115) Na₂SO₃
116) H₂NCHRCO₂Et
117) EtO₂CCHRNCO
118) RCNHNH₂.
120) RCHO, KOAc.
121) 2-Fluoronitrobenzene.
122) SnCl₂, EtOH, DMF.
123) RCHO, NaBH₃CN, HOAc.
124) NH₃, MeOH.
125) 2,4,6-Me₃PhSO₂NH₂.
126) Et₂NH, CH₂Cl₂
127) MeOC(O)Cl, Et₃N, CH₂Cl₂
128) R₂NH₂, EDC, HOBT, Et₃N, CH₂Cl₂
129) DBU, PhCH₃
130) BocNHCH(CH₂STr)CH₂NH₂, EDC, HOBT, Et₃N, CH₂Cl₂
131) R₂NHCH₂CO₂Me, HBTU, HOBT, Et₃N, CH₂Cl₂
132) BocNHCH(CH₂STr)CH₂OMs, LiHMDS, THF
133) R₂NHCH₂CO₂Me, NaBH(OAc)₃, CICH₂CH₂Cl or THF
134) R₂NHCH₂CH(OEt)₂, HBTU, HOBT, Et₃N, CH₂Cl₂
135) NaBH(OAc)₃, CICH₂CH₂Cl or THF, AcOH.
136) Piperidine, DMF.
137) Pd(Ph₃P)₄, Bu₃SnH.
138) ROCCO₂H, EDC, HOBT, Et₃N, DCM.
139) RNH₂, neutral solvents.
140) RCHO, NaBH₃CN, HOAc.
141) RNCO, solvent.
142) ROCCO₂H, EDC or HBTU, HOBT, DIEA, CH₂Cl₂ or DMF.
143) ROCOCI, Triethylamine
144) RSO₂Cl, Et₃N, CH₂Cl₂.
145) SnCl₂, EtOH, DMF.
146) RNH₂, EDC, HOBT, DIEA, CH₂Cl₂ or DMF.
147) Dibromoethane, Et₃N, CH₂Cl₂
148) Oxalyl chloride, neutral solvents.
149) LiOH, THF-MeOH.
150) Carbonylidiimidazole, neutral solvents (e.g., DCM, DMF, THF, toluene).
151) RNH₂, Et₃N, CH₂Cl₂.
152) Base, RX.
b. Illustrative combinatorial libraries

The compounds of the present invention, particularly libraries of variants having various representative classes of substituents, are amenable to combinatorial chemistry and other parallel synthesis schemes (see, for example, PCT WO 94/08051). The result is that large libraries of related compounds, e.g., a variegated library of compounds represented by formula I above, can be screened rapidly in high throughput assays in order to identify potential antifungal lead compounds, as well as to refine the specificity, toxicity, and/or cytotoxic-kinetic profile of a lead compound. For instance, simple turbidimetric assays (e.g., measuring the A600 of a culture), or spotting compounds on fungal lawns, can be used to screen a library of the subject compounds for those having inhibitory activity toward a particular fungal strain.

Simply for illustration, a combinatorial library for the purposes of the present invention is a mixture of chemically related compounds which may be screened together for a desired property. The preparation of many related compounds in a single reaction greatly reduces and simplifies the number of screening processes which need to be carried out. Screening for the appropriate physical properties can be done by conventional methods.

Diversity in the library can be created at a variety of different levels. For instance, the substrate aryl groups used in the combinatorial reactions can be diverse in terms of the core aryl moiety, e.g., a variegation in terms of the ring structure, and/or can be varied with respect to the other substituents.

variety of libraries on the order of about 100 to 1,000,000 or more diversomers of the subject antifungals can be synthesized and screened for particular activity or property.

In an exemplary embodiment, a library of candidate antifungal diversomers can be synthesized utilizing a scheme adapted to the techniques described in the Still et al. PCT publication WO 94/08051, e.g., being linked to a polymer bead by a hydrolyzable or photolyzable group e.g., located at one of the positions of the candidate antifungals or a substituent of a synthetic intermediate. According to the Still et al. technique, the library is synthesized on a set of beads, each bead including a set of tags identifying the particular diversomer on that bead. The bead library can then be "plated" on a lawn of fungi for which an inhibitor is sought. The diversomers can be released from the bead, e.g., by hydrolysis. Beads surrounded by areas of no, or diminished, fungal growth, e.g., a "halo", can be selected, and their tags can be "read" to establish the identity of the particular diversomer.

A) Direct Characterization

A growing trend in the field of combinatorial chemistry is to exploit the sensitivity of techniques such as mass spectrometry (MS), for example, which can be used to characterize sub-femtomolar amounts of a compound, and to directly determine the chemical constitution of a compound selected from a combinatorial library. For instance, where the library is provided on an insoluble support matrix, discrete populations of compounds can be first released from the support and characterized by MS. In other embodiments, as part of the MS sample preparation technique, such MS techniques as MALDI can be used to release a compound from the matrix, particularly where a labile bond is used originally to tether the compound to the matrix. For instance, a bead selected from a library can be irradiated in a MALDI step in order to release the diversomer from the matrix, and ionize the diversomer for MS analysis.

B) Multipin Synthesis

The libraries of the subject method can take the multipin library format. Briefly, Geysen and co-workers (Geysen et al. (1984) PNAS 81:3998-4002) introduced a method for generating compound libraries by a parallel synthesis on polyacrylic acid-grated polyethylene pins arrayed in the microtitre plate format. The Geysen technique can be used to synthesize and screen thousands of compounds per week using the multipin method, and the tethered compounds may be reused in many assays. Appropriate linker moieties can also been appended to the pins so that

C) Divide-Couple-Recombine

In yet another embodiment, a variegated library of compounds can be provided on a set of beads utilizing the strategy of divide-couple-recombine (see, for example, Houghten (1985) PNAS 82:5131-5135; and U.S. Patents 4,631,211; 5,440,016; 5,480,971). Briefly, as the name implies, at each synthesis step where degeneracy is introduced into the library, the beads are divided into separate groups equal to the number of different substituents to be added at a particular position in the library, the different substituents coupled in separate reactions, and the beads recombined into one pool for the next iteration.

In one embodiment, the divide-couple-recombine strategy can be carried out using an analogous approach to the so-called "tea bag" method first developed by Houghten, where compound synthesis occurs on resin sealed inside porous polypropylene bags (Houghten et al. (1986) PNAS 82:5131-5135). Substituents are coupled to the compound-bearing resins by placing the bags in appropriate reaction solutions, while all common steps such as resin washing and deprotection are performed simultaneously in one reaction vessel. At the end of the synthesis, each bag contains a single compound.

D) Combinatorial Libraries by Light-Directed, Spatially Addressable Parallel Chemical Synthesis

A scheme of combinatorial synthesis in which the identity of a compound is given by its locations on a synthesis substrate is termed a spatially addressable synthesis. In one embodiment, the combinatorial process is carried out by controlling the addition of a chemical reagent to specific locations on a solid support (Dower et al. (1991) Annu Rep Med Chem 26:271-280; Fodor, S.P.A. (1991) Science 251:767; Pirrung et al. (1992) U.S. Patent No. 5,143,854; Jacobs et al. (1994) Trends Biotechnol 12:19-26). The spatial resolution of photolithography affords miniaturization. This technique can be carried out through the use protection/deprotection reactions with photolabile protecting groups.

The key points of this technology are illustrated in Gallop et al. (1994) J Med Chem 37:1233-1251. A synthesis substrate is prepared for coupling through the covalent attachment of photolabile nitroveratryloxycarbonyl (NVOC) protected
amino linkers or other photolabile linkers. Light is used to selectively activate a specified region of the synthesis support for coupling. Removal of the photolabile protecting groups by light (deprotection) results in activation of selected areas. After activation, the first of a set of amino acid analogs, each bearing a photolabile protecting group on the amino terminus, is exposed to the entire surface. Coupling only occurs in regions that were addressed by light in the preceding step. The reaction is stopped, the plates washed, and the substrate is again illuminated through a second mask, activating a different region for reaction with a second protected building block. The pattern of masks and the sequence of reactants define the products and their locations. Since this process utilizes photolithography techniques, the number of compounds that can be synthesized is limited only by the number of synthesis sites that can be addressed with appropriate resolution. The position of each compound is precisely known; hence, its interactions with other molecules can be directly assessed.

In a light-directed chemical synthesis, the products depend on the pattern of illumination and on the order of addition of reactants. By varying the lithographic patterns, many different sets of test compounds can be synthesized simultaneously; this characteristic leads to the generation of many different masking strategies.

E) Encoded Combinatorial Libraries

In yet another embodiment, the subject method utilizes a compound library provided with an encoded tagging system. A recent improvement in the identification of active compounds from combinatorial libraries employs chemical indexing systems using tags that uniquely encode the reaction steps a given bead has undergone and, by inference, the structure it carries. Conceptually, this approach mimics phage display libraries, where activity derives from expressed peptides, but the structures of the active peptides are deduced from the corresponding genomic DNA sequence. The first encoding of synthetic combinatorial libraries employed DNA as the code. A variety of other forms of encoding have been reported, including encoding with sequenceable bio-oligomers (e.g., oligonucleotides and peptides), and binary encoding with additional non-sequenceable tags.

1) Tagging with sequenceable bio-oligomers

The principle of using oligonucleotides to encode combinatorial synthetic libraries was described in 1992 (Brenner et al. (1992) PNAS 89:5381-5383), and an example of such a library appeared the following year (Needles et al. (1993) PNAS 90:10700-10704). A combinatorial library of nominally \(7^7\) (= 823,543) peptides
composed of all combinations of Arg, Gln, Phe, Lys, Val, D-Val and Thr (three-letter amino acid code), each of which was encoded by a specific dinucleotide (TA, TC, CT, AT, TT, CA and AC, respectively), was prepared by a series of alternating rounds of peptide and oligonucleotide synthesis on solid support. In this work, the amine linking functionality on the bead was specifically differentiated toward peptide or oligonucleotide synthesis by simultaneously preincubating the beads with reagents that generate protected OH groups for oligonucleotide synthesis and protected NH₂ groups for peptide synthesis (here, in a ratio of 1:20). When complete, the tags each consisted of 69-mers, 14 units of which carried the code. The bead-bound library was incubated with a fluorescently labeled antibody, and beads containing bound antibody that fluoresced strongly were harvested by fluorescence-activated cell sorting (FACS). The DNA tags were amplified by PCR and sequenced, and the predicted peptides were synthesized. Following such techniques, compound libraries can be derived for use in the subject method, where the oligonucleotide sequence of the tag identifies the sequential combinatorial reactions that a particular bead underwent, and therefore provides the identity of the compound on the bead.

The use of oligonucleotide tags permits exquisitely sensitive tag analysis. Even so, the method requires careful choice of orthogonal sets of protecting groups required for alternating co-synthesis of the tag and the library member. Furthermore, the chemical lability of the tag, particularly the phosphate and sugar anomeric linkages, may limit the choice of reagents and conditions that can be employed for the synthesis of non-oligomeric libraries. In preferred embodiments, the libraries employ linkers permitting selective detachment of the test compound library member for assay.

Peptides have also been employed as tagging molecules for combinatorial libraries. Two exemplary approaches are described in the art, both of which employ branched linkers to solid phase upon which coding and ligand strands are alternately elaborated. In the first approach (Kerr et al. (1993) JACS 115:2529-2531), orthogonality in synthesis is achieved by employing acid-labile protection for the coding strand and base-labile protection for the compound strand.

In an alternative approach (Nikolaiev et al. (1993) Pept Res 6:161-170), branched linkers are employed so that the coding unit and the test compound can both be attached to the same functional group on the resin. In one embodiment, a cleavable linker can be placed between the branch point and the bead so that cleavage releases a molecule containing both code and the compound (Ptek et al. (1991) Tetrahedron Lett 32:3891-3894). In another embodiment, the cleavable linker
can be placed so that the test compound can be selectively separated from the bead, leaving the code behind. This last construct is particularly valuable because it permits screening of the test compound without potential interference of the coding groups. Examples in the art of independent cleavage and sequencing of peptide library members and their corresponding tags has confirmed that the tags can accurately predict the peptide structure.

2) Non-sequenceable Tagging: Binary Encoding

An alternative form of encoding the test compound library employs a set of non-sequenceable electrophoretic tagging molecules that are used as a binary code (Ohlmeyer et al. (1993) *PNAS* 90:10922-10926). Exemplary tags are haloaromatic alkyl ethers that are detectable as their trimethylsilyl ethers at least than femtomolar levels by electron capture gas chromatography (ECGC). Variations in the length of the alkyl chain, as well as the nature and position of the aromatic halide substituents, permit the synthesis of at least 40 such tags, which in principle can encode $2^{40}$ (e.g., upwards of $10^{12}$) different molecules. In the original report (Ohlmeyer et al., supra) the tags were bound to about 1% of the available amine groups of a peptide library via a photocleavable o-nitrobenzyl linker. This approach is convenient when preparing combinatorial libraries of peptide-like or other amine-containing molecules. A more versatile system has, however, been developed that permits encoding of essentially any combinatorial library. Here, the compound would be attached to the solid support via the photocleavable linker and the tag is attached through a catechol ether linker via carbene insertion into the bead matrix (Nestler et al. (1994) *J Org Chem* 59:4723-4724). This orthogonal attachment strategy permits the selective detachment of library members for assay in solution and subsequent decoding by ECGC after oxidative detachment of the tag sets.

Although several amide-linked libraries in the art employ binary encoding with the electrophoric tags attached to amine groups, attaching these tags directly to the bead matrix provides far greater versatility in the structures that can be prepared in encoded combinatorial libraries. Attached in this way, the tags and their linker are nearly as unreactive as the bead matrix itself. Two binary-encoded combinatorial libraries have been reported where the electrophoric tags are attached directly to the solid phase (Ohlmeyer et al. (1995) *PNAS* 92:6027-6031) and provide guidance for generating the subject compound library. Both libraries were constructed using an orthogonal attachment strategy in which the library member was linked to the solid support by a photolabile linker and the tags were attached through a linker cleavable only by vigorous oxidation. Because the library members can be repetitively partially
photoeluted from the solid support, library members can be utilized in multiple assays. Successive photoelution also permits a very high throughput iterative screening strategy: first, multiple beads are placed in 96-well microtiter plates; second, compounds are partially detached and transferred to assay plates; third, a metal binding assay identifies the active wells; fourth, the corresponding beads are rearrayed singly into new microtiter plates; fifth, single active compounds are identified; and sixth, the structures are decoded.

The structures of the compounds useful in the present invention lend themselves readily to efficient synthesis. The nature of the structures, as generally described by formula I, allows the combinatorial assembly of subject inhibitors as shown in an exemplary scheme below. Many suitable reactions, including those depicted below, are both mild and reliable, and are thus well suited for combinatorial chemistry. The nature of such a combinatorial approach towards the generation of a library of test compounds is apparent in the exemplary scheme below, wherein main subunits are linked combinatorially (e.g., using one of the methods described above), with potential combinatorial functionalization of the subunits bestowing additional diversity on the library.

Many variations on the above and related pathways permit the synthesis of widely diverse libraries of compounds which may be tested as inhibitors of prenyltransferases. For example, the isocyanate or chloroformate could be replaced by an isothiocyanate, R₃XS(O)Cl, R₃XS(O)₂Cl, R₃XCH₂Br or another electrophilic reagent. Where X is N, a second R₃ group may be attached, e.g., by deprotonating the amide and treating the resulting anion with a ‘soft’ electrophile, e.g., benzyl iodide, benzyl bromide, etc. Alternatively, the piperazine ring may be treated with phosgene or an equivalent thereof to form a formamyl chloride (NCOCI), which can react with a thiol, amine, or alcohol to form the corresponding urea, urethane, or thiocarbamate. In the above scheme, QM₄₄X (where X represents a leaving group)
may, for example, be a sulfonyl chloride, an acyl chloride, an isocyanate, in isothiocyanate, a chloroformate, an alkyl bromide, or another electrophilic reagent. Alternatively, the group QM₄ may be attached by a reductive amination reaction, a palladium-mediated aryl (or heteroaryl) amine coupling, or any other suitable reaction. In place of Q, a cysteine-like residue, or a protected variant thereof, may be employed to access derivatives of Formulas I and III. In this way, a wide variety of related compounds and derivatives according to Formulas I-IV may be prepared for testing. Analogous strategies may be employed to prepare compounds of the other structures, which differ primarily in the structure of the starting core.

c. Illustrative identification of other compounds of the present invention

The schemes below depict representative synthetic pathways by which such compounds may be accessed, although many other pathways will be known to those of skill in the art. The following references describe reactions which may be useful in preparing compounds active as prenyltransferase inhibitors: Gaare, K. Repstad, T.; Bannache, T.; Undheim, K. *Acta Chemica Scandinavica* 1993, 47, 57-62; Yang, Y.; Wong, H.N.C. *Tetrahedron* 1994, 50, 9583-9608; *J. Am. Chem. Soc.* 1986, 108, 2662; *J. Am. Chem. Soc.* 1970, 92, 6644; *J. Org. Chem.* 1974, 39, 2778. One of ordinary skill in the art will readily be able to synthesize variants wherein, for example, a different aryl or heteroaryl ring is used instead of the methylimidazole ring, a different aryl or heteroaryl ring is used in place of the phenyl ring of the benzyl amides, or linker or spacer groups are added to any of the reagents below.
**Synthesis of 1,4-Piperidine Compounds.**

**Compound 2:** The commercially available 3-methoxycarbonyl-4-piperidone hydrochloride (I) (22.6 g, 0.117 mol) was dissolved in dichloromethane (500 mL). Diisopropylamine (61.1 mL, 0.351 mol) was added, and the mixture was stirred. Di-tert-butyl dicarbonate (30.6 g, 0.140 mol) was added. The mixture was stirred at room temperature for 18 hours. The dichloromethane was stripped off under vacuum and the remaining residue was partitioned between ethyl acetate (200 mL) and 10% citric acid (500 mL). The aqueous layer was extracted a second time with ethyl acetate (100 mL). The ethyl acetate layers were combined and washed with brine (3 x 100 mL). The ethyl acetate was dried over MgSO₄, filtered and concentrated under
vacuum. The residue was columned by flash chromatography (5% ethyl acetate: hexanes followed by 10% and 20%). A clear oil was isolated (30 g, 100% yield).

**Compound 3**: Compound 2 (5 g, 0.019 mol) was dissolved in acetone (170 mL). 4-Bromomethylbiphenyl (5.28 g, 0.021 mol) was added followed by potassium carbonate (10.2 g, 0.073 mol). The reaction was stirred at reflux for 18 hours. The mixture was cooled and filtered by gravity filtration. The filtrate was stripped off under vacuum and the remaining residue was columned by flash chromatography (5% ethyl acetate, followed by 10% then 30%). The desired product was isolated in 65% yield (5.21 g).

**Compound 4**: Compound 3 (5.21 g, 0.012 mol) was dissolved in a minimum of dioxane. 30% H₂SO₄ (61.5 mL) was added and the mixture was refluxed for 18 hours. The mixture was cooled to room temperature. The pH was adjusted to 14 with 50% NaOH. Extraction with methylene chloride was carried out (3 x 70 mL). The methylene chloride layers were combined and washed with brine (3 x 60 mL). The methylene chloride was dried with MgSO₄, filtered and concentrated under vacuum. The desired product was isolated in 96% yield (3.14 g).

**Compound 5**: Triphosgene (2.50 g, 0.00848 mol) was dissolved in toluene (40 mL) and cooled to 0 °C under a nitrogen atmosphere. Bis-(3-chlorobenzyl) amine (2.25 g, 0.00848 mol) was dissolved in a small amount of toluene containing diisopropylethylamine (5.9 mL). This was slowly added via syringe to the triphosgene solution. The mixture was stirred at 0°C for 2 hours. The reaction was purged with nitrogen and properly ventilated until no more gas evolution was observed. Gravity filtration of the reaction mixture into a separatory funnel was carried out. The toluene was washed with brine (3 x 40 mL). The organic layer was dried over MgSO₄, filtered and concentrated under vacuum. The residue was added to a solution of compound 4 (1.5 g, 0.00565 mol) which was dissolved in dichloromethane (40 mL) that contained diisopropylethylamine (5.9 mL). The reaction was stirred for 18 hours at room temperature. The mixture was stripped
down under vacuum and the residue was partitioned between ethyl acetate (80 mL) and 10% citric acid (150 mL). The aqueous layer was extracted again with ethyl acetate (50 mL). The ethyl acetate layers were combined and washed with brine (3 x 40 mL). The ethyl acetate was dried with MgSO₄, filtered and concentrated. Purification was carried out by flash chromatography (10% ethyl acetate: hexanes, followed by 30%). Desired product was isolated in 41% yield (1.30 g).

**Compound 6**: 5-Iodo-1-methylimidazole (0.44 g, 0.00215 mol) was dissolved in anhydrous tetrahydrofuran (6.0 mL) and cooled to 0 °C. Ethyl magnesium bromide (3M, 0.71 mL, 0.00215 mol) was slowly added to the THF solution via syringe while vigorous stirring ensued. After complete addition of the ethyl magnesium bromide, stirring continued for 40 minutes. A THF solution (1 mL) of compound 5 (0.3 g, 0.000538 mol) was prepared and slowly added to the Grignard solution. The reaction mixture was stirred for 18 hours. The reaction was partitioned between ethyl acetate (30 mL) and saturated aqueous sodium bicarbonate (40 mL). A second ethyl acetate extraction was carried out on the aqueous layer. The ethyl acetate layers were combined and washed with brine (2 x 20 mL). The ethyl acetate layers were dried with MgSO₄, filtered and concentrated. Purification was carried out by flash chromatography (100% CH₂Cl₂, followed by 5% MeOH:CH₂Cl₂ then 10% MeOH:CH₂Cl₂).

**Compound 7**: Trimethylsulfoxonium iodide (0.3 g, 0.00134 mol) was dissolved in anhydrous dimethyl sulfoxide (9.0 mL) and kept under a nitrogen atmosphere. Sodium hydride (60% in mineral oil, 0.05 g, 0.00134 mol) was added to the DMSO solution. The ylide was made over a period of two hours at room temperature. Compound 5 was dissolved in a minimum of anhydrous DMSO and added via syringe to the ylide solution. The mixture was stirred at room temperature for 18 hours. The DMSO solution was partitioned between ethyl acetate (30 mL) and 10% citric acid (50 mL). A second extraction of the aqueous layer was carried out and the ethyl acetate layers were combined. Brine washes (3 x 50 mL) were done on the organic layer. The ethyl acetate was then dried over MgSO₄, filtered and
concentrated under vacuum. The residue was columned by flash chromatography (100% hexanes, followed by 5% ethyl acetate: hexanes, 10%, then 30%). A quantitative yield was isolated.

**Compound 8**: Imidazole (0.24 g, 0.00359 mol) was dissolved in anhydrous tetrahydrofuran (7 mL) and kept under a nitrogen atmosphere. Sodium hydride (60% in mineral oil, 0.14 g, 0.00359 mol) was added to the THF solution. The suspension was stirred for 10 minutes at room temperature. Compound 7 (0.000897 mol) was dissolved in a minimum of anhydrous THF and added to the imidazole/NaH solution. The reaction mixture was refluxed for 18 hours. The mixture was partitioned between ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate (50 mL). Another extraction of the aqueous layer was carried out and the ethyl acetate layers were combined. The ethyl acetate was washed with brine (3 x 40 mL). The ethyl acetate was dried over MgSO₄, filtered and concentrated under vacuum.

Purification was carried out by flash chromatography (5% MeOH: CH₂Cl₂, followed by 10% MeOH: CH₂Cl₂). Desired product was isolated in 17% yield (0.101 g).
**Synthesis of 4-Amino-1-piperidine Compounds.**

**Compound 10:** Triphosgene (1.50 g, 0.00505 mol) was dissolved in toluene (30 mL) and cooled to 0 °C under a nitrogen atmosphere. Bis-(3-chlorobenzyl) amine (1.3 g, 0.00505 mol) was dissolved in a small amount of toluene containing diisopropylethylamine (4.0 mL). This was slowly added via syringe to the triphosgene solution. The mixture was stirred at 0 °C for 2 hours. The reaction was purged with nitrogen and properly ventilated until no more gas evolution was observed. Gravity filtration of the reaction mixture into a separatory funnel was carried out. The toluene was washed with brine (3 x 30 mL). The organic layer was dried over MgSO₄, filtered and concentrated under vacuum. The residue was added to a solution of the commercially available 4-N-boc-amino piperidine 9 (1.0 g, 0.00499 mol) which was dissolved in dichloromethane (30 mL) that contained diisopropylethylamine (4.0 mL). The reaction was stirred for 18 hours at room temperature. The mixture was stripped down under vacuum and the residue was partitioned between ethyl acetate (60 mL) and 10% citric acid (90 mL). The aqueous
layer was extracted again with ethyl acetate (40 mL). The ethyl acetate layers were combined and washed with brine (3 x 30 mL). The ethyl acetate was dried with MgSO₄, filtered and concentrated. Purification was carried out by flash chromatography (10% ethyl acetate: hexanes, followed by 30%). Desired product was isolated in 58% yield (1.43 g).

**Compound 11:** Compound 10 (1.42 g, 0.0029 mol) was dissolved in dichloromethane (10 mL). Trifluoroacetic acid (3.0 mL) was added and the mixture was stirred at room temperature for 3 hours. The volatile substances were stripped off under vacuum. The residue was partitioned between dichloromethane (20 mL) and 1.0 N NaOH (80 mL). The aqueous layer was extracted with methylene chloride (3 x 10 mL). The methylene chloride layers were combined and washed with brine (30 mL). The methylene chloride was dried over MgSO₄, filtered and concentrated under vacuum. No further purification was carried out. The desired material was isolated in a 75% yield (0.855 g).

**Compound 12:** Compound 11 (2.32 g, 0.006 mol) was dissolved in 1,2-dichloroethane (60 mL). 1-Methyl-1-H-imidazole-5-carboxaldehyde (0.68 g, 0.0062 mol) was added followed by glacial acetic acid (0.69 mL). Sodium triacetoxyborohydride (2.54 g, 0.0062 mol) was added last. The suspension was stirred for 18 hours. The reaction mixture was stripped of volatile substances and the residue was partitioned between ethyl acetate (50 mL) and 1.0 N NaOH (100 mL). The aqueous layer was extracted a second time and the ethyl acetate layers were combined. The organic layer was washed with brine (3 x 40 mL) and then dried over MgSO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography (5% MeOH: CH₂Cl₂ followed by 10%). The desired material was isolated in a 71% yield (2.16 g).

**Compound 13:** Compound 12 (0.150 g, 0.000309 mol) was dissolved in 1,2-dichloroethane (3.0 mL). 4-Biphenylycarboxaldehyde (0.225 g, 0.00123 mol) was added followed by glacial acetic acid (0.035 mL). Sodium triacetoxyborohydride
(0.13 g, 0.000613 mol) was added last. The suspension was stirred for 18 hours. The reaction mixture was stripped of volatile substances and the residue was partitioned between ethyl acetate (10 mL) and 1.0 N NaOH (20 mL). The aqueous layer was extracted a second time and the ethyl acetate layers were combined. The organic layer was washed with brine (3 x 5 mL) and then dried over MgSO₄, filtered and concentrated under vacuum. The residue was purified by preparatory high performance liquid chromatography. The desired material was isolated in a 41% yield (0.0819 g).
15. To a solution of BocSer(OtBu)OH (15.0 g, 57.4 mmol) in DMF (200 mL) was added HOBT (86.1 mmol, 13.2 g), DIPEA (143.5 mmol, 18.5 g), N-Benzylglycine ethyl ester (63.2 mmol, 12.2 g), and HBTU (86.1 mmol, 32.6 g). The reaction mixture was stirred at room temperature for 16 hours and then poured into 1 N HCl (200 mL). The mixture was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with sat. aq NaHCO₃ (1 x 200 mL) and brine
(1 X 200 mL), dried (MgSO₄) and concentrated. The crude residue was purified by flash chromatography to give 15 (16.2 g, 97% yield) as a white foam.

16. 15 (55.7 mmol, 16.2 g) was dissolved in EtOAc (550 mL) containing anhydrous HCl (550 mmol, 20 g). The reaction mixture was stirred for 2 hours at room temperature and then concentrated. The residue was dissolved in Et₂O (300 mL). Sat. aq NaHCO₃ (300 mL) was added and the reaction mixture was stirred at room temperature for 4 hours. The organic layer was separated, washed with brine (1 X 200 mL), dried (MgSO₄) and concentrated. The crude residue was purified by recrystallization from EtOAc to give 16 (11.5 g, 71% yield) as a crystalline white solid.

17. To a suspension of LiAlH₄ (159.0 mmol, 6.0 g) in THF (300 mL) was added 16 (39.5 mmol, 11.5 g); portion wise over 15 min. The reaction mixture was heated at reflux for 16 hours, then cooled to 0 °C. H₂O (6 mL) was added followed by 15% NaOH (6 mL) and then H₂O (18 mL). The white ppt was removed by filtration and the filtrate was concentrated to give 17 (8.4 g, 81% yield) as a viscous oil.

18. To a solution of 17 (8.4 g, 32.1 mmol) in CH₂Cl₂ (100 mL) was added TEA (64.3 mmol, 6.5 g) followed by di-tert-butyl dicarbonate (48.2 mmol, 10.5 g). The reaction mixture was stirred at room temperature for 16 hours and then poured into sat aq NH₄Cl (100 mL). The organic layer was separated, washed with brine (1 X 100 mL), dried (MgSO₄), and concentrated to give 18 (10.3 g, 92% yield) as a white foam.

19. 10 % Pd/C (1.0 mmol, 1.0 g) was added to a solution of 18 in MeOH (100 mL) under an atmosphere of argon. The chamber was flushed with H₂ and shaken on a Parr apparatus at 60 psi for 16 hours. The catalyst was removed by filtration and the filtrate was concentrated to give 19 (7.9 g, 98% yield) as a white foam.

20. A solution of bis-(3-Cl-benzyl)amine (23.5 mmol, 6.3 g) in CH₂Cl₂ (100 mL)
containing DIPEA (25.9 mmol, 3.3 g) was added to a solution of triphosgene (8.7 mmol, 2.6 g) in CH₂Cl₂ (50 mL) over a period of 30 min. The reaction mixture was stirred an additional 15 min and a solution of 19 (23.5 mmol, 6.4 g) in CH₂Cl₂ (50 mL) containing DIPEA (25.9 mmol, 3.3 g) was added. The reaction was stirred for 3 hours and then concentrated. The residue was dissolved in EtOAc (200 mL) and washed with 10% aq KH₂PO₄ (1 X 100 mL), sat. aq NaHCO₃ (1 X 100 mL), and brine (1 X 100 mL). The organic layer was dried (MgSO₄) and concentrated. The crude residue was purified by flash chromatography to give 20 (10.9 g, 82% yield) as a white foam.

21. 20 (19.3 mmol, 10.9 g) was dissolved in EtOAc (190 mL) containing anhydrous HCl (190.0 mmol, 7.0 g). The reaction mixture was stirred for 2 hours at room temperature and then poured into 2 N NaOH (125 mL). The organic layer was separated, washed with brine (1 X 100 mL), dried (MgSO₄) and concentrated to give 21 (7.9 g, 88% yield) as a viscous oil.

22. To a solution of BocCys(STrOH) (8.9 g, 19.2 mmol) in CH₂Cl₂ (100 mL) was added HOBT (32.0 mmol, 4.9 g), DIPEA (42.5 mmol, 10.0 g), 21 (17.0 mmol, 7.9 g), and HBTU (32.0 mmol, 12.1 g). The reaction mixture was stirred at room temperature for 16 hours and then poured into 1 N HCl (100 mL). The mixture was extracted with EtOAc (3 X 100 mL) and the combined organic layers were washed with sat. aq NaHCO₃ (1 X 100 mL) and brine (1 X 100 mL). The organic layer was dried (MgSO₄) and concentrated. The crude residue was purified by flash chromatography to give 22 (12.3 g, 79% yield) as a white foam.

23. 22 (13.5 mmol, 12.3 g) was dissolved in CH₂Cl₂ (200 mL) and triethylsilane (33.8 mmol, 3.9 g) was added followed by TFA (200 mL). The reaction mixture was stirred for 3 hours and then concentrated. The crude residue was triturated with Et₂O (2 X 50 mL) and then purified by prep. reverse phase HPLC to give 23 as a white solid.
25. To a solution of 24 (21.6 mmol, 12.2 g) in CICH₂CH₂Cl (100 mL) was added 1-methyl-1H-imidazole-5-carboxaldehyde (23.7 mmol, 2.6 g). The reaction was stirred for 15 min and NaHBO₃(OAc)₂ (30.2 mmol, 6.4 g) was added. The reaction was stirred at room temperature for 16 hours, and poured into 1 N NaOH (100 mL). The mixture was extracted with EtOAc (3 X 100 mL) and the combined organic layers were washed with brine (1 X 100 mL), dried (MgSO₄) and concentrated. The crude residue was dissolved in conc. HCl (125 mL) and stirred until the solution became clear. The mixture was cooled to 0°C and brought to pH 12 by the addition of 10 N NaOH and extracted with EtOAc (3 X 200 mL). The combined organic layers were washed with brine (1 X 150 mL), dried (MgSO₄) and concentrated to give 25 (11.0 g, 78% yield) as a white foam.
26. A solution of bis-(3-Cl-benzyl)amine (64.4 mmol, 17.2 g) in CH₂Cl₂ (215 mL) containing DIPEA (70.9 mmol, 9.2 g) was added to a solution of triphosgene (23.8 mmol, 7.1 g) in CH₂Cl₂ (125 mL) over a period of 30 min. The reaction mixture was stirred an additional 15 min and a solution of (3S)-(−)-3-(t-butoxycarbonylamino)pyrrolidine (64.4 mmol, 12 g) in CH₂Cl₂ (125 mL) containing DIPEA (70.9 mmol, 9.2 g) was added. The reaction was stirred for 3 hours and then concentrated. The residue was dissolved in EtOAc (400 mL) and washed with 10% aq KHSO₄ (1 X 150 mL), sat. aq NaHCO₃ (1 X 150 mL), and brine (1 X 100 mL). The organic layer was dried (MgSO₄) and concentrated. The crude residue was purified by flash chromatography to give 26 (27.0 g, 87% yield) as a white foam.

27. TFA (75 mL) was added to a solution of 26 (56.2 mmol, 27.0 g) in CH₂Cl₂ (400 mL). The reaction was stirred for 3 hours and then concentrated. The crude residue was dissolved in EtOAc (300 mL), washed with 1 N NaOH (1 X 150 mL) and brine (1 X 150 mL), dried (MgSO₄), then concentrated to give 27 (21.5 g, 100% yield) as a white solid.

28. To a solution of 27 (28.8 mmol, 11.2 g) in CICH₂CH₂Cl (115 mL) was added 1-methyl-1H-imidazole-5-carboxaldehyde (31.6 mmol, 3.5 g). The reaction was stirred for 15 min and NaHB(OAc)₃ (40.3 mmol, 8.5 g) was added. The reaction was stirred at room temperature for 16 hours and poured into 1N NaOH (150 mL). The mixture was extracted with EtOAc (3 X 100 mL) and the combined organic layers were washed with brine (1 X 100 mL), dried (MgSO₄) and concentrated. The crude residue was dissolved in conc. HCl (125 mL) and stirred until the solution became clear. The mixture was cooled to 0 °C and brought to pH 12 by the addition of 10 N NaOH and extracted with EtOAc (3 X 200 mL). The combined organic layers were washed with brine (1 X 150 mL), dried (MgSO₄) and concentrated to give 28 (12.6 g, 91% yield) as a white foam.

29. To a solution of 28 (13.0 mmol, 6.3 g) in CICH₂CH₂Cl (50 mL) was added 4-phenylbenzaldehyde (14.33 mmol, 2.6 g) followed by AcOH (52.1 mmol, 3.1 g).
The reaction was stirred for 15 min and NaHB(OAc)₃ (18.2 mmol, 3.9 g) was added. The reaction was stirred at room temperature for 16 hours and poured into 1N NaOH (50 ml). The mixture was extracted with EtOAc (3 X 50 mL) and the combined organic layers were washed with brine (1 X 50 mL), dried (MgSO₄) and concentrated. The crude residue was dissolved in conc. HCl (75 mL) and stirred until the solution became clear. The mixture was cooled to 0 °C and brought to pH 12 by the addition of 10 N NaOH and extracted with EtOAc (3 X 100 mL). The combined organic layers were washed with brine (1 X 50 mL), dried (MgSO₄) and concentrated to give 29 (6.5 g, 77% yield) as a white foam.

*Inhibition of prenyltransferases*

a. SAR of prenyltransferase inhibitors

As described below, a variety of different compounds were tested for inhibitory activity against human and *Candida* GGPTase.
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b. Demonstration of the effect of GGPTase inhibitors on the prenylation state of newly synthesized CaRHO1.

(i) Methodology.

To look at the effect of GGPTase I inhibitors *in vivo*, a recombinant *C. albicans* strain engineered to express a Myc tagged CaRHO1 under the control of the
C. albicans PCK1 promoter is used. This promoter is repressed by glucose and derepressed by gluconeogenic carbon sources such as succinate. It should also be possible to look at the endogenous substrates of the GGPTase I. Cells are treated with a sublethal dose of compound for a period of time which has been established from a kill curve analysis in the appropriate media. After the treatment time, cells are harvested and whole cell extracts (WCE) made, these extracts are then resolved by high speed centrifugation into cytosolic and membrane fractions. Visualisation of the localisation of the MycCaRHO1 is achieved by SDS-PAGE and Western blotting. MycCaRHO1 that has been geranylgeranylated will be localised to the membrane whereas ungeranylgeranylated protein should be found in the cytosolic fraction.

Treatment of cells with DMSO (mock) and GGPTase I inhibitor MycCaRHO1 will be apparent in the WCE and pellet fractions. In mock treated cells, MycCaRHO1 should be absent from the cytosolic fraction, whereas in GGPTase I inhibitor treated cells, some MycCaRHO1 should be apparent in the cytosolic fraction indicating that a proportion of the newly synthesized MycCaRHO1 has not been geranylgeranylated. Figure 32 shows that this prediction is borne out.

(ii) Generation of the CaRHO1 replacement construct.

The 5' and 3' non-coding regions of CaRHO1 were generated by PCR and cloned into pBluescript KS- in which the CaRHO1 ORF was exactly replaced with a BamHI site. Into this vector (pSCaRHO1.5c23) a PCK1.CaURA3 cassette was inserted from pSCaPCK1.3c1 to generate pSCaRHO1.19c1. This vector was mutagenised to destroy one of the two BamHI sites (pSCaRHO1.22c22) into which the Myc tagged CaRHO1 ORF (from pSCaRHO1.20c58) was inserted. The sequence of the oligos used to generate the Myc tagged CaRHO1 ORF are:

CaRHO1.13: 5' CCCGGATCCCTTACAAGACACACATTTCTT 3'
CaRHO1.13: 5' CCGGGATCCCTTACATAATGTCTGAACAAAAATTGATATCA GAAGAAGATTTGGTTAACGG 3'

the sequence of the Myc tag is underlined and corresponds to the amino acid sequence EQKLISEEDL. This epitope is recognized by the commercially available 9E10 monoclonal antibody. The final vector designated pSCaRHO1.23c21, harbours of the 5' non-coding region of CaRHO1, the CaURA3 selectable marker, the C. albicans PCK1 promoter directing the expression of the Myc-tagged CaRHO1 and
the 3' untranslated region of CaRHO1. The presence of the CaRHO1 5' and 3' regions should direct this cassette to one of the 2 WT alleles of CaRHO1 by homologous recombination.

(iii) Generation of the C. albicans PCK1-MycCaRHO1 strain

The PCK1-MycCaRHO1 replacement construct was excised by a BssHII digest from the parent plasmid pSCaRHO1.30c21. The desired fragment was gel purified prior to being transformed into the C. albicans strain CAF3-1. The method used for CAF3-1 transformation is a lithium acetate protocol (from U. of Minnesota C. albicans web site: http://alces.med.umn.edu/ candida/liac.html). The transformation mixture is then plated onto selective (-Ura glucose) plates and incubated at 30 °C for 3 days. Individual transformants that appear are restreaked for singles and then preserved as a glycerol stock. To ensure that the correct integrative event has occurred, southern analysis was carried out on several colonies. Those colonies that exhibited the correct genotype were retained.

The strain used for the work described here is referred to as DIY-BL2-058.

(iv) Growth and treatment of cells

Cells of strain DIY-BL2-058 were grown overnight in YNB supplemented with 1 µg/ml histidine, 2 µg/ml methionine, 2 µg/ml tryptophan, 200 µg/ml glutamine and 2% glucose at 220 rpm at 31 °C. The cell number was then determined, cells were pelleted by centrifugation and resuspended in fresh media at a density of 1x10^7 cells/ml and incubated as above. Cells were either treated with 14 µl DMSO alone or 14 µl of a 25.6 mg/ml stock of inhibitor in DMSO (3 µg/ml final concentration). After 3 hrs incubation cells were pelleted, washed twice and resuspended to the original volume with the following media: YNB supplemented with 1 µg/ml histidine, 2 µg/ml methionine, 2 µg/ml tryptophan, 200 µg/ml glutamine, 2% succinate and 0.05% glucose. The PCK1 promoter is repressed in the media containing 2% glucose. The switch in media to 2% succinate, 0.05% glucose partially derepresses the PCK1 promoter such that the MycCaRHO1 protein is not overproduced. DMSO or inhibitor is then again added to this new media and the cells incubated for a further 5hrs. After the required incubation the cells are pelleted and frozen at -80 °C.
(v) Generation and fractionation of cellular extracts

To generate cellular extracts, 10x TE supplemented with a protease inhibitors cocktail was added at 3-4 volumes of the pellet size (about 200 μl) and glass beads (425-600 microns; Sigma) were added to the meniscus. This mixture was then subjected to 5 1' pulses in a bead beater with 2' on ice between pulses. The mixture was then centrifuged at 3000 rpm to pellet cellular debris and the supernatant removed. The beads were washed with an equal volume of buffer and the supernatant added to the initial sample. This whole cell extract (WCE) was again centrifuged at 3000 rpm and the supernatent removed into a fresh tube. 50 μl of this WCE was subjected to high speed centrifugation (54000 rpm for 1 hr in a TI120.1 rotor) to resolve the membrane and cytosolic fractions. The cytosolic fraction was carefully removed. The membrane pellet fraction was washed with buffer and resuspended in 1x loading buffer. All fractions were frozen at -80 °C.

(vi) SDS-PAGE and Western Blotting

Fractions were thawed on ice. The protein concentration was determined using the standard Bradford method for the WCEs and cytosolic fraction. 30 μg of protein were loaded for both the WCE and cytosolic fractions. For the membrane fraction, a volume equal to that loaded for the cytosolic fraction was loaded. Prior to loading, all fractions were boiled for 3' with loading dye. Standard procedures were employed for the SDS-PAGE and Western blotting.

To analyse the Western blot, the blot was pre-blocked with 4% fat free milk in PBST. The 9E10 monoclonal anti-myc epitope antibody (available from Calbiochem) was incubated with the blot overnight at 4 °C at a concentration recommended by the manufacturers. The primary antibody was removed and the blot was washed 3x 15' with PBST. The blot is then incubated with 2° antibody which was goat anti-mouse HRP conjugated antibody for 1 hr at room temperature. The 2° antibody is removed and the blot washed again with 3x 15' with PBST and developed using the Pierce luminescent kit according to the manufacturers instructions.

As shown in Figure 32, exposure of cells to a GGPTase I inhibitor increases the abundance of MycCaRHO1 in the cytosolic fraction (inhibitor-treated cells) but not of mock (DMSO) treated cells. Numbers 1-6 indicate the lanes of the gel which are denoted as W, whole cell extract, C, cytosolic fraction and P, pellet fraction. Protein molecular weight markers are indicated.
c. In vitro assays of fungal GGPTase inhibitors

(i) Assay protocol for determining IC50

Plate test compounds (10 μL per well) at predetermined concentration in 50% DMSO. For background control (blank) and reaction control (negative), add 10 μL of 200 μM GGPP and 10 μL 50% DMSO, respectively. Prepare assay buffer: 50 mM Tris, pH7.5, 20 mM KCl, 5 mM MgCl₂, 5 μM ZnCl₂, 0.5 mM Zw(3-14), 2 mM DTT and 0.1 mg/mL BSA.

Add 20 μL of *C. albicans* GGPTase and ³H-GGPP in assay buffer to test compound. Preincubate enzyme and ³H-GGPP with test compound for 15 minutes at room temperature. Add 20 μL *C. albicans* Rho in assay buffer. Incubate for 30 minutes at room temperature. Final assay conditions are 2 nM *C. albicans* GGPTase, 250 nM ³H-GGPP and 250 nM *C. albicans* Rho.

Add 100 μL 15 mM GGPP, 50 mM Tris, pH 7.0 and 2% BSA to quench reaction. Transfer reaction to Nickel chelate FlashPlate. Allow his-tagged *C. albicans* Rho to capture onto plate. Rinse plate 1x with 200 μL 20 mM Tris, pH 7.0. Read in TOPCOUNT.

(ii) *In vitro* susceptibility testing of compounds in *C. albicans*

1: Innoculate strain *C. albicans* strain such as SC5314 into 20 mL of the appropriate medium and incubate at 35 °C with shaking (220 rpm) overnight

2: Count the *C. albicans* cells in a 1:10 dilution of the overnight culture using a haemocytometer.

3: Work out the dilution factor required to bring the cell number to 1x10³ cells/100 μL (equivalent to 1x10⁴ cells/mL) then add the required volume of the overnight culture to 25 mL media in a falcon tube.

4: Vortex the diluted cells and immediately pipette 100 μL of the cell suspension to each of the required rows of a 96 well plate using the multipipettor

5: Prepare each of the 100x stock solutions for the compounds to be tested in DMSO in the required concentration range in Eppendorf tubes.

6: The dilution series for each of the compounds may now be prepared in sequence:
For each compound - start with highest dilution. Add 10 μL compound in DMSO to the 490 μL of appropriate media. Immediately vortex and add 100 μL to the appropriate row of cells on the 96-well plate. Repeat this process for the next and subsequent concentrations of this compound before starting on the dilution series for additional compounds.

7: When complete cover the 96-well plate with an acetate sheet and incubate at 35 °C. Inspect visually and record results for both plates at 24 hr and 48 hr. The MIC corresponds to the concentration of compound where no visible growth is observed.

(iii) Determination of Minimum fungicidal concentrations (MFC)

After the required time course for the MIC determination, the minimum fungicidal concentration can then be determined by plating out the entire contents of the well of the microtitre plates onto YPD or Sabourand plates. These plates are then incubated at 35 °C for 24-48 hrs. The MFC corresponds to the concentration of compound where no cellular growth is observed on the plate.

(iv) Assay protocol for determining cytotoxicity of GGPTase inhibitors in human cells

(A) Plate out cells at predetermined concentration in a volume of 150 μL.

(B) Allow cells to adhere to plate for twenty four hours

(C) Add compounds to cells at predetermined concentration (62.5 μg/mL down four-fold, 8 dilutions) n=2

(D) Cells are exposed to drug for 7 days for the IMR90 Cell Line, and a period of 3 days for the H460 Cell Line.

(E) 1. H460 Cells are fixed in TCA, rinsed, stained with Sulforhodamine B stain, and the stain is solubilized for a final OD read.

2. IMR90 Cells have 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) added to them for three hours prior to final read out. After the three hours, media and MTT are removed and MTT crystals are solubilized in 100% DMSO for final OD read.
All of the references and publications cited herein and U.S. Application No. 09/182,845 are hereby incorporated by reference.

_Equivalents_

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.
Claims:

1. A compound having a structure represented by general formula II:

   \[
   \begin{array}{c}
   Q \overset{M}{\longrightarrow} \overset{N}{\longrightarrow} \overset{W}{\longrightarrow} \overset{Ar}{\longrightarrow} \\
   R_4 \quad \quad \quad \quad \quad \quad \quad \quad \quad R_3 \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad W \\
   \end{array}
   \]

   wherein

   Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure;

   Ar represents an aryl or heteroaryl ring;

   W represents \(-\text{C}(=\text{Y})\), \(-\text{S}(-\text{O})\), or \(-\text{S}(-\text{O})_2\);

   Y is O or S;

   X represents O, S, or NR_3, preferably NR_3;

   R_3, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or a natural or unnatural amino acid residue, or two R_3 taken together form a 4- to 8-membered ring, which ring includes one or more carbonyls and/or heteroatoms;

   R_4 represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R_8)_2, OR_8, SR_8, C(=O)R_8, COOR_8, CON(R_8)_2, or an amino acid residue;

   R_8, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

   M represents, independently for each occurrence, a substituted or unsubstituted methylene group, a heteroatom selected from O, S, or NR_8, a subunit selected from \(-\text{C}(=\text{Y})\), \(-\text{S}(-\text{O})\), \(-\text{CH}_2\), or \(-\text{S}(-\text{O})_2\), or two M taken together represent substituted or unsubstituted ethene or ethyne; and

   q represents an integer from 0 to 3.
2. A compound having a structure represented by general formula IV:

![Structure IV](image)

wherein

- Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure;
- Ar represents an aryl or heteroaryl ring;
- Y is O or S;
- R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, or a natural or unnatural amino acid residue, or two R₃ taken together form a 4- to 8-membered ring, which ring optionally includes one or more carbonyls and/or heteroatoms;
- R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroarylalkyl;
- M represents, independently for each occurrence, a substituted or unsubstituted methylene group, a heteroatom selected from O, S, or NR₈, a subunit selected from \(-C(=Y)\)-, \(-S(O)\)-, \(-CH₂\)-, or \(-S(O)₂\)-, or two M taken together represent substituted or unsubstituted ethene or ethyne; and
- r represents an integer from 0 to 2.

3. A compound having a structure represented by general formula VI:

![Structure VI](image)

wherein
Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure;

Ar represents an aryl or heteroaryl ring;

W represents –C(=Y)–, -S(O)–, or –S(O)₂–;

Y is O or S;

Z is H or OH;

R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or a natural or unnatural amino acid residue, or two R₃ taken together form a 4- to 8-membered ring, which ring includes one or more carbonyls and/or heteroatoms;

R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl, heterocyclyl, aralkyl, heteroaryl, heteroaralkyl, N(R₈)₂, OR₈, SR₈, C(=O)R₈, COOR₈, CON(R₈)₂, or an amino acid residue;

R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, a heteroatom selected from O, S, or NR₈, a subunit selected from –C(=Y)–, -S(O)–, -CH₂–, or –S(O)₂–, or two M taken together represent substituted or unsubstituted ethene or ethyne;

x and y, independently, represent 0, 1, or 2; and

q represents an integer from 0 to 3.

4. A compound having a structure represented by general formula X:

wherein
Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure;
Ar represents an aryl or heteroaryl ring;
W represents \(-\text{C}(\equiv\text{Y})\), \(-\text{S(O)}\), or \(-\text{S(O)}_2\);
V is H or OH;
X represents O, S, or NR\(_3\), preferably NR\(_3\);
Y is O or S;
Z is H or OH;
R\(_1\) represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
R\(_2\), independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
R\(_3\), independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or a natural or unnatural amino acid residue or two R\(_3\) taken together form a 4- to 8-membered ring, which ring includes one or more carbonyls and/or heteroatoms;
R\(_8\), independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;
M represents, independently for each occurrence, a substituted or unsubstituted methylene group, a heteroatom selected from O, S, or NR\(_8\), a subunit selected from \(-\text{C}(\equiv\text{Y})\), \(-\text{S(O)}\), \(-\text{CH}_2\), or \(-\text{S(O)}_2\), or two M taken together represent substituted or unsubstituted ethene or ethyne; and
x and y, independently, represent 0, 1, or 2;
q represents an integer from 0 to 3.

5. A compound having a structure represented by general formula XIII:
wherein
Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure;
W represents \(-\text{C(=Y)}\), \(-\text{S(O)}\), or \(-\text{S(O)}_2\);
Y is O or S;
X represents O, S, or NR, preferably NR; 
R₃, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl,
heterocyclyl, heterocyclylalkyl, aryl, aralkyl, heteroaryl, heteroarylalkyl, or a natural or unnatural amino acid residue, or two R₃ taken together form a 4- to 8-membered ring, which ring includes one or more carbonyls and/or heteroatoms;
R₄ represents, as valency permits, from 0 to 8 substituents on the ring to which it is attached, selected from H, or substituted or unsubstituted alkyl, aryl,
heterocyclyl, aralkyl, heteroaryl, heteroarylalkyl, \(\text{N(R₈)}_2\), \(\text{OR₈}\), \(\text{SR₈}\), \(\text{C(=O)R₈}\),
\(\text{COOR₈}\), \(\text{CON(R₈)}_2\), or an amino acid residue;
R₈, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, ary, heteroaryl, heteroarylalkyl;
M represents, independently for each occurrence, a substituted or unsubstituted methylene group, a heteroatom selected from O, S, or NR, a subunit selected from \(-\text{C(=Y)}\), \(-\text{S(O)}\), \(-\text{CH}_2\), or \(-\text{S(O)}_2\), or two M taken together represent substituted or unsubstituted ethene or ethyne; and
q represents an integer from 0 to 3.

6. A compound having a structure represented by general formula XV:
wherein

Q represents a substituted or unsubstituted heteroaryl moiety containing at least one nitrogen atom in the ring structure;

Y is O or S;

$R_3$, independently for each occurrence, represents H, substituted or unsubstituted lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or a natural or unnatural amino acid residue, or two $R_3$ taken together form a 4- to 8-membered ring, which ring optionally includes one or more carbonyls and/or heteroatoms;

$R_8$, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl;

M represents, independently for each occurrence, a substituted or unsubstituted methylene group, a heteroatom selected from O, S, or NR$_8$, a subunit selected from $-C(=Y)-$, $-S(O)-$, $-CH_2-$, or $-S(O)_2-$, or two M taken together represent substituted or unsubstituted ethene or ethyne; and

r represents an integer from 0 to 2.

7. The compound of any preceding claim, wherein the compound inhibits a prenyltransferase activity with an IC$_{50}$ of 1 µM or less.

8. The compound of any preceding claim, wherein the compound inhibits a prenyltransferase activity of a fungal pathogen with an IC$_{50}$ at least 1 order of magnitude lower than for a prenyltransferase activity of a human.

9. The compound of any preceding claim, wherein the compound inhibits growth of a fungal pathogen with a MIC of less than 7 µg/mL.
10. A method for inhibiting fungal cell growth, comprising treating fungal cells with a compound of any preceding claim.

11. A method for disinfecting an inanimate surface, comprising treating the surface with the compound of any of claims 1-9.


14. The method of claim 13, wherein the compound has a therapeutic index of at least 10 in the animal being treated.

15. A pharmaceutical preparation for treating or preventing growth of a fungal pathogen, comprising: (i) a compound of any of claims 1-9; and (ii) a pharmaceutically suitable excipient.

16. A pharmaceutical preparation for treating or preventing unwanted cell proliferation, comprising: (i) a compound of any of claims 1-9, and (ii) a pharmaceutically suitable excipient.

17. An antiseptic preparation for disinfecting an inanimate surface, comprising a compound of any of claims 1-9 which inhibits a prenyltransferase activity of a fungal pathogen.

18. An agricultural product for application for preventing or treating fungal infection of plants, comprising a compound of any of claims 1-9 which inhibits a prenyltransferase activity of a fungal pathogen.

19. A feedstock comprising a compound of any of claims 1-9 which inhibits a prenyltransferase activity of a fungal pathogen.
20. A method for treating an animal having an infection with a fungal pathogen comprising
diagnosing an animal as having a fungal infection or as being at risk of developing a
fungal infection, and
administering to the animal a compound of any of claims 1-9 which inhibits a
prenyltransferase activity of the pathogen in an amount effective to reduce or
eliminate the fungal infection.

21. A compound of claim 2 or 6, wherein R₃ does not include a phenethyldiazaryl
moiety.

22. A compound of claim 1 or 3-5, wherein neither R₃ nor R₄ includes a
phenethyldiazaryl moiety.
Fig. 3
Fig. 5
Fig. 6
Fig. 7
\[
\begin{align*}
J-L-OH & \quad 62 \quad \rightarrow \quad J-L-O-\text{NR}_2 \\
& \quad 63 \quad \rightarrow \quad J-L-O-CO \quad \text{OR}
\end{align*}
\]

Fig. 11
Fig. 12
Fig. 24
Fig. 25
Fig. 27
Fig. 28
Fig. 29
Fig. 30
Fig. 31
## INTERNATIONAL SEARCH REPORT

### Document Summary

**International application No.**

- **PCT/US02/38511**

**A. CLASSIFICATION OF SUBJECT MATTER**

- **IPC(7)**: A61K 31/40, 31/4178, 31/445, 31/454, 31/495, 31/496; C07D 207/14, 211/56, 241/04, 401/04, 401/06, 401/12, 403/06, 403/12.
- **US CL**: 514/253.01, 254.05, 255.01, 326, 329, 343, 397, 544/360, 370, 388, 546/194, 210, 224, 548/314, 7, 538.

### Fields Searched

Minimum documentation searched (classification system followed by classification symbols)

- **U.S.:** Please See Continuation Sheet

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- **CAS ONLINE STRUCTURE SEARCH**

### Documents Considered to be Relevant

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 00/51611 A1 (MERCK &amp; CO., INC.) 08 September 2000(08.09.00), see entire document especially pages 30-34 and carboxamide species on pages 37-38 and 42 and pages 84-85 for a list of uses.</td>
<td>1,2,7,10,21-22</td>
</tr>
<tr>
<td>X,P</td>
<td>US 6,358,956 B1 (HARTMAN et al.) 19 March 2002(19.03.02), see especially columns 13-18 and carboxamide species in columns 20-22 and list of uses in columns 49-50.</td>
<td>1,2,7,10,21-22</td>
</tr>
</tbody>
</table>

- **»** Special categories of cited documents:
  - **»T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - **»X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - **»Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  - **»A** document member of the same patent family

**Date of the actual completion of the international search**

11 February 2003 (11.02.2003)

**Name and mailing address of the ISA/US**

- **Commissioner of Patents and Trademarks**
- **Box PCT**
- **Washington, D.C. 20231**

**Authorized officer**

- **Teresa D. Roberts**

**Telephone No.** (703) 308-1235

**Facsimile No.** (703) 308-2330

**Date of mailing of the international search report**

13 Mar 2003

**Form PCT/ISA/210** (second sheet) July 1998
INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claim Nos.: 1-7, 10, 21-22 (all in part) because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
   Please See Continuation Sheet

3. □ Claim Nos.: 8, 9, 11-20 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
□ The additional search fees were accompanied by the applicant’s protest.
□ No protest accompanied the payment of additional search fees.
Continuation of Box 1 Reason 2:
The claims relate to an extremely large number of permutations based on the scope of variables as generically set forth in the claims which are not all adequately supported in the description within the meaning of Article 6. The claims have been searched based on the classification and/or structural makeup of examples listed in Tables 1-3 in the description.

Continuation of B. FIELDS SEARCHED Item 1:
514/253.01,254.05,254.07,255.01,318,326,329,343,397,423; 544/360,366,370,388,546/194,210,224,274,7,548/314,7,538.