INHIBITORS OF YEAST FILAMENTOUS GROWTH AND METHOD OF THEIR MANUFACTURE

FIELD OF THE INVENTION

The invention relates to the use of α,β-unsaturated fatty acids to inhibit the filamentous growth of fungi and yeasts and to a method for producing same. In particular the invention relates to the use of optionally substituted C_8 to C_{15} fatty acids or salts, esters or amides thereof for inhibiting or retarding the yeast-to-mycelium transition of organisms having a dimorphic life cycle.

BACKGROUND OF THE INVENTION

Candida albicans

The dimorphic fungus *Candida albicans* is one of the most significant human fungal pathogens, particularly in immuno-compromised patients. This fungus can infect and colonize in a wide range of micro-environments in the body including the blood stream, superficial sites in the mucosa and all of the major internal organs, during systemic infections. [1,2]

A distinguishing feature of *C. albicans* is the yeast-mycelium dimorphism. The yeast-to-mycelium morphogenic transition is one of several essential virulence attributes in human pathogenesis. The yeast-to-mycelium morphogenic transition is triggered by various signals, including serum, high temperature, neutral pH, nutrient poor media, and certain chemicals such as *N*-acetylglucosamine. It appears that many of the responses to these signals reflect normal interactions between the fungus and its host *in vivo*.

The yeast-to-mycelium transition has been shown to be one of several virulence attributes that enable *C. albicans* to invade human tissues. [3-5] Patients with serious disease generally have filaments of *C. albicans* penetrating the infected tissue. Various genes involved in hyphal morphogenesis have been identified, including *cek1*, *cla4*, *cpp1*, *cst20*, and *tup1*, encoding MAP kinase, PAK, phosphatase, MEKK kinase, and

transcription factor, respectively. Mutation of these genes blocked *C. albicans* yeast-to-mycelium transition and attenuated its virulence against mouse animal models. [6,7]

Several categories of natural and synthetic chemicals have been evaluated in recent years against *C. albicans* cell growth and dimorphism transition. A novel cyclic β-amino acid that inhibits isoleucyl-tRNA synthetase was shown to inhibit cell growth of *C. albicans*. [8] Carvone and perillaldehyde were found to interfere with the formation of filamentous structures of *C. albicans*. [9]

An autoregulatory substance (ARS) from the eukaryote *C. albicans*, characterized as 3,7,11-trimethyl-2,6,10-dodecatrienoic acid (farnesoic acid), inhibits *C. albicans* germ tube (mycelium) formation and appears to play a key role in the regulation of the morphological transition in *C. albicans*. [10] Its derivatives have been evaluated for their activity in regulation of morphological transitions in *C. albicans* and it was concluded that the *trans* isomer of farnesoic acid is essential for its potent inhibition of the yeast-to-mycelium transition. [11]

Farnesol, a close derivative of ARS, was shown to prevent mycelial development of *C. albicans* in both growth morphology assay and differentiation assay using three chemically distinct activators for germ tube formation (L-proline, N-acetylglucosamine, and serum). [12]

Farnesol and its metabolite farnesoic acid (ARS) is also found in higher life forms, including humans. They are generated intracellularly and some of the farnesyl derivatives are required for synthesis of cholesterol, steroids, retinoids, and farnesylated proteins. [16,17] Moreover, farnesol metabolites have been implicated as signalling molecules in the regulation of cholesterol degradation through activation of FXR, a nuclear receptor which represses cholesterol metabolism pathway. [16,18] Conceivably, tipping over the subtle balance of these important intermediates and cellular signals may lead to substantial changes in normal physiological processes.

A diffusible signal factor (DSF) from the plant pathogen *Xanthomonas campestris* is a prokaryotic cell-cell communication signal and required for bacterial virulence, [13] but little is known about its structure and scope of function.

Rapid emergence of antibiotic resistance demands development of alternative approaches to prevent and control infectious diseases. As the lethal effect of antibiotics is the common cause to force microbes to mutate and survive, non-antimicrobial means of controlling *C. albicans* and other microorganisms could be useful to avoid or delay the development of antibiotic-resistance. Inhibiting the yeast-to-mycelium transition avoids selection of resistant organisms.

Synthesis of α,β-unsaturated Fatty Acids

Methods for producing short chain (less than 8 carbons) *cis*-α,β-unsaturated fatty acids using the Favorsky rearrangement of corresponding 1,3-dibromo-2-ones in alkaline solution at room temperature are reported in the art which also reports that best yields are obtained when using potassium bicarbonate. [14,15]

It is an object of the present invention to provide an alternative means for controlling *Candida albicans* infection or to at least provide the public with a useful choice.

SUMMARY OF THE INVENTION

According to a first aspect of the present invention there is provided use of at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position in the manufacture of a formulation for inhibiting or retarding the yeast-to-mycelium transition of an organism having a yeast-to-mycelium dimorphic life cycle.

A second aspect of the invention provides a method of inhibiting or retarding the yeast-to-mycelium transition of an organism having a yeast-to-mycelium dimorphic life cycle comprising administering to a subject at least one optionally substituted C_8 to C_{15}

fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position.

A third aspect of the invention provides a formulation for inhibiting or retarding the yeast-to-mycelium transition of an organism having a yeast-to-mycelium dimorphic life cycle comprising at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position.

A fourth aspect of the present invention provides use of at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position in the manufacture of a formulation for treating or preventing an infection by an organism having a yeast-to-mycelium dimorphic life cycle.

A fifth aspect of the invention provides a method treating or preventing an infection by an organism having a yeast-to-mycelium dimorphic life cycle comprising administering to a subject at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position.

A sixth aspect of the invention provides a formulation treating or preventing an infection by an organism having a yeast-to-mycelium dimorphic life cycle comprising at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position.

The following statements relate to the above aspects of the invention.

In one embodiment, the organism having a yeast-to-mycelium dimorphic life cycle is selected from the group comprising *Aspergillus fumigatus, Aureobasidium pullulans, Benjaminiella poitrasii, Blastomyces dermatitidis, Ceratocystis ulmi, Debaryomyces hansenii, Histoplasma capsulatum, Paracoccidioides brasiliensis,*

Phaeococcomyces exophiale, Sporothrix schenckii, Ustilago maydis, Yarrowia lipolytica and organisms of the *Candida* genus. A preferred organism of the *Candida* genus is *Candida albicans*.

In one embodiment, preferably the fatty acid is a C_8 to C_{13} fatty acid. Preferably the fatty acid is a C_{12} fatty acid.

In another embodiment, preferably the fatty acid has at least one substituent. In one embodiment the substituent is a branched alkyl group. Preferably the substituent is an alkyl group, preferably a group selected from the group comprising methyl, ethyl, propyl, butyl, and pentyl, and isomeric forms thereof. It will be apparent to a skilled worker that the fatty acid may have other substituents.

In another embodiment, preferably the fatty acid is selected from the group consisting of:

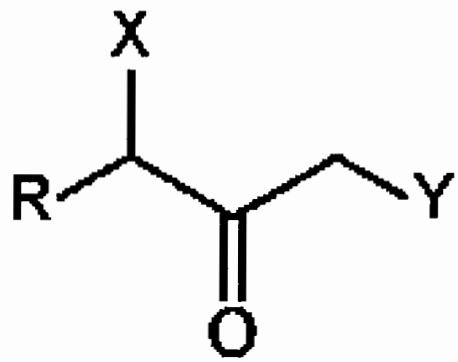
$(CH_3)_2CH(CH_2)_7CH=CHCOOH$;
$CH_3(CH_2)_4CH=CHCOOH$;
$CH_3(CH_2)_6CH=CHCOOH$;
$CH_3(CH_2)_8CH=CHCOOH$;
$CH_3(CH_2)_9CH=CHCOOH$; and
$CH_3(CH_2)_{11}CH=CHCOOH$.

In another embodiment, preferably the fatty acid is a *trans* fatty acid.

In another embodiment, preferably the fatty acid is a *cis* fatty acid.

In another embodiment, preferably the formulation comprises a food, drink, food additive, drink additive, dietary supplement, nutraceutical or pharmaceutical composition. Preferably the pharmaceutical composition comprises a fatty acid of the invention and a pharmaceutically acceptable carrier.

A seventh aspect of the present invention provides a process for producing a *cis* α,β-unsaturated fatty acid of the formula R-CH=CH-COOH comprising reacting a compound of Formula I:



(I)

with:

(1) NaOH; followed by

(2) an acid,

wherein R is an optionally substituted alkyl group and X and Y are independently selected from the group comprising Cl and Br.

In one embodiment, preferably R is optionally substituted $CH_3(CH_2)_{3-10}CH_2$- or $(CH_3)_2CH(CH_2)_{2-9}CH_2$-.

In one embodiment, preferably R is has at least one substituent. In a preferred embodiment the substituent is a branched alkyl group. Preferably the substituent is an alkyl group, preferably a group selected from the group comprising methyl, ethyl, propyl, butyl, and pentyl, and isomeric forms thereof. It will be apparent to a skilled worker that R may have other substituents.

In one embodiment, preferably a compound of formula (I) is reacted with about 0.5, 1, 2, 3, 4, 5 or 6 equivalent NaOH.

In one embodiment, preferably a compound of formula (I) is reacted with NaOH for about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, or 9 hours or longer.

In one embodiment, preferably a compound of formula (I) is reacted with NaOH for about 4 to about 8 hours.

In one embodiment, preferably the acid comprises about 0.5, 1, 1.5 or 2 N HCl.

In one embodiment, preferably a compound of formula (I) is reacted with 4 equivalent NaOH and H_2O for about 6 to about 8 hours followed by neutralization with 1N HCl.

In one embodiment, preferably the process is carried out at about 20 to 30 degrees C.

An eighth aspect of the invention provides a *cis* α,β-unsaturated fatty acid produced by a process of the invention.

A ninth aspect of the invention provides *cis*-11-methyl-2-dodecenoic acid.

A tenth aspect of the invention provides *trans*-11-methyl-2-dodecenoic acid.

An eleventh aspect of the invention provides a pharmaceutical composition comprising *cis* and/or *trans* 11-methyl-2-dodecenoic acid and a pharmaceutically acceptable carrier.

A twelfth aspect of the invention provides a pharmaceutical composition comprising a *cis and/*or *trans* fatty acid selected from the group comprising $CH_3(CH_2)_4CH=CHCOOH$; $CH_3(CH_2)_6CH=CHCOOH$; $CH_3(CH_2)_8CH=CHCOOH$; $CH_3(CH_2)_9CH=CHCOOH$; and $CH_3(CH_2)_{11}CH=CHCOOH$ and a pharmaceutically acceptable carrier.

This invention may also be said broadly to consist in the part, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or combinations of any two or more said parts, elements or features, and where specifically integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a shows HPLC analysis of the efficiency of synthesising *cis*-2-dodecenoic acid using a previously reported method.

Figure 1b shows HPLC analysis of the efficiency of synthesising *cis*-2-dodecenoic acid using a method of the invention.

Figure 2 shows the *in vitro* inhibitory activity of selected fatty acids against the yeast-to-mycelium transition of *Candida albicans*.

Figures 3a and 3b show that DSF does not affect yeast growth but inhibits the yeast-to-mycelium transition of a culture of *Candida albicans* (the bar indicates 30 μm).

DETAILED DESCRIPTION OF THE INVENTION

The applicants have found that α,β-unsaturated fatty acids that are structurally dissimilar to ARS have efficacy in inhibiting or retarding the yeast-to-mycelium transition of organisms having a yeast-to-mycelium dimorphic life cycle and that *cis* isomers also have efficacy contrary to previous reports. Exemplary organisms having a yeast-to-mycelium dimorphic life cycle include organisms of the *Candida* genus such as *Candida albicans*. The invention is also useful in respect of other organisms having a yeast-to-mycelium dimorphic life cycle including but not limited to *Aspergillus fumigatus, Aureobasidium pullulans, Benjaminiella poitrasii, Blastomyces dermatitidis, Ceratocystis ulmi, Debaryomyces hansenii, Histoplasma capsulatum, Paracoccidioides brasiliensis, Phaeococcomyces exophiale, Sporothrix schenckii, Ustilago maydis,* and

Yarrowia lipolytica. The fatty acids of the invention are thus useful to treat or prevent infection by these organisms.

Fatty acids useful herein include optionally substituted C_8 to C_{15} α,β-unsaturated fatty acids or salts, esters or amides thereof containing one double bond wherein the double bond is at the α,β-position. In one embodiment, preferably the fatty acid is a C_8 to C_{13} fatty acid. Preferably the fatty acid is a C_{12} fatty acid. Fatty acids useful herein may be substituted or unsubstituted, and *cis* or *trans* fatty acids. Fatty acids useful herein include $(CH_3)_2CH(CH_2)_7CH=CHCOOH$; $CH_3(CH_2)_4CH=CHCOOH$; $CH_3(CH_2)_6CH=CHCOOH$; $CH_3(CH_2)_8CH=CHCOOH$; $CH_3(CH_2)_9CH=CHCOOH$; and $CH_3(CH_2)_{11}CH=CHCOOH$. It will be apparent to a skilled worker that the fatty acid may comprise alkyl or other substituents.

The applicants have also elucidated the structure of DSF to be *cis*-11-methyl-2-dodecenoic acid.

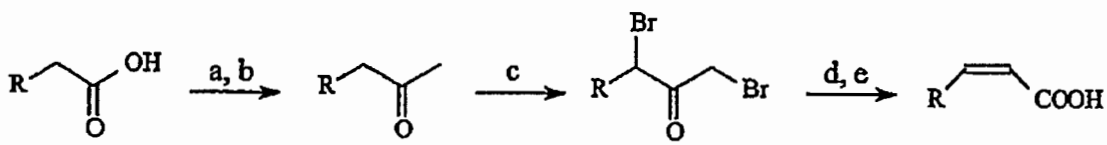
The applicants have also elucidated a novel reaction scheme for high yield production of *cis*-α,β-unsaturated fatty acids.

The previously reported methods for synthesis of α,β-unsaturated fatty acids can be used for preparation of short-chain *cis*-α,β-unsaturated fatty acids (less than 8 carbons). [15]

However, it was found that these methods were not suitable for synthesising long-chain *cis*-α,β-unsaturated fatty acids. For example, under the reported optimal reaction conditions, the yield of *cis*-2-dodecenoic acid (Figure 2, compound 7) was found to be less than 5%, even after a prolonged reaction time of up to 96 h (Figure 1a – first peak *cis*-2-dodecenoic acid; second peak 1,3-dibromo-2-dodecanone).

It was found that replacing potassium bicarbonate with sodium hydroxide increased the yield of *cis*-2-dodecenoic acid up to 98% (Scheme 1 and Figure lb – first peak *cis*-2-dodecenoic acid; second peak 1,3-dibromo-2-dodecanone).

Scheme 1

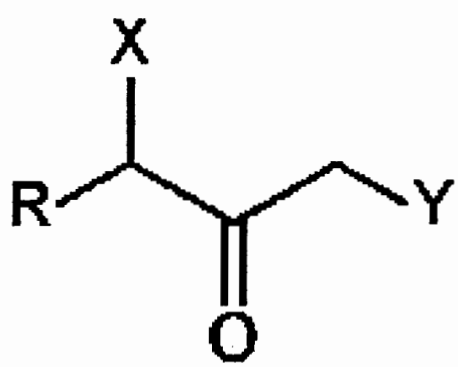


$R = CH_3(CH_2)_{3\text{-}10}CH_2$, $(CH_3)_2CH(CH_2)_6CH_2$

In Scheme 1, (a) LiMe, CH_3OCH_3; (b) H_2O; (c) Br_2, HBr, H_2O; (d) NaOH, H_2O; (e) HCl.

In preferred embodiment of Scheme 1, (a) LiMe, CH_3OCH_3, 4 h; (b) H_2O, ~90% yield; (c) Br_2, HBr, H_2O, 2 h, ~50% yield; (d) NaOH, H_2O, 6-8 h; (e) 1N HCl, >85% yield.

Accordingly, in one aspect, the invention provides a process for producing a *cis* α,β-unsaturated fatty acid of the formula R-CH=CH-COOH comprising reacting a compound of Formula I:



(I)

with:

(1) NaOH; followed by

(2) an acid,

wherein R is an optionally substituted alkyl group and X and Y are independently selected from the group comprising Cl and Br.

In one embodiment, preferably R is optionally substituted $CH_3(CH_2)_{3-10}CH_2$- or $(CH_3)_2CH(CH_2)_{2-9}CH_2$-.

In one embodiment, preferably R is substituted. In a preferred embodiment the substitution is a branched alkyl group. Preferably the substituted is an alkyl group, preferably a group selected from the group comprising methyl, ethyl, propyl, butyl, and pentyl, and isomeric forms thereof. It will be apparent to a skilled worker that R may have other substituents.

In one embodiment, preferably a compound of formula (I) is reacted with about 0.5, 1 2, 3, 4, 5 or 6 equivalent NaOH.

In one embodiment, preferably a compound of formula (I) is reacted with NaOH for about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, or 9 hours or longer.

In one embodiment, preferably a compound of formula (I) is reacted with NaOH for about 4 to about 8 hours.

In one embodiment, preferably the acid comprises about 0.5, 1, 1.5 or 2 equivalent HCl.

In one embodiment, preferably a compound of formula (I) is reacted with 4 equivalent NaOH and H_2O for about 6 to about 8 hours followed by neutralization with 1N HCl.

In one embodiment, preferably the process is carried out at about 20 to 30 degrees C.

To conduct the HPLC analyses depicted in Figures 1a and 1b, the reaction products were extracted with hexane, dried, then analyzed using HPLC on a C_{18} reverse-phase column (4.6 x 250 mm) and eluted with methanol-water (80:20, vol/vol) at a flow rate of 1 ml/min. The retention time was 9.5 min for *cis*-2-dodecenoic acid, and 23.2 min for 1,3-dibromo-2-dodecanone, respectively.

The other long-chain *cis*-α,β-unsaturated fatty acids of Figure 2 were also synthesized in high yields using the modified method of Scheme 1 (Table 1).

Table 1 - Yields of *cis*-α,β-unsaturated fatty acids produced according to Scheme 1

Compound*	1	5	6	7	8	9
Yield (%)	88.3	93.9	96.5	98.1	97.2	95.5

* Compounds are numbered according to Figure 2. The yield was calculated as a percentage of product/substrate based on HPLC analysis.

The purified reaction products were examined by NMR. In all cases, the chemical shifts and the coupling constants of the protons on the double bond (~11.5 Hz) indicate the products were *cis*-acids (data not shown).

Figure 2 shows the *in vitro* inhibitory activity of selected fatty acids against the yeast-to-mycelium transition of *Candida albicans*, including the *cis* and *trans* isomers of DSF (compounds 1 and 2), ARS (compound 3), and compounds produced according to a process of the invention (*cis* compounds 5 to 9).

Contrary to the previous reports that the *trans* configuration of the α,β-double bond of ARS is essential for the inhibitory effect against *C. albicans* filamentous growth,[11] DSF and compound 2 (the *trans*-isomer of DSF) showed an identical inhibitory activity. Moreover, the other *cis*- and *trans*-isomer pairs of α,β-unsaturated fatty acids (compounds 5 and 11, 6 and 12, 7 and 13, 8 and 14, 9 and 15) also each showed very similar or identical inhibitory activity (Figure 2).

As yeast-to-mycelium transition appears to be necessary for the pathogenicity of *C. albicans*, the compounds disclosed herein have utility for the control of the fungal disease. Rapid emergence of antibiotic resistance demands development of alternative approaches to prevent and control infectious diseases. As the lethal effect of antibiotics is the common cause to force microbes to mutate and survive, non-antimicrobial means of controlling *C. albicans* and other microorganisms could be useful to avoid or delay the development of antibiotic-resistance. Inhibiting the yeast-to-mycelium transition provides such a possibility.

The fatty acids useful herein may be incorporated into a food, drink, food additive, drink additive, dietary supplement, neutraceutical or pharmaceutical.

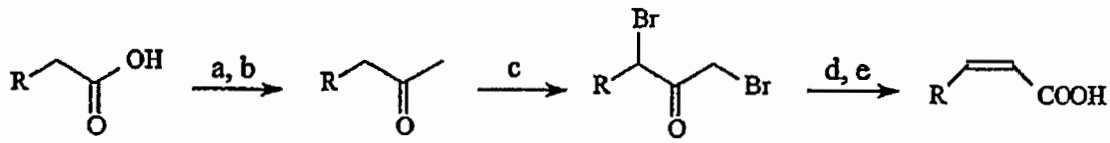
The pharmaceutical compositions provided by the invention comprise a fatty acid of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the invention can be administered in any suitable manner and thus may be formulated for administration orally, topically, subcutaneously, intramuscularly, intravenously, or parenterally, for example.

The invention consists in the foregoing and also envisages constructions of which the following gives examples only.

EXAMPLE 1

Scheme 1 and Scheme 2 set out below were employed to synthesize α,β-unsaturated fatty acids listed in Figure 2. As shown in Scheme 1, *cis*-α,β-unsaturated fatty acids were synthesized using the Favorsky rearrangement of corresponding 1,3-dibromo-2-ones in alkaline solution at room temperature by modifying a previously reported method. [15,16] Commercially available fatty acids (Sigma-Aldrich, USA) were converted to corresponding methyl ketones in the presence of LiMe. Methyl ketones were reacted with bromide reagents to produce 1,3-dibromo-2-ones which were then rearranged to produce *cis*-α,β-unsaturated fatty acids in the presence of 4 equivalent NaOH.

Scheme 1

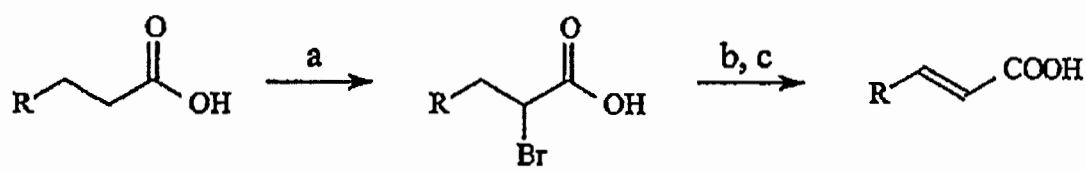


$R = CH_3(CH_2)_{3\text{-}10}CH_2$, $(CH_3)_2CH(CH_2)_6CH_2$

In Scheme 1, (a) LiMe, CH_3OCH_3, 4 h; (b) H_2O, ~90% yield; (c) Br_2, HBr, H_2O, 2 h, ~50% yield; (d) NaOH, H_2O, 6-8 h; (e) 1N HCl, >85% yield.

Trans-α,β-unsaturated fatty acids were synthesized through elimination of 2-bromo fatty acids prepared by brominating saturated fatty acids, using potassium *tert*-butoxide as a base in *tert*-butanol (Scheme 2). The methyl ester of ARS (3,7,11-trimethyl-2,6,10-dodecatrienoic acid) was synthesized as previously reported[16] and then hydrolyzed to produce ARS.

Scheme 2



$R = CH_3(CH_2)_{3\text{-}10}CH_2$, $(CH_3)_2CH(CH_2)_6CH_2$

In Scheme 2, (a) Br_2, HBr, H_2O, 2 h, ~55% yield; (b) $KOBu^{tert}$, tert-BuOH, 3 h; (c) 1N HCl, >90% yield.

Referring to Figure 2, compounds 1 and 5-9 were synthesized according to Scheme 1, compounds 2 and 11-15 were synthesized according to Scheme 2, compound 3 was synthesized as previously reported[16], and the others were obtained commercially

from Sigma-Aldrich, USA. All the synthetic compounds were characterized by ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

EXAMPLE 2

An *in vitro* bioassay of the inhibitory activity of the fatty acids of Figure 2 against *C. albicans* yeast-to-mycelium transition was carried out.

C. albicans strain CAI4 (ATCC MYA-684) was incubated at 28 °C for 24 h with shaking in a glucose salts (GS) medium (5 g of glucose, 0.26 g of $Na_2HPO_4 \cdot 12H_2O$, 0.66 g of KH_2PO_4, 0.88 g of $MgSO_4 \cdot 7H_2O$, 0.33 g of NH_4Cl, 16 μg of biotin per litre) supplemented with 0.1 mg/ml of uridine.

All the *C. albicans* cells remained in the yeast form under these conditions. The yeast cells were washed three times with GS medium before use and then were dispersed in GS medium to give a solution with a final OD_{600} of 1.0.

The test compounds set out in Figure 2 were diluted to the indicated concentrations with GS medium and were added to 100 μl of the washed *C. albicans* culture in 96-well microtitre plates, mixed and incubated at 37 °C for 6 hours (h).

The percentages of yeast cells and germ tubes (filamentous form) were determined microscopically by counting about 300 cells from three replicates. Inhibitory activity was defined as the mole concentration that gave a minimum of 90% inhibition (IC_{90}) relative to control.

Compounds with useful yeast-to-mycelium inhibitory activity were compounds 1, 2, 5 to 8 and 11 to 14 as shown in Figure 2.

EXAMPLE 3

Compound 1 was not observed to have a toxic effect on yeast cell growth even at concentrations up to 1 mM, as shown in Figure 3b (the bar indicates 30 μm).

A starter culture of *C. albicans* strain CAI4 was prepared by growing the fungus at a low temperature (28 °C) in GS medium to maintain the yeast cell morphology. The culture was diluted with the same fresh medium with or without supplement of DSF and incubated at a high temperature (37 °C) which promotes yeast-to-mycelium conversion. Figure 3a (the bar indicates 30 μm) shows that within 6 hours of incubation, the majority of *C. albicans* cells formed germ tubes in the absence of DSF, whereas addition of 30 μM DSF to the culture of *C. albicans* completely abolished yeast to-mycelium transition (Figure 3b). No toxic effect on yeast cell growth was noticed even when DSF concentration was increased up to 1 mM. The synthetic DSF was indistinguishable with the natural DSF in inhibition of germ tube formation.

The above describes some preferred embodiments of the present invention and indicates several possible modifications but it will be appreciated by those skilled in the art that other modifications can be made without departing from the scope of the invention.

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WHAT WE CLAIM IS:

1. Use of at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position in the manufacture of a formulation to inhibit or retard the yeast-to-mycelium transition of an organism having a yeast-to-mycelium dimorphic life cycle.

2. Use of at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position in the manufacture of a formulation to treat or prevent an infection by an organism having a yeast-to-mycelium dimorphic life cycle.

3. A use of claim 1 or 2 wherein the organism is selected from the group comprising *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Benjaminiella poitrasii*, *Blastomyces dermatitidis*, *Ceratocystis ulmi*, *Debaryomyces hansenii*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Phaeococcomyces exophiale*, *Sporothrix schenckii*, *Ustilago maydis*, *Yarrowia lipolytica* and organisms of the *Candida* genus.

4. A use of claim 1 or 2 wherein the organism is *Candida albicans*.

5. A use of any one of claims 1 to 4 wherein the fatty acid is a C_8 to C_{13} fatty acid.

6. A use of any one of claims 1 to 4 wherein the fatty acid is a C_{12} fatty acid.

7. A use of any one of the preceding claims wherein the fatty acid is substituted.

8. A use of claim 7 wherein the substituent comprises a branched alkyl group.

9. A use of claim 7 wherein the substituent comprises an alkyl group selected from the group comprising methyl, ethyl, propyl, butyl, and pentyl, and isomeric forms thereof.

10. A use of any one of claims 1 to 4 wherein the fatty acid is selected from the group consisting of:

$(CH_3)_2CH(CH_2)_7CH=CHCOOH$;
$CH_3(CH_2)_4CH=CHCOOH$;
$CH_3(CH_2)_6CH=CHCOOH$;
$CH_3(CH_2)_8CH=CHCOOH$;
$CH_3(CH_2)_9CH=CHCOOH$; and
$CH_3(CH_2)_{11}CH=CHCOOH$.

11. A use of any one of claims 1 to 10 wherein the fatty acid is a *trans* fatty acid.

12. A use of any one of claims 1 to 10 wherein the fatty acid is a *cis* fatty acid.

13. A use of any one of the preceding claims wherein the formulation comprises a food, drink, food additive, drink additive, dietary supplement, nutraceutical or pharmaceutical.

14. A method of inhibiting or retarding the yeast-to-mycelium transition of an organism having a yeast-to-mycelium dimorphic life cycle comprising administering to a subject at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position.

15. A method treating or preventing an infection by an organism having a yeast-to-mycelium dimorphic life cycle comprising administering to a subject at least one optionally substituted C_8 to C_{15} fatty acid or a salt, ester or amide thereof containing only one double bond wherein the double bond is at the α,β-position.

16. A method of claim 14 or 15 wherein the organism is selected from the group comprising *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Benjaminiella poitrasii*, *Blastomyces dermatitidis*, *Ceratocystis ulmi*, *Debaryomyces hansenii*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Phaeococcomyces exophiale*, *Sporothrix schenckii*, *Ustilago maydis*, *Yarrowia lipolytica* and organisms of the *Candida* genus.

17. A method of claim 14 or 15 wherein the organism is *Candida albicans*.

18. A method of any one of claims 14 to 17 wherein the fatty acid is a C_8 to C_{13} fatty acid.

19. A method of any one of claims 14 to 17 wherein the fatty acid is a C_{12} fatty acid.

20. A method of any one of claims 14 to 19 wherein the fatty acid is substituted.

21. A method of claim 20 wherein the substituent comprises a branched alkyl group.

22. A method of claim 20 wherein the substituent comprises an alkyl group selected from the group comprising methyl, ethyl, propyl, butyl, and pentyl, and isomeric forms thereof.

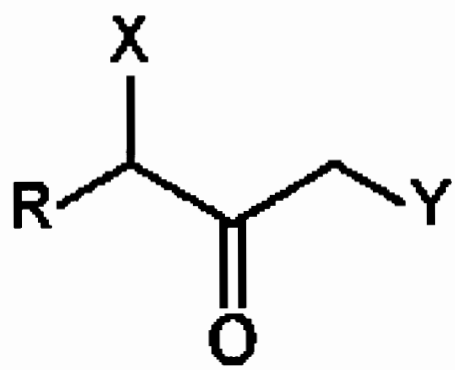
23. A method of any one of claims 14 to 17 wherein the fatty acid is selected from the group consisting of:

$(CH_3)_2CH(CH_2)_7CH=CHCOOH$;
$CH_3(CH_2)_4CH=CHCOOH$;
$CH_3(CH_2)_6CH=CHCOOH$;
$CH_3(CH_2)_8CH=CHCOOH$;
$CH_3(CH_2)_9CH=CHCOOH$; and
$CH_3(CH_2)_{11}CH=CHCOOH$.

24. A method of any one of claims 14 to 23 wherein the fatty acid is a *trans* fatty acid.

25. A method of any one of claims 14 to 23 wherein the fatty acid is a *cis* fatty acid.

26. A process for producing a *cis* α,β-unsaturated fatty acid of the formula R-CH=CH-COOH comprising reacting a compound of Formula I:



(I)

with:

(1) NaOH; followed by

(2) an acid,

wherein R is an optionally substituted alkyl group and X and Y are independently selected from the group comprising Cl and Br.

27. A process of claim 26 wherein R is optionally substituted $CH_3(CH_2)_{3-10}CH_2$- or $(CH_3)_2CH(CH_2)_{2-9}CH_2$-.

28. A process of claim 26 or 27 wherein R is substituted.

29. A process of claim 28 wherein the substituent comprises a branched alkyl group.

30. A process of claim 28 wherein the substituent comprises an alkyl group selected from the group comprising methyl, ethyl, propyl, butyl, and pentyl, and isomeric forms thereof.

31. A process of any one of claims 26 to 30 wherein a compound of formula (I) is reacted with about 0.5, 1, 2, 3, 4, 5 or 6 equivalent NaOH.

32. A process of any one of claims 26 to 31 wherein a compound of formula (I) is reacted with NaOH for about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, or 9 hours.

33. A process of any one of claims 26 to 30 wherein a compound of formula (I) is reacted with NaOH for about 6 to about 8 hours.

34. A process of anyone of claims 26 to 33 wherein the acid comprises about 0.5, 1, 1.5 or 2 N HCl.

35. A process of any one of claims 26 to 30 wherein a compound of formula (I) is reacted with 4 equivalent NaOH and H_2O for about 4 to about 8 hours followed by 1N HCl.

36. A process of any one of claims 26 to 35 carried out at about 20 to 30 degrees C.

37. A *cis* α,β-unsaturated fatty acid when produced by a process as claimed in any one of claims 26 to 36.

38. *cis* or *trans* 11-methyl-2-dodecenoic acid.

39. A pharmaceutical composition comprising *cis* or *trans* 11-methyl-2-dodecenoic acid and a pharmaceutically acceptable carrier.