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Evaluation of Antimicrobial Activity of Farnesoic Acid Derivatives

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Abstract The biological activities of farnesoic acid derivatives against pathogenic fungi and bacteria were investigated. Farnesoic acid and its derivatives showed growth inhibitory activities against various bacteria. Among the compounds tested, geranylgeranoic acid (**3**) had potent antibacterial activity against *Salmonella typhimurium*, *Proteus vulgaris*, and *Bacillus subtilis* with minimum inhibitory concentration (MIC) in the range of 6.25–12.5 μg/ml. On the other hand, amide derivatives of farnesoic acid showed some antifungal activities. In particular, 3,7,11-trimethyl-dodeca-2,6,10-trienoic acid amide (**5a**) had a potent antifungal activity against *Aspergillus niger*, *Candida albicans*, and *Trichophyton* sp. with MIC in the range of 6.25–25 μg/ml.

Key words: Farnesoic acid derivatives, antimicrobial activity, MIC

Compounds derived from the isoprenoid/cholesterol biosynthetic pathway have recently been shown to have novel biological activities [11–14]. These bioactive compounds include certain sterols, oxysterols, farnesol, and geranylgeraniol, as well as diphosphate derivatives of these compounds [3–5]. *Candida albicans* has a distinguished feature, dimorphism, which is known to have an ability to switch between two morphological forms: a budding yeast form and a invasive filamentous form. This ability has been postulated to contribute to the virulence of this organism [2, 7, 10]. We recently reported that *C. albicans* excretes an autoregulatory substance capable of regulating the morphological transition in a culture medium [9]. The structure of this compound was identified as 3,7,11-trimethyl-dodeca-2,6,10-trienoic acid (farnesoic acid), which is an isoprenoid compound. In order to understand the structure-activity relationship of farnesoic acid, we synthesized a series of farnesoic acid derivatives and evaluated their morphogenic regulatory activities [6]. Some of these derivatives were found to possess yeast-to-hypha transition inhibitory activity against *C. albicans* dimorphism. These findings have implications for developmental signaling by the fungus which could have some medicinal value regarding the development of antifungal therapies.

In the present study, in order to determine the scope of the biological activity of farnesoic acid-related compounds, we investigated their antimicrobial activities upon various bacteria and fungi. *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC14028, *Proteus vulgaris* ATCC3851, *Bacillus subtilis* ATCC6633, and *Staphylococcus aureus* ATCC6538p were used for the antibacterial activity test, and *Aspergillus niger* ATCC9642, *Candida albicans* ATCC10231 and IFO1594, *Trichophyton mentagrophytes* IFO5812 and *Trichophyton rubrum* IFO6204 were used for the antifungal activity test. Those microorganisms are

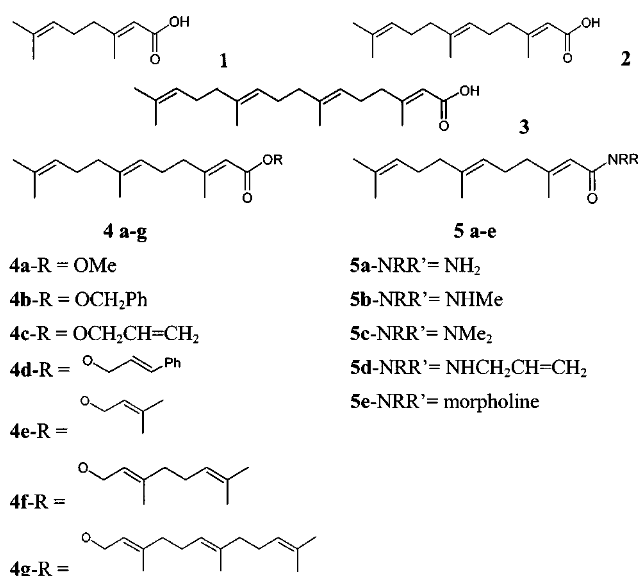

Fig. 1. Chemical structures of farnesoic acid (**2**) and its derivatives (**1**, **3**, **4a–4g**, and **5a–5e**).

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well-known pathogenic bacteria and fungi against humans and animals.

The chemical structures of farnesoic acid and its derivatives [6] are shown in Fig. 1. (*E*,*E*)-Farnesoic acid (**2**) was prepared by the oxidation of commercial (*E*,*E*)-farnesol to farnesal with DMSO-sulfur trioxide/pyridine, followed by the oxidation of farnesal with $NaClO_2$ without causing *cis*-*trans* isomerization of the α,β-unsaturated double bond. The esterification of farnesoic acid (**2**) by using various alcohols was accomplished with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) to provide derivatives **4a–g** with no *E/Z* isomerization problems. The amide analogues **5a–e** were also obtained without isomerization by using *N*-hydroxysuccinimidyl ester as an intermediate. Farnesoic acid (**2**) was condensed with *N*-hydroxysuccinimide in the presence of EDCI to give rise to *N*-hydroxysuccinimidyl ester. Treatment of *N*-hydroxysuccinimidyl ester with commercially available amines in THF gave **5a–e** in satisfactory yields. (*E*)-Geranoic acid (**1**) along with (*E*,*E*,*E*)-geranylgeranoic acid (**3**), which are shorter and longer, respectively, than farnesoic acid (**2**) by one isoprene unit, were prepared from the corresponding alcohol by using the preparative method that was applied to farnesoic acid (**2**).

The antifungal activity test for farnesoic acid derivatives on yeast *Candida albicans* was carried out by following the macrobroth dilution method M27-T which was proposed by the National Committee for Clinical Laboratory Standards (NCCLS) [8]. Briefly, 0.3 ml of serial two-fold dilutions of the test compounds in DMSO was mixed with 2.7 ml of RPMI 1640 broth (0.165 M MOPS buffer at pH 7.0) (Sigma, St. Louis, MO, U.S.A.) containing fresh inoculum of 10^3 cells/ml. The assay tubes were incubated at 28°C for 3 days, and the range of concentrations tested were 100 µg/ml to 0.1 µg/ml. The antifungal spectrum against filamentous fungi was determined by the macrobroth dilution method of Association of Official Analytical Chemists (AOAC) [1]. A spore suspension was collected with 0.1% Tween-80 solution from potato dextrose agar plates that had been incubated at 28°C for 2 weeks. Standardization adjustments were made to the spore suspension in order to achieve an initial inoculum size of 10^5 spores/ml. YM (1.0% glucose, 0.5% polypeptone, 0.3% yeast extract, and 0.3% malt extract) broth (0.165 M MOPS buffer at pH 7.0) was used as the antifungal assay medium. The minimum inhibitory concentration (MIC) was taken as the concentration at which no growth was observed after being incubated for 4 days at 28°C. The antibacterial activity of test compounds was determined by a two-fold microtiter broth dilution method [15]. Bacteria were grown overnight and diluted 10^{-5} into fresh Standard Methods (SM) broth medium (Difco Lab., Detroit, MI, U.S.A.). One-hundred ml of SM broth containing approximately 10^4–10^5 CFU/ml of test bacteria was added to each well of a 96-well microtiter plate containing prescribed concentrations of the test compound. The plate was incubated for 24 h at 37°C. The MIC was taken as the concentration at which no growth was observed.

The antibacterial activity of farnesoic acid derivatives against five kinds of pathogenic bacteria was investigated by the broth dilution method. As shown in Table 1, farnesoic acid (**2**) showed a broad but weak antibacterial activity toward five kinds of pathogenic bacteria with an exception of *E. coli*. In regards to the effect of chain length

Table 1. Antibacterial activity of farnesoic acid derivatives.

Compd[a]	MIC (µg/ml)[b]				
	E. coli ATCC25922	*S. typhimurium* ATCC14028	*P. vulgaris* ATCC3851	*B. subtilis* ATCC6633	*S. aureus* ATCC6538p
1	>100	>100	>100	100	>100
2	>100	50	25	50	50
3	>100	12.5	6.25	6.25	>100
4a	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100
4e	>100	>100	>100	>100	>100
4f	>100	>100	>100	>100	>100
4g	>100	>100	>100	>100	>100
5a	>100	50	25	12.5	25
5b	>100	>100	>100	>100	>100
5c	>100	100	50	50	100
5d	>100	>100	>100	>100	>100
5e	>100	>100	100	50	100

[a]All compounds tested were characterized by ^{1}H-NMR, ^{13}C-NMR, and MS.
[b]MIC is defined in the text.

Table 2. Antifungal activity of farnesoic acid derivatives.

Compd[a]	MIC (μg/ml)[b]				
	A. niger ATCC9642	*C. albicans* ATCC10231	*C. albicans* IFO1594	*T. mentagrophytes* IFO5812	*T. rubrum* IFO6204
1	>100	>100	>100	>100	>100
2	>100	>100	>100	> 50	50
3	>100	>100	>100	>100	>100
4a	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100
4e	>100	>100	>100	>100	>100
4f	>100	>100	>100	>100	>100
4g	>100	>100	>100	>100	>100
5a	12.5	25	25	6.25	12.5
5b	>100	>100	>100	>100	>100
5c	100	>100	100	25	50
5d	>100	>100	>100	>100	>100
5e	>100	>100	>100	50	100

[a]All compounds tested were characterized by ^{1}H-NMR, ^{13}C-NMR, and MS.
[b]MIC is defined in the text.

for a given farnesoic acid (**2**), shortening the chain length by one isoprene unit, geranoic acid (**1**), resulted in a dramatic decrease in inhibitory effect upon bacteria. Extension of the chain by one isoprene unit, geranylgeranoic acid (**3**), resulted in a modest increase of inhibitory activity upon *S. typhimurium*, *P. vulgaris*, and *B. subtilis*, where the minimum inhibitory concentration (MIC) was in the range of 6.25–12.5 μg/ml. These results suggest that isoprenoid chain length is closely related to the antibacterial activity. 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 **4a–g** were inactive against bacterial cell growth. However, the primary amide **5a** and tertiary amides **5c** and **5e** showed weak antibacterial activities on various bacteria.

The antifungal activity of farnesoic acid derivatives against five kinds of pathogenic fungi was investigated and summarized in Table 2. In the previous study [6], **2** had no detectable effect on yeast cell growth but had a potent inhibitory activity upon the yeast-to-hypha transition (filamentous growth) in *C. albicans*. Interestingly, in the present study, **2** showed a weak antifungal activity against *Trichophyton* spp. Shortening or extening the chain length by one isoprene unit (**1** and **3**, respectively) led to a loss of activity when compared with **2**. In contrast, the modification of the acid moiety of **2** to amides **5a–e** exhibited some antifungal activity. In particular, primary amide **5a** had a potent antifungal activity against *A. niger, C. albicans*, *T. mentagrophytes*, and *T. rubrum*, where the MIC was in the range of 6.25–25 μg/ml. 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 weak activities when compared to the primary amide **5a**. These results suggest that the amide group is closely related to the enhanced antifungal activity of farnesoic acid derivatives.

In summary, we evaluated farnesoic acid derivatives as antimicrobial agents. From these results, it is apparent that the antibacterial activity of farnesoic acid derivatives is significantly dependent on the chain length as well as on substitutions of the isoprenoid template (compounds **1–3**). The importance of amide functionality for the inhibition of fungal growth is demonstrated by comparing compound **2** with compounds **5a–e**. Unfortunately, we can not fully understand this result at this time. Furthermore, we are not yet able to elucidate the structure-activity relationship of these amide derivatives. We should synthesize more analogues, and then demonstrate the possible relationship between the antimicrobial activity of these isoprene derivatives and some specific regulatory function. Therefore, further studies on the antimicrobial activities of many farnesoic acid derivatives are urgently in need.

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