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Evaluation of Morphogenic Regulatory Activity of Farnesoic Acid and Its Derivatives Against *Candida albicans* Dimorphism

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Abstract—A series of farnesoic acid derivatives was prepared and their morphogenic regulatory activities were evaluated. Their inhibitory activities against yeast cell growth and yeast-to-hypha transition examined in *Candida albicans* cells are dependent upon the chain length as well as the substitution patterns on the isoprenoid template. The preliminary structure–activity relationship of these compounds is described to elucidate the essential structural requirements.

Introduction

Candida albicans is a major pathogen in humans, particularly in immunocompromised patients. This fungus can colonize and infect a wide range of microenvironments in the body, including the blood stream, superficial sites in the mucosa and all of the major internal organs, during systemic disease.[1] *C. albicans* has a distinguishing feature, dimorphism, which is the ability to switch between two morphological forms: a budding yeast form and a multicellular invasive filamentous form. This ability has been postulated to contribute to the virulence of the organism.[2,3]

Morphogenesis is triggered by various signals in vitro. Serum, high temperature, neutral pH, and poor media stimulate yeast cells to sprout true hyphae.[4] Conversely, low temperature, air, acidic pH, and enriched media promote yeast cell growth. Molecular studies of dimorphism have led to the identification of several genes that regulate hyphal morphogenesis.[5,6] These include the two genes, *CPH1* and *EFG1*, whose products play positive roles in promoting the formation of filamentous forms. *C. albicans CPH1* is regulated by an MAP kinase cascade that includes homologues of *S. cerevisiae STE20*, *STE7* and an MAP kinase. *EFG1* has a complex role in the regulation of filamentous growth in *C. albicans*, having both positive and negative effects.[7,8] Two other *C. albicans* genes, *TUP1* and *RBF1,* act negatively to repress the ability to grow in filamentous forms.[9]

The regulatory networks that control the morphogenesis of *C. albicans* are being elucidated. However, despite these investigations, the mechanisms of dimorphism remain unclear, possibly because the environmental factors that induce the dimorphic transition have many effects on cells, making it difficult to identify which factors are essential for the transition.

We recently reported that *C. albicans* excretes an autoregulatory substance capable of regulating the morphological transition in culture medium.[10] Moreover, growth experiments revealed that this compound does not inhibit yeast cell growth but inhibits filamentous growth. We identified this compound as 3,7,11-trimethyl-dodeca-2,6,10-trienoic acid (farnesic acid or farnesoic acid), an isoprenoid compound.[10] In this report, we designed and prepared a series of derivatives of farnesoic acid, and evaluated their morphogenic regulatory activities to elucidate the structure–activity relationship.

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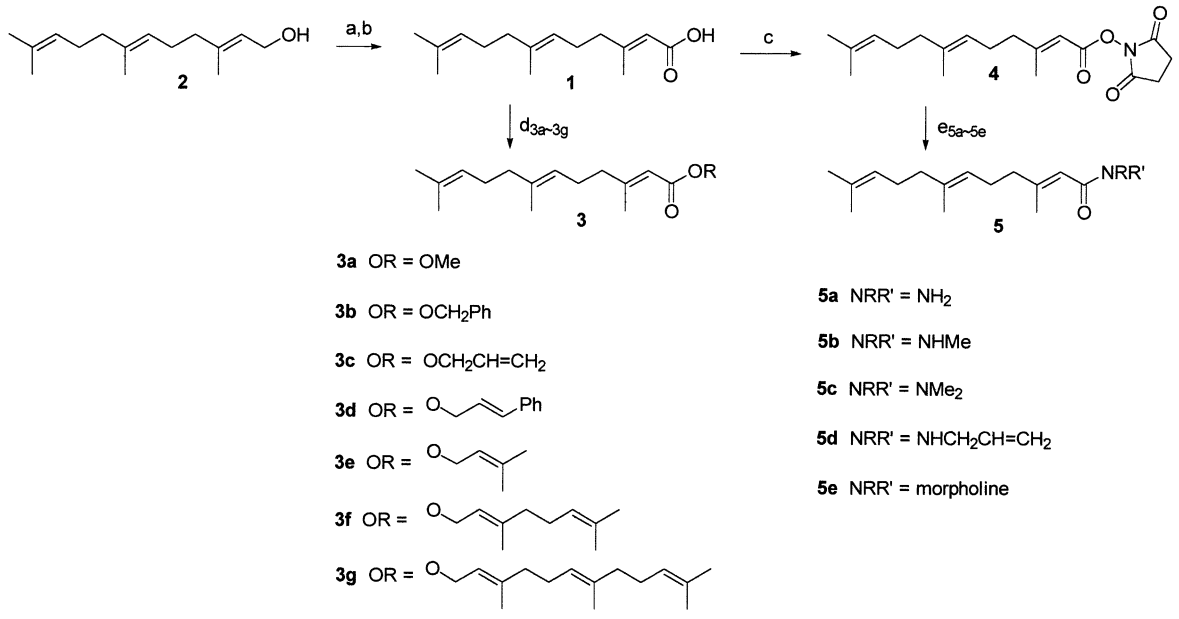
Synthesis

The synthesis of derivatives of farnesoic acid is outlined in Scheme 1. (*E*,*E*)-Farnesoic acid (**1**) was prepared by the oxidation of commercial (*E*,*E*)-farnesol (**2**) to farnesal with DMSO–sulfur trioxide/pyridine, followed by the oxidation of farnesal to **1** with $NaClO_2$ without causing *cis–trans* isomerization of the α,β-unsaturated double bond.[11] The esterification of farnesoic acid (**1**) by various alcohols was accomplished with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) to afford derivatives **3** with no *E*/*Z* isomerization problems.[12] The amide analogues **5** were also obtained without isomerization by using *N*-hydroxysuccinimidyl ester **4** as an intermediate. Compound **1** was condensed with *N*-hydroxysuccinimide in the presence of EDCI to furnish **4**. Treatment of **4** with commercially available amines in THF gave **5** in good yields.

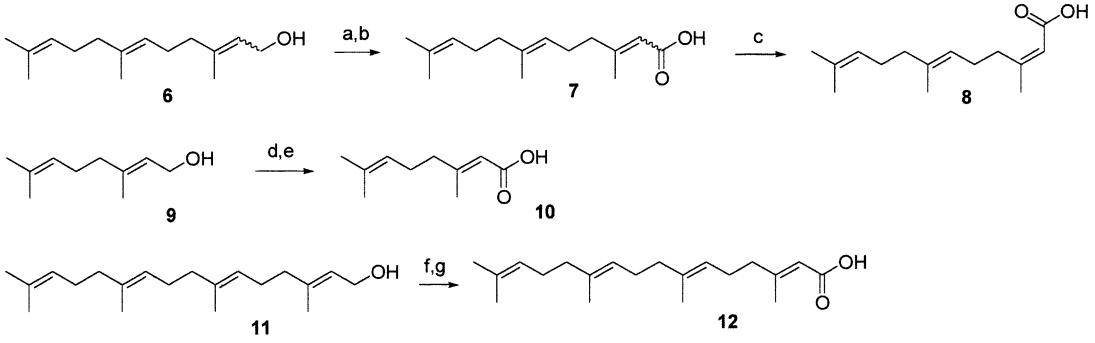
As shown in Scheme 2, the two-step oxidation of commercial farnesol **6** (mixture of isomers) to acid **7**, followed by silica gel column chromatography, provided the (2*Z*,6*E*)-farnesoic acid (**8**) with spectral data identical to those previously reported.[11] (*E*)-Geranoic acid (**10**) and (*E*,*E*,*E*)-geranylgeranoic acid (**12**), which are shorter and longer, respectively, than farnesoic acid (**1**) by one isoprene unit, were prepared from the corresponding alcohols (**9**, **11**) using the preparative method used for compound **1**.

Morphogenic Regulatory Activity and Discussion

The compounds were evaluated in terms of their inhibitory activities against yeast cell growth and yeast-to-hypha transition (filamentous growth) using *C. albicans* cells according to the procedure previously described.[10,13]



Scheme 1. Reagents and conditions: (a) DMSO, Et_3N, SO_3·pyr, CH_2Cl_2, 3 h, 80%; (b) 2-methyl-2-butene, $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, H_2O, *t*-BuOH, 2 h, 77%; (c) *N*-hydroxysuccinimide, EDCI, CH_2Cl_2, 6 h, 76%; (d_{3a}) MeOH, EDCI, DMAP, CH_2Cl_2, 2 h, 73%; (d_{3b}) benzyl alcohol, EDCI, DMAP, CH_2Cl_2, 1 h, 52%; (d_{3c}) allyl alcohol, EDCI, DMAP, CH_2Cl_2, 3 h, 48%; (d_{3d}) cinnamyl alcohol, EDCI, DMAP, CH_2Cl_2, 3 h, 80%; (d_{3e}) 3-methyl-2-butene-1-ol, EDCI, DMAP, CH_2Cl_2, 3 h, 83%; (d_{3f}) geraniol, EDCI, DMAP, CH_2Cl_2, 1 h, 85%; (d_{3g}) farnesol, EDCI, DMAP, CH_2Cl_2, 1.5 h, 25%; (e_{5a}) NH_4OH, THF, 2 h, 96%; (e_{5b}) CH_3NH_2, THF, 20 min, 87%; (e_{5c}) $(CH_3)_2NH$, THF, 20 min, 84%; (e_{5d}) allyl amine, THF, 1 h, 77%; (e_{5e}) morpholine, THF, 1 h, 87%.



Scheme 2. Reagents and conditions: (a) DMSO, Et_3N, SO_3·pyr, CH_2Cl_2, 3 h, 91%; (b) 2-methyl-2-butene, $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, H_2O, *t*-BuOH, 4 h; (c) silica gel column chromatography (14% for **8**, 66% for **1**); (d) DMSO, Et_3N, SO_3·pyr, CH_2Cl_2, 3 h, 77%; (e) 2-methyl-2-butene, $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, H_2O, *t*-BuOH, 3 h, 90%; (f) DMSO, Et_3N, SO_3·pyr, CH_2Cl_2, 4 h, 30%; (g) 2-methyl-2-butene, $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, H_2O, *t*-BuOH, 2 h, 72%.

The yeast-to-hypha transition of a low-density population of *C. albicans* cells (10^5 cells/mL) was inhibited by the addition of test compounds to cells in glucose salts (GS) medium. This inhibition was used to assay morphogenic regulatory activity, and was quantified by counting the number of germ tubes observed after 4 h of incubation at 37 °C. Cells in control experiments usually developed germ tubes only after about 1–2 h at the earliest. Inhibitory activity is defined as the concentration (μg/mL) that gave 50% inhibition (IC_{50}) relative to the control (0.5% DMSO). Results obtained are summarized in Table 1.

Synthetic (*E*,*E*)-farnesoic acid (**1**) exhibited almost the same inhibitory activity ($IC_{50} = 4.56$ μg/mL) upon the yeast-to-hypha transition as the farnesoic acid isolated from *C. albicans* excretions ($IC_{50} = 3.12$ μg/mL),[10] whereas the (2*Z*,6*E*)-isomer (**8**) showed only weak activity (18% at 100 μg/mL). Isomers **1** and **8** did not display any inhibitory effect upon yeast cell growth at the highest concentration tested (100 μg/mL). These biological results along with comparison of NMR spectra of **1** and **8** with those of isolated farnesoic acid let us confirm that the isolated autoregulatory substance capable of regulating the morphological transition is (*E*,*E*)-farnesoic acid among four possible isomers. These results also indicate that the *trans*-configuration of farnesoic acid is essential for its potent inhibitory activity upon the yeast-to-hypha transition.

With regard to the effect of chain length, for a given farnesoic acid, shortening the chain length by one isoprene unit (**10**) resulted in a dramatic decrease in inhibitory effect upon yeast-to-hypha transition (0% at 100 μg/mL) and a modest inhibitory activity increase upon yeast cell growth (57% at 100 μg/mL). Extension of the chain by one isoprene unit (**12**) led to a loss of activity on both yeast-to-hypha transition and yeast cell growth at the highest concentration tested (100 μg/mL).

Having established the effect of chain length, we then examined the effect of modifying the acid moiety to an ester or amide. As shown in Table 1, all of the ester derivatives **3a**–**g** were inactive against yeast cell growth and yeast-to-hypha transition. However, the amide derivative **5a** showed antifungal activities on *C. albicans*, and inhibited both yeast cell and filamentous growth. The inhibitory activity of **5a** against yeast-to-hypha transition was somewhat diminished compared with **1**. Modifications to the amide NH position were performed in order to determine the role this group has on inhibition. Interestingly, whereas the secondary amides **5b** and **5d** were not active at the highest concentration tested, the tertiary amides **5c** and **5e** were found to have similar activities compared to the primary amide **5a**. Currently we cannot fully understand this result, and are not in a position to elucidate the structure–activity relationship of amide derivatives **5** and farnesoic acid (**1**). We also evaluated the morphogenic regulatory activity of farnesol (**2**), which showed a potent inhibitory effect on filamentous growth ($IC_{50} = 2.82$ μg/mL), and to a lesser extent, on yeast cell growth (35% at 100 μg/mL).

Table 1. In vitro inhibitory activity of farnesoic acid and its derivatives upon *C. albicans* cell growth

Compd[a]	IC_{50} (μg/mL)[b]	
	Yeast cell growth	Yeast-to-hypha transition
1	>100[c]	4.56±0.42
2	35% at 100 μg/mL[d]	2.82±0.23
3a	>100	>100
3b	>100	>100
3c	>100	>100
3d	>100	>100
3e	>100	>100
3f	>100	>100
3g	>100	>100
5a	26.3±4.12	20.71±3.56
5b	>100	>100
5c	43.7±5.43	16.95±2.07
5d	>100	>100
5e	87.5±8.94	41.01±3.79
8	>100	18% at 100 μg/mL[d]
10	57% at 100 μg/mL[d]	>100
12	>100	>100

[a]All compounds tested were characterized by ^{1}H NMR, ^{13}C NMR, and MS.[14] Compounds **1**,[11,15] **2**,[15] **3a**,[15] **3b**,[16] **5a**,[17] **5b**,[18] **5c**,[19] **5e**,[20] **8**,[11,15] **10**[15] and **12**[21] are previously known.
[b]All determinations were performed in triplicate.
[c]No inhibitory activity was observed at the highest concentration tested (100 μg/mL).[13]
[d]Inhibition at the highest concentration tested.

From these results, it is apparent that the inhibitory potency and selectivity of farnesoic acid derivatives are sensitively dependent upon the chain length, as well as to substitutions on the isoprenoid template. The importance of acid functionality for the selective inhibition of yeast cell growth and yeast filamentous growth is demonstrated by comparing compound **1** with compounds **2** and **3**. Changes to the chain (**10** and **12**) resulted in a loss of activity, indicating the sensitive nature of the chain length.

In this study, we synthesized and evaluated farnesoic acid derivatives as morphogenic regulatory agents, and confirm that the substance capable of regulating the yeast-to-hypha morphological transition is (*E*,*E*)-farnesoic acid. Moreover, (*E*,*E*)-farnesol was found to be more active than (*E*,*E*)-farnesoic acid against this transition, but to be less selective, because it had an inhibitory effect on yeast cell growth as well. Concerning the structure–activity relationships in the farnesoate series, the most significant finding was that the selective regulatory ability and inhibitory potency were found to be very dependent upon the chain length and the acid functionality.

Acknowledgements

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13. (a) For the yeast cell growth inhibition assay, *C. albicans* ATCC 10231 cells were grown at 30 °C in YPD medium (10 g of yeast extract, 20 g of polypeptone, and 20 g of glucose per liter) with vigorous shaking. After 48 h of incubation, cells were harvested and washed twice with sterile distilled water, and inoculated into fresh YPD medium to give an initial cell density of approximately 10^7 cells/mL. Stock solutions of test compounds were prepared in 100% dimethyl sulfoxide (DMSO) and stored at −20 °C. Each stock solution was diluted with YPD medium to prepare serial 2-fold dilutions in the range of 100–0.1 μg/mL before use. The final concentration of DMSO was less than 0.5%. Cells were then grown with or without a prescribed concentration of test compound with vigorous shaking. After 4 h of incubation at 30 °C, the cell density at A_{610} was measured. A cell suspension of 10^7 cells/mL gave an A_{610} value of approximately 1.0. (b) For the yeast-to-hypha transition assay, stock solutions of test compounds were prepared and diluted with a glucose salts (GS) medium as described above. GS medium containing a prescribed concentration of the test compound was added to 10^5 cells/mL of washed yeast cells in Petri dishes (diameter, 30 mm), mixed, and incubated for 4 h at 37 °C. The percentages of yeast and hypha were determined microscopically by counting 200 cells from triplicate dishes. Inhibitory activity was defined to be the concentration (μg/mL) giving 50% inhibition (IC_{50}) relative to the control (GS medium containing 0.5% DMSO).
14. A representative example: compound **1**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.63 (d, J = 1.2 Hz, 1H), 5.01 (m, 2H), 2.10–2.13 (m, 7H), 1.89–2.03 (m, 5H), 1.61 (d, J = 1.2 Hz, 3H), 1.53 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.81, 161.99, 135.31, 130.43, 123.18, 121.70, 114.09, 40.20, 38.64, 25.65, 24.92, 24.65, 18.14, 16.66, 15.01. **3e**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.61 (d, J = 1.5 Hz, 1H), 5.30 (m, 1H), 4.98–5.03 (m, 2H), 4.52 (d, J = 7.2 Hz, 2H), 2.08–2.10 (m, 7H), 1.88–2.02 (m, 5H), 1.65 (td, J = 12.8, 1.0 Hz, 8H), 1.53 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.85, 158.88, 137.57, 135.10, 130.38, 123.21, 121.90, 118.01, 114.53, 59.46, 39.95, 38.65, 25.66, 24.95, 24.76, 24.65, 17.82, 16.99, 16.66, 14.99. **3g**: ^{1}H NMR ($CDCl_3$, 300 Hz) δ 5.61 (d, J = 1.2 Hz, 1H), 5.30 (td, J = 7.2, 1.3 Hz, 1H), 4.99–5.03 (m, 4H), 4.54 (d, J = 7.2 Hz, 2H), 2.08–2.10 (m, 7H), 1.88–2.03 (m, 12H), 1.64 (d, J = 0.9 Hz, 3H), 1.61 (d, J = 0.9 Hz, 6H), 1.53 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.86, 159.83, 141.78, 136.10, 135.39, 131.38, 131.28, 124.33, 124.21, 123.69, 122.90, 118.72, 115.56, 83.18, 60.48, 40.95, 39.68, 39.65, 39.55, 26.72, 26.66, 26.22, 25.97, 25.67 (2C), 18.82, 17.67, 16.91, 16.47, 16.00. **5d**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.79 (dddd, J = 15.9, 10.8, 5.7, 5.7 Hz, 1H), 5.50 (s, 1H), 5.41 (bs, 1H), 4.99–5.16 (m, 4H), 3.84–3.91 (m, 2H), 2.06–2.09 (m, 7H), 1.88–2.02 (m, 4H), 1.61 (d, J = 0.9 Hz, 3H), 1.53 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.05, 153.37, 134.90, 133.55, 130.33, 123.23, 122.13, 116.90, 115.09, 65.47, 39.79, 38.67, 25.70, 25.08, 24.67, 17.35, 16.67, 15.01. **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.61 (d, J = 1.5 Hz, 1H), 4.98–5.11 (m, 2H), 2.58 (dd, J = 8.1, 7.5 Hz, 2H), 2.11 (dd, J = 15.3, 7.5 Hz, 2H), 1.91–2.03 (m, 4H), 1.85–1.87 (m, 3H), 1.60 (d, J = 1.2 Hz, 3H), 1.53–1.54 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.79, 163.51, 135.99, 131.31, 124.31, 123.30, 115.74, 39.68, 33.64, 26.70, 26.65, 25.70, 25.65, 17.64, 15.86.
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