Antifungal Activity of 1,4-Dialkoxynaphthalen-2-Acy1
Imidazolium Salts by Inducing Apoptosis of Pathogenic
Candida spp.

Jisue Lee 1,†, Jae-Goo Kim 2,†, Haena Lee 1, Tae Hoon Lee 1, Ki-Young Kim 2,** and Hakwon Kim 1,*

1 Department of Applied Chemistry, Global Center for Pharmaceutical Ingredient Materials,
Kyung Hee University, Seocheon, Giheung, Yongin, Gyeonggi-do 1732, Korea; cwltn7552@naver.com (J.L.);
dlgosk97@naver.com (H.L.); thlee@khu.ac.kr (T.H.L.)
2 Graduate School of Biotechnology, Kyung Hee University, Seocheon, Giheung, Yongin,
Gyeonggi-do 1732, Korea; zxcv913@naver.com
* Correspondence: kiyoung@khu.ac.kr (K.-Y.K.); hwkim@khu.ac.kr (H.K.); Tel.: +82-312012633 (K.-Y.K.);
+82-312012459 (H.K.)
† These authors contributed equally to this work.

Abstract: Even though Candida spp. are staying commonly on human skin, it is also an opportunistic pathogenic fungus that can cause candidiasis. The emergence of resistant Candida strains and the toxicity of antifungal agents have encouraged the development of new classes of potent antifungal agents. Novel naphthalen-2-acyl imidazolium salts (NAIMSs), especially 1,4-dialkoxy-NAIMS from 1,4-dihydroxynaphthalene, were prepared and evaluated for antifungal activity. Those derivatives showed prominent anti-Candida activity with a minimum inhibitory concentration (MIC) of 3.125 to 6.26 µg/mL in 24 h based on microdilution antifungal susceptibility test. Among the tested compounds, NAIMS 7c showed strongest antifungal activity with 3.125 µg/mL MIC value compared with miconazole which showed 12.5 µg/mL MIC value against Candida spp., and more importantly >100 µg/mL MIC value against C. auris. The production of reactive oxygen species (ROS) was increased and JC-1 staining showed the loss of mitochondrial membrane potential in C. albicans by treatment with NAIMS 7c. The increased release of ultraviolet (UV) absorbing materials suggested that NAIMS 7c could cause cell bursting. The expression of apoptosis-related genes was induced in C. albicans by NAIMS 7c treatment. Taken together, the synthetic NAIMSs are of high interest as novel antifungal agents given further in vivo examination.

Keywords: 1,4-Dialkoxynaphthalen-2-acyl imidazolium salts; Candida sp.; antifungal agent; apoptosis

1. Introduction

Infections by invasive fungal pathogens result from immunosuppression, long-term broad-spectrum antimicrobials, endocrinopathies, organ transplantation and use of indwelling catheters [1,2]. Candida spp. is a critical invasive fungal pathogen causing disease in humans, normally responsible for 90% of mucosal infections and 60% of candidiasis episodes [3]. Although various compounds are currently used to control Candida infectious diseases, including well-known azoles such as fluconazole, miconazole and others, the mortality of patients with Candida infection is above 15% [4,5]. Antibiotic-resistant Candida spp. have also arisen. The drugs currently used against fungal pathogens have limitations because of their toxicity [6,7]. For instance, Acetaminophen (APAP), amphotericin B deoxycholate (DAMB) and the triazoles may cause hepatic toxicity [8,9]. Therefore, there is an urgent need for a new drug to treat Candida infection.

Imidazolium salts (IMS) have been reported to exhibit fungicidal activity [10]. Due to its ionicity, IMS provides properties that are unusual and highly interesting for pharmaceutical formulation including potential efficacy against some bacteria and fungi [11].
In addition, tuning the toxicity of IMSs as antitumor agents has attracted much attention [12]. Considering these and our previous results of antifungal compound study, we decided to explore in further detail the potential activity of a new hybrid compound formed by attaching an imidazole moiety to 1,4-dialkoxynaphthalene-2-acyl compound to enhance antifungal activity.

Alagebrum, known as an advanced glycation end-products (AGES) breaker that reverse one of the main mechanisms of ageing, has a structure of phenacyl thiazolium salt [13]. The phenacyl moiety is known to play an important role in biological activity [14,15]. Therefore, we were interested in the naphthalencyl moiety, similar to the phenacyl moiety, as a pharmacophore of antifungal agents. The 1,4-dialkoxy naphthalencyl compound, derived from 1,4-naphthoquinone, became of particular interest (Figure 1).

![Figure 1. Schematic design for synthesis of acynaphthalene-imidazolium hybrid using pharmacophore-hybridization approach.](image)

Induced endogenous fungal apoptotic responses could provide a basis for antifungal therapies. Environmental stress (acetic acid and hydrogen peroxide) and an antifungal agent (amphotericin B, hibiscuslide C and coumarin) have been known to induce apoptosis in *C. albicans* [16–18]. Apoptosis is a kind of programmed cell death. Multicellular organisms and even single-celled organisms, such as yeast, can exhibit many features of apoptosis, including DNA fragmentation, reactive oxygen species (ROS) production and the loss of mitochondrial membrane potential [19–21].

With the above considerations, a series of novel IMSs linked to a 2-acetyl-1,4-dialkoxy naphthalene moiety were efficiently synthesized through pharmacophore-hybridization strategy (Figure 1) to find promising drugs for dealing with *Candida* spp. infection. Through microdilution antifungal susceptibility, NAIMS 7c showed the highest antifungal activity among them by inducing *Candida* apoptosis and cell bursting.

2. Materials and Methods
2.1. General Remarks

The reactions were monitored by thin-layer chromatography (TLC) on Merck Silica gel 60F254. Column chromatography was performed on Merck silica gel 200–300 mesh. Melting points were determined on the melting point apparatus electrothermal A9100X1 and were uncorrected. We recorded $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra on a JEOL FT-NMR spectrometer (Tokyo, Japan), respectively. Spectra are referenced relative to the chemical shift of tetramethylsilane (TMS). High-resolution mass spectra were obtained with a JEOL JMS-700 mass spectrometer. All solvents and reagents were commercially available from Acros Organics (Brookline, MA, USA), Aldrich (St. Louis, MO, USA) and TCI (Tokyo, Japan) and were used as received. The chemicals 1-Methylimidazolide (6a) from Sigma-Aldrich (St. Louis, MO, USA) and 1-benzylimidazole (6b) from Aldrich are commercially available. We readily obtained 1,4-Diacetoxy naphthalene (1a), 1,4-diacetoxy-5-methoxynaphthalene (1b), 1,4-diisooamyloxynaphthalene (8), 4-acetoxy-2-acetyl-1-isooamyloxynaphthalene (9') and 2-acetyl-1,4-diisooamyloxynaphthalene (9) from 1,4-naphthoquinone...
or 5-hydroxy-1,4-naphthoquinone [22]. We prepared 2-Bromoacetyl-1-naphthalene and 2-bromoacetyl-1-methoxynaphthalene from 2-acetyl-1-naphthalene and 2-acetyl-1-methoxynaphthalene, respectively, according to the α-bromination procedure.

2.2. Synthetic Procedures and Analytical Data

2.2.1. Synthesis of 3-acetyl-4-hydroxynaphthalen-1-yl acetate (2a)

1,4-Diacetoxynaphthalene 1a (5 g, 20.4 mmol) was dissolved in boron trifluoride acetic acid complex (20 mL, 144 mmol) and heated under reflux for 1 h. Then, it was cooled to room temperature, quenched with water, extracted by ethyl acetate and washed with water and brine. The combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography to give the compound 2a as a pale-yellow solid. (4.9 g, 99%): 

$^1$H NMR (300 MHz, CDCl$_3$) δ ppm 13.92 (s, 1H), 8.44 (d, 1H, J = 8.2 Hz), 7.71 (d, 1H, J = 8.0 Hz), 7.65–7.60 (m, 1H), 7.55–7.50 (m, 1H), 7.35 (s, 1H), 2.59 (s, 3H), 2.42 (s, 3H); 

$^{13}$C NMR (75 MHz, CDCl$_3$) δ ppm 203.6, 169.6, 160.3, 137.6, 130.8, 130.4, 126.4, 125.8, 124.7, 120.9, 116.3, 111.8, 26.7, 20.7.

2.2.2. Synthesis of 3-acetyl-4-hydroxy-5-methoxynaphthalen-1-yl acetate (2b)

Following the procedure described above for the preparation of 2a, compound 2b was obtained from 5-methoxy-1,4-diacetoxynaphthalene (1b; 0.11 g, 0.4 mmol) as a pale-yellow solid. (0.1 g, 92%): 

$^1$H NMR (300 MHz, CDCl$_3$) δ ppm 14.33 (br s, 1H), 7.58 (dd, 1H, J = 8.0 Hz, 8.2 Hz), 7.46 (s, 1H), 7.34 (d, 1H, J = 8.2 Hz), 6.95 (d, 1H, J = 8.0 Hz), 4.06 (s, 3H), 2.68 (s, 3H), 2.45 (s, 3H). 

$^{13}$C NMR (75 MHz, CDCl$_3$) δ ppm 202.5, 169.7, 162.1, 159.7, 137.2, 133.3, 131.0, 117.7, 116.7, 113.4, 112.9, 107.0, 56.2, 27.7, 20.8.

2.2.3. Synthesis of 3-acetyl-4-alkyloxynaphthalen-1-yl acetate (3a–3d)

3-Acetyl-4-methoxynaphthalen-1-yl acetate (3a)

Compound 2a (0.49 g, 2 mmol) was dissolved in dimethylformamide (DMF, 4 mL). Then, iodomethane (0.19 mL, 3 mmol) and cesium carbonate (0.98 g, 3 mmol) were added to the solution. The mixture was heated under reflux for 1.5 h and was cooled to room temperature. It was quenched with water, extracted by ethyl acetate, and washed with water and brine. The combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography to afford the compound 3a as a yellow solid. (0.41 g, 80%): 

$^1$H NMR (300 MHz, CDCl$_3$) δ ppm 8.25–8.21 (m, 1H), 7.86–7.81 (m, 1H), 7.67–7.58 (m, 2H), 7.54 (s, 1H), 4.01 (s, 3H), 2.78 (s, 3H), 2.46 (s, 3H). 

$^{13}$C NMR (75 MHz, CDCl$_3$) δ ppm 198.0, 169.1, 155.3, 142.5, 129.8, 128.7, 128.5, 123.5, 121.4, 117.2, 76.6, 63.6, 30.3, 20.4.

3-Acetyl-4-(isoamyl)oxynaphthalen-1-yl acetate (3b)

Following the procedure described above for the preparation of 3a, compound 3b was obtained from 2a (0.49 g, 2 mmol) and 1-bromo-3-methyl butane (0.36 mL, 3 mmol) as a yellow oil. (0.44 g, 86%): 

$^1$H NMR (300 MHz, CDCl$_3$) δ ppm 8.25–8.21 (m, 1H), 7.86–7.81 (m, 1H), 7.67–7.58 (m, 2H), 7.54 (s, 1H), 4.01 (s, 3H), 2.78 (s, 3H), 2.46 (s, 3H). 

$^{13}$C NMR (75 MHz, CDCl$_3$) δ ppm 199.2, 169.4, 154.6, 142.5, 130.0, 129.5, 128.4, 126.6, 124.1, 121.6, 117.5, 76.2, 39.0, 30.5, 25.0, 22.6, 20.8.

3-Acetyl-4-isopropoxynaphthalen-1-yl acetate (3c)

Following the procedure described above for the preparation of 3a, compound 3c was obtained from 2a (0.49 g, 2 mmol) and 2-bromopropane (0.28 mL, 3 mmol) as a yellow oil. (0.36 g, 63%): 

$^1$H NMR (300 MHz, CDCl$_3$) δ ppm 8.23–8.18 (m, 1H), 7.84–7.79 (m, 1H), 7.66–7.56 (m, 2H), 7.49 (s, 1H), 4.07–4.01 (m, 2H), 2.76 (s, 3H), 2.45 (s, 3H). 

$^{13}$C NMR (75 MHz, CDCl$_3$) δ ppm 200.8, 169.3, 152.2, 142.3, 130.0, 129.5, 129.1, 128.4, 126.6, 124.1, 121.4, 117.2, 79.5, 60.2, 30.4, 22.2, 20.7.
3-Acetyl-4-(isoamyloxy)-5-methoxynaphthalen-1-yl acetate (3d)

Following the procedure described above for the preparation of 3a, compound 3d was obtained from 2b (0.1 g, 0.36 mmol) as a yellow solid. (0.98 g, 82%): \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) ppm 7.51 (dd, 1H, \( J = 8.2 \) Hz, 7.6 Hz), 7.45 (s, 1H), 7.41 (d, 1H, \( J = 7.6 \) Hz), 6.95 (d, 1H, \( J = 7.6 \) Hz), 4.03 (s, 3H), 3.90 (t, 2H, \( J = 7.2 \) Hz), 2.76 (s, 3H), 2.44 (s, 3H), 1.87–1.75 (m, 3H), 0.97 (d, 6H, \( J = 6.2 \) Hz).

13C NMR (75 MHz, CDCl\(_3\)) \( \delta \) ppm 200.5, 169.3, 157.2, 154.9, 142.4, 132.2, 129.7, 128.9, 120.8, 118.3, 114.0, 106.9, 56.1, 56.0, 38.9, 31.3, 29.6, 25.1, 22.7, 20.9.

2.2.4. General Synthesis of 2-acetyl-1,4-dialkoxynaphthalene (4)

2-Acetyl-1,4-dimethoxynaphthalene (4a; CAS Registry Number 65131-13-7)

3a (0.29 g, 1.12 mmol) was dissolved in methanol (6 mL) and was cooled to 0 °C. Then 1 wt % potassium hydroxide solution in methanol (5.4 mL) was added. After stirring for 2 h, the mixture was neutralized by adding Amberlite IR-120(H) and stirred for additional 15 min. Amberlite IR-120(H) was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was dissolved in DMF (2 mL), and iodomethane (0.11 mL, 1.68 mmol) and cesium carbonate (0.5 g, 1.68 mmol) were added. The mixture was heated under reflux. After monitoring the reaction complete by TLC, it was cooled to room temperature, extracted with dichloromethane (DCM), washed with water and dried over anhydrous magnesium sulfate. It was concentrated in vacuo and purified by flash column chromatography to give the product 4a as a yellow oil. (0.26 g, quantitative yield): \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) ppm 8.28–8.23 (m, 1H), 8.19–8.15 (m, 1H), 7.63–7.57 (m, 2H), 7.08 (s, 1H), 4.01 (s, 3H), 3.96 (s, 3H), 2.81 (s, 3H).

13C NMR (75 MHz, CDCl\(_3\)) \( \delta \) ppm.

2-Acetyl-1,4-diisoamyloxynaphthalene (4b)

Following the procedure described above for the preparation of 4a, compound 4b was obtained from 3b (1 g, 3.3 mmol) and 1-bromo-3-methylbutane (0.6 mL, 5 mmol) as a yellow oil. (0.47 g, 42%): \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) ppm 8.29–8.24 (m, 1H), 8.16–8.10 (m, 1H), 7.60–7.55 (m, 2H), 7.01 (s, 1H), 4.17 (t, 2H, \( J = 6.3 \) Hz), 3.97 (t, 2H, \( J = 6.6 \) Hz), 2.78 (s, 1H), 1.00 (d, 6H, \( J = 6.3 \) Hz), 0.98 (d, 6H, \( J = 5.4 \) Hz).

13C NMR (75 MHz, CDCl\(_3\)) \( \delta \) ppm 200.5, 151.0, 150.3, 128.9, 127.7, 127.4, 126.8, 122.9, 122.5, 102.6, 75.8, 66.6, 39.1, 37.8, 30.7, 25.1, 24.9, 22.6, 22.5.

2-Acetyl-1,4-diisopropoxynaphthalene (4c)

Following the procedure described above for the preparation of 4a, compound 4c was obtained from 3c (0.12 g, 0.42 mmol) as a yellow oil. (0.1 g, 83%): \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) ppm 8.28–8.25 (m, 1H), 8.15–8.11 (m, 1H), 7.60–7.53 (m, 2H), 6.95 (s, 1H), 4.80–4.72 (m, 1H), 4.31–4.25 (m, 1H), 2.76 (s, 3H), 1.45 (s, 3H), 1.43 (s, 3H), 1.31 (d, 6H, \( J = 6.0 \) Hz).

13C NMR (75 MHz, CDCl\(_3\)) \( \delta \) ppm 202.5, 149.6, 147.7, 130.0, 129.5, 129.4, 127.7, 127.4, 126.8, 122.9, 122.5, 102.6, 75.8, 66.6, 39.1, 37.8, 30.7, 25.1, 24.9, 22.6, 22.5.

2-Acetyl-1,4-diisoamyloxy-8-methoxynaphthalene (4d)

Following the procedure described above for the preparation of 4a, compound 4d was obtained from 3d (0.09 g, 0.27 mmol) as a yellow solid. (0.09 g, 77%): \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) ppm 7.90 (d, 1H, \( J = 8.2 \) Hz), 7.46 (dd, 1H, \( J = 8.2 \) Hz, 7.8 Hz), 7.00 (s, 1H), 6.95 (d, 1H, \( J = 7.6 \) Hz), 4.15 (t, 2H, \( J = 6.4 \) Hz), 4.01 (s, 3H), 3.85 (t, 2H, \( J = 7.1 \) Hz), 2.78 (s, 3H), 1.98–1.75 (m, 6H), 1.00 (d, 6H, \( J = 6.4 \) Hz), 0.96 (d, 6H, \( J = 6.2 \) Hz).

13C NMR (75 MHz, CDCl\(_3\)) \( \delta \) ppm 202.1, 156.7, 150.7, 150.3, 131.1, 129.7, 127.6, 126.3, 123.5, 122.6, 104.4, 78.8, 70.5, 30.8, 22.3, 22.1.

2.2.5. General Synthesis of naphthalenacyl bromide (5a–5e)

2-Bromoacetyl-1,4-dimethoxynaphthalene (5a)

4a (0.2 g, 0.86 mmol) was dissolved in DCM (10 mL). Tetraethylammonium tribromide (TBA-Br\(_3\); 0.36 g, 1.12 mmol) was added and the resulting solution was stirred under argon atmosphere for 3 h. It was quenched with water, extracted with DCM and washed with...
water, 1M sodium bicarbonate solution and brine, successively. The combined organic phase was dried over anhydrous sodium sulfate and concentrated in vacuo. It was purified by flash column chromatography to give the compound 5a as a yellow solid. (0.18 g, 67%) \[ 23\]:

1H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 8.30 (d, 1H, \( J = 8.3 \) Hz), 8.20 (d, 1H, \( J = 7.9 \) Hz), 7.74 (t, 1H, \( J = 7.7 \) Hz), 7.65 (t, 1H, \( J = 7.3 \) Hz), 6.82 (s, 1H), 4.82 (s, 2H), 3.99 (s, 3H).

\[ 13 \]C NMR (75 MHz, (CD\textsubscript{3})\textsubscript{2}SO) \( \delta \) ppm 192.8, 152.0, 151.8, 129.3, 128.1, 127.2, 124.4, 123.2, 122.6, 102.1, 64.2, 55.7, 36.4, 36.4.

2-Bromoacetyl-1,4-diisamyloxynaphthalene (5b)

Following the procedure described above for the preparation of 5a, compound 5b was obtained from 4b (3.76 g, 11 mmol) as a yellow oil. (2.8 g, 62%):

1H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 8.31–8.27 (m, 1H), 8.12–8.09 (m, 1H), 7.63–7.57 (m, 2H), 6.99 (s, 1H), 4.79 (s, 2H), 4.19–4.15 (m, 2H), 4.02–3.97 (m, 2H), 1.99–1.88 (m, 2H), 1.86–1.78 (m, 4H), 1.01 (d, 6H, \( J = 6.6 \) Hz), 1.99 (d, 6H, \( J = 6.3 \) Hz).

\[ 13 \]C NMR (75 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 193.9, 151.4, 150.4, 129.4, 128.5, 128.0, 127.2, 125.1, 123.0, 122.7, 102.7, 76.4, 66.8, 39.0, 37.8, 36.2, 25.2, 25.0, 22.6, 22.6.

2-Bromoacetyl-1,4-diisopropoxynaphthalene (5c)

Following the procedure described above for the preparation of 5a, compound 5c was obtained from 4c (0.17 g, 0.63 mmol) as a yellow oil. (0.13 g, 56%):

1H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 8.30–8.27 (m, 1H), 8.10–8.08 (m, 1H), 7.60–7.55 (m, 2H), 6.92 (s, 1H), 4.79 (s, 2H), 4.79–4.76 (m, 1H), 4.32–4.26 (m, 1H), 1.55–1.43 (m, 6H), 1.33–1.30 (m, 6H).

\[ 13 \]C NMR (75 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 195.7, 150.0, 147.7, 129.8, 129.4, 127.6, 126.9, 123.4, 122.9, 104.3, 100.5, 70.6, 36.0, 22.3, 22.0.

2-Bromoacetyl-1,4-diisoamyloxy-8-methoxynaphthalene (5d)

Following the procedure described above for the preparation of 5a, compound 5d was obtained from 4d (0.67 g, 1.8 mmol) as a yellow solid. (0.53 g, 65%):

1H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 7.91 (d, 1H, \( J = 8.4 \) Hz), 7.50 (dd, 1H, \( J = 7.8 \) Hz, 8.4 Hz), 6.97 (d, 1H, \( J = 7.8 \) Hz), 6.96 (s, 1H), 4.81 (s, 2H), 4.15 (t, 2H, \( J = 6.2 \) Hz), 4.02 (s, 3H), 3.86 (t, 2H, \( J = 7.1 \) Hz), 1.98–1.72 (m, 6H), 1.00 (d, 6H, \( J = 6.5 \) Hz), 0.96 (d, 6H, \( J = 6.2 \) Hz).

\[ 13 \]C NMR (CDCl\textsubscript{3}, 75 MHz) \( \delta \) ppm 195.1, 156.7, 151.1, 150.4, 131.5, 128.2, 127.0, 120.0, 115.1, 107.4, 103.9, 76.8, 66.9, 56.0, 38.9, 37.9, 37.1, 25.2, 25.1, 22.7, 22.6.

2-Bromoacetyl-1-methoxynaphthalene (5e)

Following the procedure described above for the preparation of 5a, compound 5e was obtained from 2-acetyl-1-methoxynaphthalene (0.5 g, 2.5 mmol) as a white solid. (0.49 g, 70%):

1H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 8.21–8.18 (m, 1H), 7.88–7.85 (m, 1H), 7.74 (d, 1H, \( J = 8.6 \) Hz), 7.65 (d, 1H, \( J = 8.4 \) Hz), 7.46 (t, 2H, \( J = 6.2 \) Hz), 4.74 (s, 2H), 4.03 (s, 3H).

\[ 13 \]C NMR (CDCl\textsubscript{3}, 75 MHz) \( \delta \) ppm 193.0, 157.7, 137.2, 128.7, 128.4, 127.7, 127.6, 126.9, 123.4, 122.9, 104.3, 100.5, 76.8, 66.9, 56.0, 38.9, 37.9, 37.1, 25.2, 25.1, 22.7, 22.6.

2.2.6. General Synthesis of 1-substituted benzylimidazoles (6c and 6d)

1-(4-Methoxybenzyl)-1H-imidazole (6c)

Imidazole (0.25 g, 3.68 mmol) and potassium carbonate (0.5 g, 3.68 mmol) were dissolved in acetonitrile (15 mL). Then 4-methoxybenzyl chloride (0.5 mL, 3.68 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. After completion of the reaction, it was concentrated under reduced pressure and purified by flash column chromatography to obtain compound 6c in the form of an ivory solid. (0.4 g, 60%) \[ 24\]:

1H NMR (300 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 7.52 (s, 1H), 7.12–7.07 (m, 3H), 6.89–6.85 (m, 1H), 7.54 (s, 1H), 7.52 (s, 1H), 7.12–7.07 (m, 3H), 6.89–6.86 (m, 1H), 5.04 (s, 2H), 3.80 (s, 3H). \[ 13 \]C NMR (75 MHz, CDCl\textsubscript{3}) \( \delta \) ppm 159.4, 137.1, 129.6, 128.7, 128.0, 119.0, 114.2, 55.2, 50.1.
1-(4-Nitrobenzyl)-1H-imidazole (6d)

Following the procedure described above for the preparation of 6c, compound 6d was obtained from 4-nitrobenzyl bromide (0.5 g, 2.31 mmol) as a yellow solid. (0.46 g, >98%): 1H NMR (300 MHz, CDCl3) δ ppm 8.22 (d, 2H, J = 8.6 Hz), 7.59 (s, 1H), 7.28 (d, 2H, J = 6.5 Hz), 7.15 (s, 1H), 6.92 (s, 1H), 5.26 (s, 2H); 13C NMR (75 MHz, CDCl3) δ ppm 147.3, 143.4, 137.3, 129.9, 127.5, 123.8, 119.1, 49.5.

2.2.7. General Synthesis of NAIMSs (7a–7i, 10, 11)

3-(2-(1,4-Dimethoxynaphthalen-2-yl)-2-oxoethyl)-1-methyl-1H-imidazol-3-ium bromide (NAIMS 7a)

Compound 5a (0.05 g, 0.16 mmol) and 1-methylimidazole (6a; 0.025 mL, 0.32 mmol) were dissolved in acetonitrile (2 mL). The mixture was heated under reflux for 1 d. It was concentrated under reduced pressure and recrystallized by acetonitrile and ether to give the compound 7a as an ivory solid. (0.061 g, 98%): 1H NMR (300 MHz, (CD3)2SO) δ ppm 9.10 (s, 1H), 8.26–8.22 (m, 2H), 7.78–7.76 (m, 4H), 7.19 (s, 1H), 5.98 (s, 2H), 4.09 (s, 3H), 3.99 (s, 3H), 3.97 (s, 3H). 13C NMR (75 MHz, (CD3)2SO) δ ppm 191.1, 152.8, 151.3, 137.8, 129.1, 128.9, 127.9, 124.1, 123.2, 123.1, 122.2, 101.1, 64.1, 58.2, 55.8, 35.9. m.p. 227.6 °C decomposed. HRMS (FAB) m/z Calcd. for C18H19N2O3 [M−Br]+ 311.1396, found 311.1395.

3-(2-(1,4-Bis(isoamyloxy)naphthalen-2-yl)-2-oxoethyl)-1-methyl-1H-imidazol-3-ium bromide (NAIMS 7b)

Following the procedure described above for the preparation of 7a, compound 7b was obtained from 5b (0.12 g, 0.28 mmol) and imidazole 6a (0.045 g, 0.56 mmol) as an ivory solid. (0.1 g, 71%): 1H NMR (300 MHz, (CD3)2SO) δ ppm 9.12 (s, 1H), 8.24–4.14 (m, 4H), 3.97 (s, 3H), 1.97–1.85 (m, 4H), 1.81–1.74 (m, 2H), 0.99–0.97 (m, 12H). 13C NMR (75 MHz, (CD3)2SO) δ ppm 191.1, 151.3, 150.6, 137.8, 129.0, 128.2, 127.8, 124.0, 123.6, 123.3, 122.3, 102.0, 75.5, 66.5, 57.9, 38.4, 37.2, 35.9, 24.8, 24.6, 22.4. m.p. 68.9–70.8 °C. HRMS (FAB) m/z Calcd. for C26H35N2O3 [M−Br]+ 423.2648, found 423.2644.

1-Benzyl-3-(2-(1,4-bis(isoamyloxy)naphthalen-2-yl)-2-oxoethyl)-1H-imidazol-3-ium bromide (NAIMS 7c)

Following the procedure described above for the preparation of 7a, compound 7c was obtained from 5b (0.05 g, 0.12 mmol) and 1-benzylimidazole (6b; 0.037 g, 0.24 mmol) to give the compound 7c as an ivory solid. (0.07 g, >98%): 1H NMR (300 MHz, (CD3)2SO) δ ppm 9.30 (s, 1H), 8.23 (dd, 1H, J = 2.9, 6.0 Hz), 7.92 (s, 1H), 7.80 (s, 1H), 7.77–7.74 (m, 2H), 1.80–1.76 (m, 2H), 0.99–0.97 (m, 12H). 13C NMR (75 MHz, (CD3)2SO) δ ppm 191.0, 151.3, 150.5, 137.5, 134.8, 129.0, 128.7, 128.2, 128.1, 132.3, 122.2, 122.0, 101.9, 75.5, 66.5, 58.1, 51.9, 38.4, 37.2, 35.9, 24.8, 24.6, 22.4. m.p. 68.9–70.8 °C. HRMS (FAB) m/z Calcd. for C32H39N2O3 [M−Br]+ 499.2961, found 499.2960.

3-(2-(1,4-Bis(isoamyloxy)naphthalen-2-yl)-2-oxoethyl)-1-(4-methoxybenzyl)-1H-imidazol-3-ium bromide (NAIMS 7d)

Following the procedure described above for the preparation of 7a, compound 7d was obtained from 5b (0.06 g, 0.14 mmol) and 4-methoxybenzylimidazole (6c; 0.05 g, 0.28 mmol) as an ivory solid. (0.045 g, 55%): 1H NMR (300 MHz, CD2SO6) δ 9.17 (s, 1H), 8.23 (dd, 1H, J = 2.9, 6.0 Hz), 8.15 (dd, 1H, J = 3.3, 6.2 Hz), 7.86 (s, 1H), 7.77–7.74 (m, 3H), 7.44 (d, 2H, J = 8.4 Hz), 7.18 (s, 1H), 7.02 (d, 2H, J = 8.6 Hz), 7.18 (s, 1H), 5.92 (s, 2H), 5.46 (s, 2H), 4.21–4.12 (m, 4H), 1.90–1.87 (m, 2H), 1.80–1.74 (m, 2H), 0.98 (d, 6H, J = 2.9 Hz), 0.97 (d, 6H, J = 3.6 Hz). 13C NMR (75 MHz, CD2SO6) δ 191.0, 159.6, 151.4, 150.5, 137.5, 134.8, 129.0, 128.7, 128.2, 128.1, 124.4, 123.5, 123.3, 122.2, 122.0, 101.9, 75.5, 66.5, 58.1, 51.9, 38.4, 37.2, 24.7, 24.5, 22.5, 22.4. m.p. 87.7–88.8 °C. HRMS (FAB) m/z Calcd. for C32H30N2O3 [M−Br]+ 499.2961, found 499.2960.
24.7, 24.5, 22.5, 22.4. m.p. 113.3–114.7 °C. HRMS (FAB) m/z Calcd. for C_{33}H_{41}N_{2}O_{4} [M – Br]^+ 529.3066, found 529.3068.

3-((1,4-Bis(isoamyloxy)naphthalen-2-yl)-2-oxoethyl)-1-(4-nitrobenzyl)-1H-imidazol-3-ium bromide (NAIMS 7e)

Following the procedure described above for the preparation of 7a, compound 7e was obtained from 5b (0.06 g, 0.14 mmol) and 1-(4-nitrobenzyl)imidazole (6d; 0.04 g, 0.21 mmol) as an ivory solid. (0.06 g, 70%): 1H NMR (300 MHz, CD_{3}SO) δ 9.28 (s, 1H), 8.34 (d, 2H, J = 6.7 Hz), 8.23–8.07 (m, 2H), 7.92 (s, 1H), 7.81 (s, 1H), 7.78–7.75 (m, 2H), 7.70 (d, 2H, J = 7.1 Hz), 7.20 (s, 1H), 5.96 (s, 2H), 5.74 (s, 2H), 4.22–4.14 (m, 4H), 1.99–1.91 (m, 4H), 1.79–1.76 (m, 2H), 0.99–0.96 (m, 12H). 13C NMR (75 MHz, CD_{3}SO) δ 190.9, 151.4, 150.6, 147.6, 142.1, 137.9, 129.4, 129.0, 128.1, 127.8, 124.7, 124.1, 123.5, 123.3, 122.2, 122.1, 101.9, 75.6, 66.5, 58.2, 51.0, 38.4, 37.2, 24.7, 24.5, 22.5, 22.4.; m.p. 160.2–160.6 °C; HRMS (FAB) m/z Calcd. for C_{32}H_{36}N_{3}O_{3} [M – Br]^+ 544.2811, found 544.2812.

1-Benzyl-3-(2-(1,4-diisopropoxynaphthalen-2-yl)-2-oxoethyl)-1H-imidazol-3-ium bromide (NAIMS 7f)

Following the procedure described above for the preparation of 7a, compound 7f was obtained from 5c (0.08 g, 0.22 mmol) and 1-benzylimidazole (6b; 0.06 g, 0.38 mmol) as an ivory solid. (0.065 g, 59%): 1H NMR (300 MHz, CDCl₃) δ 11.00 (s, 1H), 8.32–8.30 (m, 1H), 8.10–8.07 (m, 1H), 7.63–7.60 (m, 2H), 7.47–7.42 (m, 5H), 7.12–7.10 (m, 2H), 7.04 (s, 1H), 6.08 (s, 2H), 5.61 (s, 2H), 4.81–4.77 (m, 1H), 4.55–4.51 (m, 1H), 1.47–1.45 (m, 12H). 13C NMR (75 MHz, CDCl₃) δ ppm 192.2, 150.2, 149.8, 139.2, 132.5, 130.7, 129.6, 129.5, 129.1, 128.9, 128.4, 127.0, 125.2, 123.8, 123.1, 123.1, 120.8, 103.1, 79.7, 70.8, 58.6, 53.5, 22.4, 22.0. m.p. 191.7–192.5 °C. HRMS (FAB) m/z Calcd. for C_{28}H_{33}N_{3}O_{3} [M – Br]^+ 443.2335, found 443.2332.

3-(1,4-Bis(isoamyloxy)-8-methoxynaphthalen-2-yl)-2-oxoethyl)-1-methyl-1H-imidazol-3-ium bromide (NAIMS 7g)

Following the procedure described above for the preparation of 7a, compound 7g was obtained from 5d (0.05 g, 0.11 mmol) and 1-methylimidazole (6a; 0.018 g, 0.22 mmol) as an ivory solid. (0.051 g, 88%): 1H NMR (300 MHz, CD_{3}SO) δ ppm 9.06 (s, 1H), 7.81 (d, 1H, J = 8.4 Hz), 7.75 (s, 1H), 7.72 (s, 1H), 7.68–7.62 (m, 1H), 7.21 (d, 1H, J = 8.0 Hz), 7.17 (s, 1H), 5.87 (s, 2H), 4.18–4.13 (m, 2H), 3.99 (s, 3H), 3.95 (s, 3H), 3.95–3.92 (m, 2H), 1.89–1.87 (m, 4H), 1.84–1.72 (m, 4H), 0.96 (d, 6H, J = 5.5 Hz), 0.95 (d, 6H, J = 5.6 Hz). 13C NMR (75 MHz, CD_{3}SO) δ ppm 191.6, 156.9, 152.5, 150.0, 137.8, 131.7, 139.7, 124.3, 123.9, 123.0, 119.3, 114.0, 108.3, 102.7, 75.8, 66.4, 58.2, 56.0, 38.2, 37.2, 35.8, 24.8, 24.7, 22.7, 22.4. m.p. 112.9–114.7 °C. HRMS (FAB) m/z Calcd. for C_{27}H_{32}N_{2}O_{4} [M – Br]^+ 453.2752, found 453.2752.

1-Benzyl-3-(2-(1,4-bis(isoamyloxy)-8-methoxynaphthalen-2-yl)-2-oxoethyl)-1H-imidazol-3-ium bromide (NAIMS 7h)

Following the procedure described above for the preparation of 7a, compound 7h was obtained from 5d (0.1 g, 0.22 mmol) and imidazole 6b (0.05 g, 0.33 mmol) as an ivory solid. (0.1 g, 80%): 1H NMR (300 MHz, CD_{3}SO) δ ppm 9.22 (s, 1H), 7.87 (s, 1H), 7.80 (d, 1H, J = 8.4 Hz), 7.74 (s, 1H), 7.67–7.62 (m, 1H), 7.48–7.42 (m, 5H), 7.20 (d, 1H, J = 7.7 Hz), 7.17 (s, 1H), 5.87 (s, 2H), 5.54 (s, 2H), 4.17–4.12 (m, 2H), 3.99 (s, 3H), 3.95–3.91 (m, 2H), 1.90–1.84 (m, 4H), 1.75–1.73 (m, 2H), 0.96 (d, 6H, J = 6.6 Hz), 0.94 (d, 6H, J = 6.5 Hz). 13C NMR (75 MHz, CD_{3}SO) δ ppm 190.9, 151.4, 150.6, 147.6, 142.1, 137.5, 134.8, 131.3, 129.7, 123.9, 123.0, 119.3, 114.0, 108.2, 102.7, 75.9, 66.4, 58.5, 56.0, 51.9, 38.1, 37.2, 24.8, 24.7, 22.6, 22.3. m.p. 121.3–122.7 °C. HRMS (FAB) m/z Calcd. for C_{31}H_{41}N_{2}O_{4} [M – Br]^+ 529.3066, found 529.3068.
3-(2-(1,4-Bis(isoamyloxy)-8-methoxynaphthalen-2-yl)-2-oxoethyl)-1-(4-nitrobenzyl)-1H-imidazol-3-ium bromide (NAIMS 7i)

Following the procedure described above for the preparation of 7a, compound 7i was obtained from 5d (0.1 g, 0.22 mmol) and imidazole 6d (0.067 g, 0.33 mmol) as an ivory solid. (0.12 g, 88%): 1H NMR (300 MHz, (CD3)2SO) δ ppm 9.27 (s, 1H), 8.34 (d, 2H, J = 8.0 Hz), 7.90 (s, 1H), 7.81 (d, 1H, J = 8.4 Hz), 7.79 (s, 1H), 7.69 (d, 2H, J = 8.6 Hz), 7.68–7.62 (m, 1H), 7.21 (d, 1H, J = 8.0 Hz), 7.17 (s, 1H), 5.90 (s, 2H), 5.73 (s, 2H), 4.17–4.13 (m, 2H), 3.99 (s, 3H), 3.94 (t, 2H, J = 7.3 Hz), 1.87–1.83 (m, 4H), 1.77–1.73 (m, 2H), 0.96 (d, 6H, J = 6.3 Hz), 0.94 (d, 6H, J = 6.2 Hz). 13C NMR (75 MHz, (CD3)2SO) δ ppm 191.4, 156.9, 152.5, 150.0, 147.6, 142.1, 137.9, 131.3, 129.7, 129.4, 124.6, 124.2, 124.0, 122.0, 119.3, 114.0, 108.2, 102.7, 75.9, 66.4, 58.5, 56.0, 51.0, 38.2, 37.2, 24.8, 24.7, 22.6, 22.4. m.p. 158.3–159.8 °C. HRMS (FAB) m/z Calcd. for C33H40N3O6 [M−Br]+ 574.2917, found 574.2916.

1-Benzyl-3-(2-(naphthalen-2-yl)-2-oxoethyl)-1H-imidazol-3-ium bromide (NAIMS 10)

Following the procedure described above for the preparation of 7a, compound 10 was obtained from 2-bromoacetylnaphthalene (0.1 g, 0.4 mmol) and imidazole 6b as a white solid. (0.09 g, 56%): 1H NMR (300 MHz, (CD3)2SO) δ ppm 9.24 (s, 1H), 8.79 (s, 1H), 8.19 (d, 1H, J = 8.0 Hz), 8.13 (d, 1H, J = 8.2 Hz), 8.06 (d, 1H, J = 8.2 Hz), 7.91 (s, 1H), 7.76 (s, 1H), 7.75–7.69 (m, 2H), 7.47–7.45 (m, 5H), 6.16 (s, 2H), 5.56 (s, 2H). 13C NMR (75 MHz, (CD3)2SO) δ ppm 191.1, 137.5, 135.4, 134.8, 131.9, 130.9, 130.4, 129.6, 129.3, 129.0, 128.8, 128.7, 128.2, 127.8, 127.4, 124.1, 124.0, 123.1, 122.2, 55.5, 52.0. m.p. 170.8 °C decomposed. HRMS (FAB) m/z Calcd. for C22H19N2O [M−Br]+ 327.1497, found 327.1497.

1-Benzyl-3-(2-(1-methoxynaphthalen-2-yl)-2-oxoethyl)-1H-imidazol-3-ium bromide (NAIMS 11)

Following the procedure described above for the preparation of 7a, compound 11 was obtained from 2-bromoacetyl-1-methoxynaphthalene 5e (0.1 g, 0.36 mmol) and imidazole 6b as an ivory solid. (0.12 g, 79%): 1H NMR (300 MHz, (CD3)2SO) δ ppm 9.39 (s, 1H), 8.29 (d, 1H, J = 7.5 Hz), 8.07 (d, 1H, J = 8.4 Hz), 7.95 (s, 1H), 7.91–7.88 (m, 2H), 7.84 (s, 1H), 7.78–7.70 (m, 2H), 7.47–7.45 (m, 5H), 6.04 (s, 2H), 5.61 (s, 2H), 4.16 (s, 3H). 13C NMR (75 MHz, (CD3)2SO) δ ppm 191.1, 137.5, 135.4, 134.8, 131.9, 130.9, 130.4, 129.6, 129.3, 129.0, 128.8, 128.7, 128.2, 127.8, 127.4, 124.1, 122.2, 55.5, 52.0. m.p. 159.5–159.8 °C. HRMS (FAB) m/z Calcd. for C23H21N2O2 [M−Br]+ 357.1603, found 357.1604.

2.3. Fungal Strains and Culture

Candida tropicalis var. tropicalis (KCTC17762), Candida glabrata (KCTC7219), Candida tropicalis (KCTC7212), Candida albicans (KCTC7270), Candida albicans (KCTC7965) and Candida auris (KCTC17810) were purchased from KCTC (Korean Collection for Type Cultures, Candida parapsilosis var. parapsilosis (KACC45480) and Candida albicans (KACC30071) were purchased from KACC (Korean Agricultural Culture Collection, Suwon, Korea). All strains were kept in 20% glycerol at −70 °C. They were cultured in YPD containing yeast extract 10 g/L (BD Difco, Franklin Lakes, NJ, USA), peptone 20 g/L (BD Difco) and 2% D-glucose (w/v) (Daejung, Gyeonggi-do, Suwon, Korea) at 30 °C for 24–48 h [26].

2.4. Antifungal Susceptibility Microdilution Assay

Microdilution assay was performed based on the description of CLSI document M27-A [26–28]. Compounds were prepared in DMSO (Dimethyl sulfoxide; Junsei, Tokyo, Japan) at a concentration of 10 mg/mL as a stock solution. A colony of each strain was inoculated in 3 mL of YPD broth at 30 °C overnight, and the medium was changed to new fresh medium. The strains were adjusted in 20% glycerol at −70 °C. They were cultured in YPD containing yeast extract 10 g/L (BD Difco, Franklin Lakes, NJ, USA), peptone 20 g/L (BD Difco) and 2% D-glucose (w/v) (Daejung, Gyeonggi-do, Suwon, Korea) at 30 °C for 24–48 h [26].
2.5. Cell Viability Assay

Cell viability was estimated according to previously reported method with slightly modification [30]. A normal cell line, HaCaT (ATCC, VA, USA), was added to a 96-well plate at 1.0 × 10^4 cells per well and incubated for 24 h. Various concentrations of NAIMS 7c (3.125–100 µg/mL) were added and further incubated for 24 h. MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO, USA) in PBS was added into each well, followed by incubation for 3 h at 37 °C. The medium was then removed, and cells were suspended in 100 µL DMSO for 10 m. Viable cells were calculated from optical density (OD_{540}) values measured using a microplate reader (BioTek Instruments, Winooski, VT, USA).

2.6. Fungal Cell Growth Test

Inoculation and culture conditions in this study were same as those in the above-mentioned antifungal susceptibility microdilution assay. The compound concentrations then ranged from 1.56 to 25 µg/mL. We cultured 96-well plates (SPL, Gyeonggi-do, Korea) at 30 °C and OD_{600} were measured using a microplate reader (BioTek Instruments) at indicated time [31].

2.7. ROS Detection

ROS detection cell-based assay kit (Cayman Chemical, Ann Arbor, MI, USA) was used according to manufacturer’s instructions. Cells were incubated with/without antimycin (positive control) or indicated concentrations of NAIMS 7c for 2 h. Cells were rinsed with ice cold cell-based assay buffer and then incubated with ROS staining buffer. Dihydroethidium (DHE) fluorescence was measured using an excitation wavelength 480 nm and an emission wavelength 580 nm according to manufacturer’s instructions by VICTOR2 (Perkin Elmer, Waltham, MA, USA). Antimycin A was used as positive control [32].

2.8. Measurement of Mitochondrial Membrane Potential (ΔΨm)

_C. albicans_ was added to the wells of a 24-well plate at a density of 5.0 × 10^6 CFU/mL. NAIMS 7c (1.56, 3.125 and 6.25 µg/mL) was added and further incubated for 2 h. Cells were stained with 5 µM of JC-1 (Biotium, CA, USA) for 30 m at 30 °C. After washing, photographic images were acquired under an inverted fluorescence microscope (EVOS FL Cell Imaging System, Thermo Fisher, Waltham, MA, USA) using the microscope program [17].

2.9. Detection for Release of UV Absorbing Materials at 260 and 280 nm

Overnight cultured _C. albicans_ was washed with PBS to stop further proliferation. Cells were treated with indicated concentration of NAIMS 7c for 1 h at 30 °C. Lysate released was measured with the Ultrospec 3000 at 260 and 280 nm. The values were adjusted by subtracting the optical density measured for the corresponding negative control, which was obtained by same compound concentrations in PBS without cells at the same wavelength [33,34].

2.10. Quantitative Reverse Transcriptase PCR (qRT-PCR) analysis

qRT-PCR was performed following the method with slight modification [35]. Total RNA was extracted with TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA). Real-time PCR was performed in 96-well PCR plates (Bio-rad, Hercules, CA, USA) using 2× RT Pre-MIX kit (Biofact, Daejeon-si, Korea) and CFX Connect Real-Time PCR Detection System (Bio-rad). Primer sequences used in this study are listed in Table 1.
μg/mL MIC values for compounds, MIC values were detected every day for μg/mL against all fungal activity compared with other derivatives; MIC values ranged from 3.125 to 6.25 μg/mL against Candida spp. After 24 h incubation. Among the NAIMS 7c showed stable antifungal activity compared with other derivatives. The MIC values of miconazole were used as a positive control and confirmed over 12.5 μg/mL to using broth microdilution assays. The MIC values of miconazole were used as a positive group in the naphthalene ring made for comparison with 1,4

2.11. Statistical Analysis

All data are expressed as the mean ± S.D. Significant differences among the groups were determined using the Student’s t-test or one-way ANOVA, and p < 0.05 was considered significant.

3. Results

3.1. Chemistry

First, we tried to synthesize 2-acetyl-1,4-dialkoxy naphthalenes 4, a key intermediate to target NAIMS 7 by direct alkylation of 1,4-dihydroxy naphthalene followed by Friedel–Crafts acylation. However, Friedel–Crafts acylation was found to be inefficient due to its low yield. Therefore, another synthetic route to produce 4 was suggested as shown in Scheme 1. The compounds 2 were synthesized in high yield from 1,4-diacetoxy naphthalene (1) by Fries rearrangement. Then, alkylation of compound 1 with the corresponding alkyl halide afforded compound 3. The removal of the acetyl group with KOH followed by O-alkylation produced key intermediate 4 in two steps with good yield. Various α-bromination reactions of compound 4 were tested to find a condition optimized for the synthesis of bromoacetyl intermediate 5. Finally, we found that compound 5 was obtained in the best yield when reacted with TBA-Br3. N-Arylimidazoles such as 6a and 6d, which are not commercially available, were synthesized from imidazole and 4-substituted benzyl halide in acetonitrile. The coupling reaction of the 2-bromoacetyl intermediates 5 with corresponding imidazoles 6 gave nine new 1,4-dialkoynaphthalen-2-acyl imidazolium salts 7a–i.

Table 1. Primer list used for qRT-PCR.

| Gene  | Oligomer                                      | References |
|-------|-----------------------------------------------|------------|
| ACT1  | F: TAGGTTCGAAGCTGCTGAC                       | [36]       |
| YPK1  | R: CCTGGAACATGGTAGTC                        | This study |
| HAC1  | F: TACAACACACACATCAACGAG                     | [37]       |
| MCA1  | R: ATTAGTGGACCGGAGATG                        | [38]       |

Scheme 1. Preparation of 1,4-dialkoxy-NAIMSs 7a–7i.

Reagents and conditions. (i) BF3, 2CH3COOH (7 eq), reflux, 1h; (ii) Cs2CO3 (1.5 eq), R1-X (1.5 eq), DMF (0.5M), 70 °C, 2.5 h; (iii) KOH (0.67 eq), MeOH (0.5 M), 0 °C, 1h, then Amberlite IR-120(H), 0 °C, 15 m; (iv) Cs2CO3 (1.5 eq), R1-X (1.5 eq), DMF (0.5M),
70 °C, 1.5 h; (v) TBA-Br3 (1.2–1.3 eq), CH2Cl2 (0.05 M), rt, 3 h; (vi) imidazole (1–2 eq), CH3CN, reflux, 1d.

To investigate a simple relationship between structure and activity of NAIMSs, we prepared compounds 8, 9, 9′, NAIMS 10 and NAIMS 11, as shown in Figure 2. Compounds 8, 9 and 9′ have no imidazolium moiety, and NAIMS 10 and NAIMS 11 have no dialkoxy substituents in a naphthalene ring. The same coupling reaction of readily available 2-bromoacetyl-naphthalene or 2-bromoacetyl-1-methoxynaphthalene (5e) with imidazole 6b gave, respectively, NAIMS 10 and NAIMS 11.

![Scheme 1. Preparation of 1,4-dialkoxy-NAIMSs 7a–7i. Reagents and conditions.](image)

**Figure 2.** Structure of naphthalene compounds without an imidazolium moiety or a dialkoxy group in the naphthalene ring made for comparison with 1,4-dialkoxy-NAIMS 7.

3.2. **Antifungal Activity of NAIMS 7c Against Candida spp.**

The growth inhibitory activity of 7a–i, 10 and 11 against Candida spp. was evaluated using broth microdilution assays. The MIC values of miconazole were used as a positive control and confirmed over 12.5 μg/mL to Candida spp. after 24 h incubation. Among the tested compounds, NAIMS 7b, NAIMS 7c, NAIMS 7d and NAIMS 7e showed stronger antifungal activity against C. albicans than miconazole. NAIMS 7c showed strongest antifungal activity compared with other derivatives; MIC values ranged from 3.125 to 6.25 μg/mL against all Candida spp. used in this study. In particular, NAIMS 7c showed 3.125 μg/mL MIC value in 24 h assay against C. auris (KCTC17810) which possessed native resistance to miconazole (Table 2). To check the stability of antifungal activity of synthetic compounds, MIC values were detected every day for 72 h. NAIMS 7c had 3.125 and 6.25 μg/mL MIC values for C. albicans (KACC30071) at 48 and 72 h, respectively. Miconazole, by contrast, showed sustainable anti-Candida activity of 25 μg/mL MIC value for 72 h (Table 3). NAIMS 7c showed stable antifungal activity compared with other derivatives. The yield of NAIMS 7c was fortunately higher than NAIMS 7b and NAIMS 7d, and this suggested that NAIMS 7c is more cost-beneficial than other derivatives (Scheme 1). In vitro cell viability of NAIMS 7c against HaCaT was performed via MTT assays. NAIMS 7c had IC50 of 21.39 μg/mL against HaCaT. Based on results, cell growth test was also evaluated for the serial diluted concentration of NAIMS 7c in C. albicans (KCTC7965, KCTC7270 and KACC30071) for 48 h (Figure 3). Regardless of the time, NAIMS 7c at 6.25 μg/mL strongly inhibited cell growth compared with miconazole.
| Compound     | C. albicans (KCTC7965) | C. albicans var. tropicalis (KCTC17762) | C. tropicalis var. parapsilosis (KACC45480) | C. glabrata (KCTC7219) | C. tropicalis (KCTC7212) | C. auris (KCTC17810) |
|--------------|------------------------|-----------------------------------------|---------------------------------------------|-----------------------|------------------------|----------------------|
| Miconazole   | 12.5                   | 12.5                                    | 12.5                                        | 12.5                  | 12.5                   | >100                 |
| NAIMS 7c     | 3.125                  | 6.25                                    | 3.125                                       | 6.25                  | 3.125                  | 3.125                |
| NAIMS 7g     | 12.5                   | 25                                      | 6.25                                        | 25                    | 6.25                   | 6.25                 |
| NAIMS 7h     | 50                     | 25                                      | 25                                          | 25                    | 50                     | 25                   |
| NAIMS 8      | >100                   | >100                                    | >100                                        | >100                  | >100                   | N/A                  |
| NAIMS 9      | >100                   | >100                                    | >100                                        | >100                  | >100                   | >100                 |
| NAIMS 10     | >100                   | >100                                    | >100                                        | >100                  | >100                   | >100                 |
| NAIMS 11     | >100                   | >100                                    | >100                                        | >100                  | 25                     | >100                 |
| NAIMS 7e     | 6.25                   | 6.25                                    | 6.25                                        | 6.25                  | 6.25                   | 3.125                |
| NAIMS 7f     | 6.25                   | 12.5                                    | 3.125                                       | 12.5                  | 3.125                  | 6.25                 |
| NAIMS 7g     | 12.5                   | 12.5                                    | 6.25                                        | 12.5                  | 6.25                   | 12.5                 |
| NAIMS 7a     | >100                   | >100                                    | >100                                        | >100                  | >100                   | >100                 |
| NAIMS 7d     | 3.125                  | 6.25                                    | 3.125                                       | 6.25                  | 3.125                  | 6.25                 |
| NAIMS 7i     | 100                    | 12.5                                    | 6.25                                        | 25                    | 25                     | 100                  |
Table 3. Changes of MIC values (µg/mL) by the time.

| Fungal Strains                  | Time  | Miconazole | NAIMS 7c | NAIMS 7g | NAIMS 7h | 8  | 9  | 9' | 9  | NAIMS 10 | NAIMS 11 | NAIMS 7e | NAIMS 7b | NAIMS 7i | NAIMS 7a | NAIMS 7d | NAIMS 7f |
|--------------------------------|-------|------------|----------|----------|----------|----|----|----|----|----------|----------|----------|----------|----------|----------|----------|----------|
| C. albicans (KCTC7965)        | 48h   | 12.5       | 6.25     | 12.5     | 100      | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     |
|                               | 72h   | 12.5       | 6.25     | 12.5     | 100      | >100| >100| >100| >100| 25       | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
| C. tropicalis var. tropicalis | 48h   | 12.5       | 6.25     | 25       | 50       | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
| (KCTC17762)                   | 72h   | 12.5       | 12.5     | 25       | 100      | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
| C. parapsilosis var. parapsilosis | 48h  | 12.5       | 6.25     | 25       | 100      | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
| (KACC45480)                   | 72h   | 12.5       | 12.5     | 25       | 100      | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
| C. glabrata (KCTC7219)        | 48h   | 12.5       | 3.125    | 25       | 50       | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
|                               | 72h   | 12.5       | 6.25     | 25       | 100      | >100| >100| >100| >100| 12.5     | 12.5     | 12.5     | >100     | >100     | >100     | >100     |
| C. tropicalis (KCTC7212)      | 48h   | 12.5       | 6.25     | 6.25     | 50       | >100| 25  | 50  | 25  | 12.5     | 6.25     | 12.5     | >100     | >100     | >100     | >100     |
|                               | 72h   | 12.5       | 6.25     | 6.25     | 100      | >100| 50  | 50  | 50  | 25       | 6.25     | 6.25     | >100     | >100     | >100     | >100     |
| C. auris (KCTC17810)          | 48h   | >100       | 3.125    | 6.25     | 25       | >100| >100| >100| >100| 12.5     | 6.25     | 12.5     | >100     | >100     | >100     | >100     |
|                               | 72h   | >100       | 3.125    | 6.25     | 25       | N/A | N/A | >100| >100| 12.5     | 6.25     | 12.5     | >100     | >100     | >100     | >100     |
Figure 3. The growth of *C. albicans* was inhibited by NAIMS 7c. Microdilution assay was performed at 3.125 µg/mL for 48 h. Data represent mean ± SD from three independent experiments. **p < 0.05 and ***p < 0.01.

3.3. Inducing ROS Level and the Loss of Mitochondria Membrane Potential by NAIMS 7c.

To identify antifungal activity of NAIMS 7c against *C. albicans*, ROS production in *C. albicans* was detected after NAIMS 7c treatment. NAIMS 7c induced higher ROS
production than did antimycin. The treatment of 0.78 μg/mL of NAIMS 7c increased the DHE fluorescence with the maximum at 1.56 μg/mL (Figure 4). For further study about the mechanism of NAIMS 7c, C. albicans was stained with JC-1 dye to detect the mitochondria membrane potential. NAIMS 7c decreased a red fluorescence at 1.56 μg/mL in C. albicans. This suggested that NAIMS 7c causes damage to mitochondria and alters the cellular state of C. albicans (Figure 5). Based on these results, NAIMS 7c induces loss of mitochondria membrane potential and increases the release of ROS in C. albicans.

![Figure 4](image-url)

**Figure 4.** NAIMS 7c induced the production of reactive oxygen species (ROS) in C. albicans. The amount of ROS of C. albicans (KCTC7965) was detected after 2 h. Antimycin was used for the positive control. Data represent mean ± SD from three independent experiments.

![Figure 5](image-url)

**Figure 5.** NAIMS 7c changed the mitochondrial membrane potential in C. albicans. We treated 1.56 μg/mL of NAIMS 7c for 2 h. Cells were detected by fluorescence microscope. Red fluorescence indicates dye aggregated in the mitochondria, and green indicates dye scattered in the cytoplasm.
3.4. The Cell Lysis and the Apoptosis of C. albicans by NAIMS 7c

To determine the induction of the loss of mitochondria membrane potential by NAIMS 7c treatment, changes in UV absorbing materials were detected by spectrophotometer with NAIMS 7c in the C. albicans culture medium supernatant. The use of 12.5 μg/mL of NAIMS 7c treatment significantly increased the absorbance at 260 and 280 nm after 1 and 2 h (Figure 6). These results show that treatment of NAIMS 7c at over the MIC value can lead to lysis of Candida spp. and might induce the apoptosis of Candida spp. Three different Candida-apoptosis related genes were used to detect the effect of NAIMS 7c in C. albicans. YPK1 is a serine/threonine protein kinase that affects diverse cellular activities, including sphingolipid homeostasis. HAC1 is a transcription factor and plays a major role in stress-related transcriptional response. MCA1 is a cysteine protease involved in apoptosis in response to stresses. The expression of these three genes was dramatically increased by NAIMS 7c treatment (Figure 7).

![Figure 6. NAIMS 7c induced the cell lysis of C. albicans. The release-detecting assay was performed after 1 or 2 h. Data represent mean ± SD from three independent experiments. ** p < 0.05.](image-url)
4. Discussion

Candida spp. are the most common organism recovered from the skin and blood of hospitalized patients. Although the need to treat them is increasing, the range of antifungal agents available is limited because of their toxicity. In addition, resistant strains and new species that show innate resistance to antifungal agents have been reported [39,40]. Therefore, it is necessary to introduce new antifungal agents to limit the spread of pathogenic fungi.

For the synthesis of 1,4-dialkoy-NAIMS, we developed a highly efficient synthesis method including the synthesis of 1,4-dialkoxy-2-acyl intermediate 4, the α-bromination reaction of compound 4, and the SN2 reaction of compound 5 and imidazole 6, such as a quaternization of imidazole. We found that compound 4 was produced in better yield by a novel synthetic method including Fries-rearrangement of compound 1 followed by alkylation than the direct alkylation of 1,4-dihydroxynaphthalene and subsequent Friedel–Crafts acylation. In addition, it was confirmed that the α-bromination reaction of compound 4 was best under the reaction conditions of TBA-Br3.

Among the 14 synthesized compounds (7a–i, 8, 9, 9’, 10, 11), NAIMS 7b, 7c, and 7d showed superior activity compared to other synthetic NAIMSs or miconazole. In particular, NAIMS 7c is best when considering its antifungal activity, stability and synthesis efficiency. As shown in Figure 8, the energy calculation (Gaussian B3LYP, 6-311 [d, p]) predicts NAIMS 7c presenting a slightly curved structure similar to phospholipids. Based on these results, we might propose a first-pass reasoning on the relationship between structure and activity as follows. For antifungal activity, the imidazolium ring must be essential. The naphthalene ring requires a 1,4-dialkoxy group, in which the optimal alkyl group is an isoamyl group. Furthermore, it seems better to have no methoxy groups in the naphthalene A ring and no electron withdrawing groups such as NO2 in the benzene ring in order for NAIMS to be activated.

Figure 7. NAIMS 7c induced the expression of apoptosis-related genes of C. albicans. mRNA of C. albicans (KCTC7965) was obtained after 2 h treatment. Miconazole and NAIMS 7c were treated at 6.25 and 1.56 μg/mL, respectively. Data represent mean ± SD from three independent experiments. *** p < 0.01.
Miconazole was used as an antifungal agent which has a fungicidal activity against planktonic Candida spp., and the cytotoxic concentration of NAIMS 7c can be significantly lower than miconazole \([41,42]\). NAIMS 7c had IC\(_{50}\) of 21.39 and 7.56 µg/mL against HaCaT and C. albicans (KACC30071), respectively. Miconazole had IC\(_{50}\) of 13.10 and 17.25 µg/mL against HaCaT and C. albicans, respectively. Accordingly, the selective index (IC\(_{50}\) HaCaT/IC\(_{50}\) C. albicans) of NAIMS 7c was higher than miconazole. This result shows that NAIMS 7c can be more effective to treat Candida spp. and safe. Additional in vivo research should be provided to determine the property of any potential candidate for therapeutic applications in the future \([43]\). Thus, NAIMS 7c could serve as a promising lead compound for further research. The 1,4-dialkoxy naphthalene-2-acyl compound 9 without the imidazolium moiety and the naphthalene-2-acyl imidazolium 10 without the dialkoxy substituent on the naphthalene ring showed no antifungal activity. However, its hybrid NAIMS 7c found an excellent antifungal agent. Therefore, as mentioned in the literature, the pharmacophore-hybridization approach is thought to be a useful tool for new drug design and development \([44]\).

NAIMS 7c led to induced ROS production, the loss of mitochondria membrane potential, the release of UV absorbing materials and up-regulated apoptotic gene expression. ROS and mitochondria play an essential part in apoptosis. These results suggest that NAIMS 7c induced apoptosis in C. albicans.

In summary, a series of novel imidazolium salts containing 2-acetylnaphthalene moiety were designed, prepared and evaluated for antifungal activity. The results presented in this study conclusively demonstrate that NAIMS 7c has a long-term enhanced antifungal activity against Candida spp., including especially C. albicans and C. auris, which possess native resistance to miconazole, as compared with other compounds including miconazole. Further studies of structure–activity relationships and a wide range of biological activities are ongoing and will be reported in the future.

**Author Contributions:** Conceptualization, K.-Y.K. and H.K.; methodology, K.-Y.K., H.K., and J.-G.K.; validation, K.-Y.K. and H.K.; formal analysis, K.-Y.K. and H.K.; investigation, J.L., H.L. and J.-G.K.; resources, J.L., K.-Y.K., H.K. and J.-G.K.; data curation, T.H.L., K.-Y.K. and H.K.; writing—original draft preparation, J.L, H.L. and J.-G.K.; writing—review and editing, T.H.L., K.-Y.K., H.K., J.L. and J.-G.K.; supervision, K.-Y.K. and H.K.; project administration, T.H.L., K.-Y.K. and H.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the GRRC program of Gyeonggi province [GRRC-kyunghee2020(B04)].

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.
References

1. Tangarife-Castano, V.; Correa-Royero, J.; Zapata-Londoño, B.; Durán, C.; Stanshenko, E.; Mesa-Arango, A.C. Anti-Candida albicans activity, cytotoxicity and interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants. *Infectious 2011*, 15, 160–167. [CrossRef]

2. Cornely, O.A.; Lass-Flörl, C.; Lagrou, K.; Arsic-Arsenijevic, V.; Hoenigl, M. Improving outcome of fungal diseases - Guiding experts and patients towards excellence. *Mycoses 2017*, 60, 420–425. [CrossRef]

3. Pfaller, M.A.; Pappas, P.G.; Wingard, J.R. Invasive Fungal Pathogens: Current Epidemiological Trends. *Clin. Infect. Dis. 2006*, 43, S3–S14. [CrossRef]

4. Pal, M. Morbidity and Mortality Due to Fungal Infections. *J. Appl. Microbiol. Biochem. 2018*, 1, 1–3. [CrossRef]

5. Chandra, J.; Long, L.; Isham, N.; Mukherjee, P.K.; Disciullo, G.; Appelt, K.; Ghannoum, M.A. In Vitro and In Vivo Activity of a Novel Catheter Lock Solution against Bacterial and Fungal Biofilms. *Antimicrob. Agents Chemother. 2018*, 62, e00722-18. [CrossRef]

6. Zida, A.; Bamba, S.; Yacouba, A.; Ouedraogo-Traore, R.; Guiguemde, R. Anti- Candida albicans natural products, sources of new antifungal drugs: A review. *J. Mycol. Méd. 2017*, 27, 1–19. [CrossRef]

7. Whaley, S.G.; Berkow, E.L.; Rybak, J.M.; Nishimoto, A.T.; Barker, K.S.; Rogers, P.D. Azole Antifungal Resistance in Candida albicans and Emerging Non-albicans Candida Species. *Front. Microbiol. 2017*, 7, 2173. [CrossRef]

8. Björnsson, E.; Jerlsted, P.; Bergqvist, A.; Olsson, R. Fulminant drug-induced hepatic failure leading to death or liver transplantation in Sweden. *J. Gastroenterol. 2005*, 40, 1095–1101.

9. Kyriakidis, I.; Tragianidis, A.; Münchens, S.; Groll, A.H. Clinical hepatotoxicity associated with antifungal agents. *Expert Opin. Drug Saf. 2016*, 16, 1–17. [CrossRef]

10. Ribas, A.; Del Ponte, E.; Dalbem, A.; Dalla-Lana, D.; Bündchen, C.; Donato, R.; Schrecker, H.; Fuentesfría, A. Imidazolium salts with antifungal potential for the control of head blight of wheat caused by Fusarium graminearum. *J. Appl. Microbiol. 2016*, 121, 445–452. [CrossRef]

11. Cornellas, A.; Perez, L.; Comelles, F.; Ribosa, I.; Manresa, A.; Garcia, M.T. Self-aggregation and antimicrobial activity of imidazolium and pyridinium based ionic liquids in aqueous solution. *J. Colloid Interface Sci. 2011*, 355, 164–171. [CrossRef] [PubMed]

12. Mersc, L.; Albrecht, M. Beyond catalysis: N-heterocyclic carbene complexes as components for medicinal, luminescent, and functional materials applications. *Chem. Soc. Rev. 2010*, 39, 1903–1912. [CrossRef]

13. Vasan, S.; Zhang, X.; Kapurniotu, A.; Bernhagen, J.; Teichberg, S.; Basgen, J.; Wagle, D.; Shih, D.; Terlecky, I.; Bucala, R.; et al. An agonist cleaving glucose-derived protein crosslinks in vitro and in vivo. *Nature 1996*, 382, 275–278. [CrossRef]

14. Domininanni, S.J.; Yen, T.T. Oral hypoglycemic agents. Discovery and structure-activity relationships of phenacylimidazolium enones from the corresponding enones using organic ammonium tribromide (OATB). *J. Org. Chem. 2000*, 65, 415–418. [CrossRef]

15. Cooper, M.E.; Thallas, V.; Forbes, J.; Scalbert, E.; Sastra, S.; Darby, I.; Soulsis, T. The cross-link breaker, N-phenacylthiazolium bromide prevents vascular advanced glycation end-product accumulation. *Diabetologia 2000*, 43, 660–664. [CrossRef]

16. Phillips, A.J.; Sugden, I.; Ramsdale, M. Apoptosis induced by environmental stresses and amphotericin B in Candida albicans. *Proc. Natl. Acad. Sci. USA 2003*, 100, 14327–14332. [CrossRef] [PubMed]

17. Hwang, J.; Choi, H.; Kim, A.; Yun, J.; Yu, R.; Woo, E.-R.; Lee, D. Hbiculside C-induced cell death in Candida albicans involves apoptosis mechanism. *J. Appl. Microbiol. 2011*, 117, 1400–1411. [CrossRef]

18. Jia, C.; Zhang, J.; Yu, L.; Wang, C.; Yang, Y.; Rong, X.; Xu, K.; Chu, M. Antifungal Activity of Coumarin Against Candida albicans Is Related to Apoptosis. *Front. Cell. Infect. Microbiol. 2019*, 8, 445. [CrossRef]

19. E Leadsham, J.; Kotsiadi, V.N.; Tarrant, D.J.; Gourlay, C.W. Apoptosis and the yeast actin cytoskeleton. *Cell Death Differ. 2009*, 17, 754–762. [CrossRef]

20. Pereira, C.; Silva, R.; Saraiva, L.; Johansson, B.; Sousa, M.J.; Côrte-Real, M. Mitochondria-dependent apoptosis in yeast. *Biochim. Biophys. Acta (BBA) Bioenerg. 2008*, 1783, 1286–1302. [CrossRef] [PubMed]

21. Simon, H.-U.; Haj-Yehia, A.; Levis-Schaffer, F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis 2000*, 5, 415–418. [CrossRef]

22. Boyer, J.L.; Krum, J.E.; Myers, M.C.; Fazal, A.N.; Wigal, C.T. Synthetic utility and mechanistic implications of the fies rearrangement of hydroquinone diesters in borin trifluoride complexes. *J. Org. Chem. 2000*, 65, 4712–4714. [CrossRef]

23. Bose, G.; Barua, P.M.B.; Chaudhuri, M.K.; Kalita, D.; Khan, A.T. A convenient and useful method of preparation of α-bromo enones from the corresponding enones using organic ammonium tribromide (OATB). *Chem. Lett. 2001*, 30, 290–291. [CrossRef]

24. Arjomand, O.K.; Kavosoi, M.; Adibi, H. Synthesis and investigation of inhibitory activities of imidazole derivatives against the metallo-β-lactamase IMP-1. *Bioorg Chem. 2019*, 92, 103277. [CrossRef] [PubMed]

25. Bahhous, M.; Bouraiou, A.; Chelghom, M.; Bouacida, S.; Roisnel, T.; Smati, F.; Bentchouala, C.; Goss, P.C.; Belfaitah, A. Synthesis, crystal structure and antibacterial activity of new highly functionalized ionic compounds based on the imidazole nucleus. *Bioorg Chem. Med. Lett. 2013*, 23, 1274–1278. [CrossRef]

26. Kim, K.; Bo, T.H.Q.; Shin, Y.-K.; Kim, K.-Y. Antifungal activity of magnoflorine against Candida strains. *World J. Microbiol. Biotechnol. 2018*, 34, 167. [CrossRef] [PubMed]

27. Balouiri, M.; Sadiki, M.; Ibnououda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal. 2016*, 6, 71–79. [CrossRef] [PubMed]
28. Clinical and Laboratory Standards Institute. M27-A3: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard—3rd ed.; CLSI: Wayne, PA, USA, 2008.

29. Shin, Y.; Kim, K. Macelignan inhibits bee pathogenic fungi Ascophaera apis growth through HOG1 pathway. Braz. J. Med Biol. Res. 2016, 49, 5313. [CrossRef] [PubMed]

30. Kim, J.; Shin, Y.K.; Kim, K.Y. Promotion of keratinocyte proliferation through ERK1/2 stimulation. Evid. Based Complement. Alternat. Med. 2018, 2018, 4580627. [CrossRef] [PubMed]

31. Mumma, J.O.; Chhay, J.S.; Ross, K.L.; Eaton, J.S.; Newell-Litwa, K.A.; Fridovich-Keil, J.L. Distinct roles of galactose-1P in galactose-mediated growth arrest of yeast deficient in galactose-1P uridylyltransferase (GALT) and UDP-galactose 4′-epimerase (GALE). Mol. Genet. Metab. 2008, 93, 160–171. [CrossRef] [PubMed]

32. Hossain, K.F.B.; Rahman, M.; Sikder, T.; Hosokawa, T.; Saito, T.; Kurasaki, M. Selenium modulates inorganic mercury induced cytotoxicity and intrinsic apoptosis in PC12 cells. Ecotoxicol. Environ. Saf. 2021, 207, 111262. [CrossRef] [PubMed]

33. Chen, C.Z.; Cooper, S.L. Interactions between dendrimer biocides and bacterial membranes. Biomaterials 2002, 23, 3359–3368. [CrossRef]

34. Alshaibani, M.; Zin, N.M.; Jail, J.; Sidik, N.; Ahmad, S.J.; Kamal, N.; Edrada-Ebel, R. Isolation, purification, and characterization of five active diketopiperazine derivatives from endophytic Streptomyces SUK 25 with antimicrobial and cytotoxic activities. J. Microbiol. Biotechnol. 2017, 27, 1249–1256. [CrossRef]

35. Li, X.; Qian, J.; Wang, C.; Zheng, K.; Ye, L.; Fu, Y.; Han, N.; Tian, H.; Pan, J.; Wang, J.; et al. Regulating Cytoplasmic Calcium Homeostasis Can Reduce Aluminum Toxicity in Yeast. PLOS ONE 2011, 6, e21148. [CrossRef]

36. Morici, P.; Fais, R.; Rizzato, C.; Tavanti, A.; Lupetti, A. Inhibition of Candida albicans biofilm formation by the synthetic lactoferricin derived peptide hLF1-11. PLoS ONE 2016, 11, e0167470. [CrossRef]

37. Haque, F.; Verma, N.K.; Alfatah, M.; Bijlani, S.; Bhattacharyya, M.S. Sophorolipid exhibits antifungal activity by ROS mediated endoplasmic reticulum stress and mitochondrial dysfunction pathways in Candida albicans. RSC Adv. 2019, 9, 41639–41648. [CrossRef]

38. Lü, H.; Zhu, Z.; Dong, L.; Jia, X.; Sun, X.; Yan, L.; Chai, Y.; Jiang, Y.; Cao, Y. Lack of Trehalose Accelerates H2O2-Induced Candida albicans Apoptosis through Regulating Ca2+ Signaling Pathway and Caspase Activity. PLOS ONE 2011, 6, e15808. [CrossRef]

39. Arendrup, M.C.; Patterson, T.F. Multidrug-Resistant Candida: Epidemiology, Molecular Mechanisms, and Treatment. J. Infect. Dis. 2017, 216, S445–S451. [CrossRef] [PubMed]

40. Forsberg, K.; Woodworth, K.; Walters, M.; Berkow, E.L.; Jackson, B.; Chiller, T.; Vallabhaneni, S. Candida auris: The recent emergence of a multidrug-resistant fungal pathogen. Med Mycol. 2018, 57, 1–12. [CrossRef] [PubMed]

41. Ganan, M.; Lorentzen, S.B.; Aam, B.B.; Eijsink, V.G.H.; Gaustad, P.; Sørlie, M. Antibiotic saving effect of combination therapy through synergistic interactions between well-characterized chito-oligosaccharides and commercial antifungals against medically relevant yeasts. PLoS ONE 2019, 14, e0227098. [CrossRef]

42. Vazquez, J.A.; Sobel, J.D. Miconazole Mucoadhesive Tablets: A Novel Delivery System. Clin. Infect. Dis. 2012, 54, 1480–1484. [CrossRef] [PubMed]

43. Espinel-Ingroff, A.; Shadomy, S. In vitro and in vivo evaluation of antifungal agents. Eur. J. Clin. Microbiol. Infect. Dis. 1989, 8, 352–361. [CrossRef] [PubMed]

44. Liu, L.-X.; Wang, X.-Q.; Yan, J.-M.; Li, Y.; Sun, C.-J.; Chen, W.; Zhou, B.; Zhang, H.-B.; Yang, X.-D. Synthesis and antitumor activities of novel dibenzo[b,d]furane imidazole hybrid compounds. Eur. J. Med. Chem. 2013, 66, 423–437. [CrossRef] [PubMed]