Article

Synthesis, *in vitro* Antifungal Activity and Molecular Modeling Studies of New Mannich Bases Derived from Lawsone

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Hydroxynaphthoquinones such as lawsone (2-hydroxy-1,4-naphthoquinone) have proven to be effective antifungal agents. These compounds were tested for antifungal activity against yeast standard and clinical strains by the broth microdilution method. Among the synthetic lawsone derivatives, 2-hydroxy-3-((2-hydroxyphenyl)(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione, 2-hydroxy-3-(((4-nitrophenyl)amino)(phenyl)methyl)naphthalene-1,4-dione and 2-hydroxy-3-((2-hydroxyphenyl)((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione showed high activity against *Candida albicans* ATCC 10231, with minimal inhibitory concentrations (MICs) and minimal fungicidal concentrations (MFCs) ranging from 20 to 330 and from 80 to 330 μg mL^{-1}, respectively. Moreover, they also showed a mechanism of action on exogenous ergosterol. Therapeutic concentrations (CC$_{50}$) of 2-hydroxy-3-((2-hydroxyphenyl)(pyrrolidin-1-yl)methyl) naphthalene-1,4-dione, 2-hydroxy-3-(((4-nitrophenyl)amino)(phenyl)methyl)naphthalene-1,4-dione and 2-hydroxy-3-((2-hydroxyphenyl)((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione were 52.81, 52.58 and 85.94 μg mL^{-1}, respectively, which can be considered moderate or low. In addition, docking studies showed that these compounds had similar binding energy to standard ketoconazole, which are recognized as the molecular target by van der Waals interactions. Furthermore, they are under Lipinski's rule of 5 with a druglikeness score better than ketoconazole and nystatin. These findings suggest that 2-hydroxy-3-((2-hydroxyphenyl)(pyrrolidin-1-yl)methyl) naphthalene-1,4-dione, 2-hydroxy-3-(((4-nitrophenyl)amino)(phenyl)methyl)naphthalene-1,4-dione and 2-hydroxy-3-((2-hydroxyphenyl)((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione have potential as leading compounds against human fungal infections.

Keywords: lawsone, Mannich bases, antifungal activity, docking

Introduction

Naphthoquinone derivatives possess continuous interest as potential therapeutic agents. Among them, hydroxynaphthoquinones such as lawsone (2-hydroxy-1,4-naphthoquinone), have proven to be effective due to their chemical and pharmacological properties.[1] The antifungal activity of certain naphthoquinone and lawsone derivatives has been reported from *in vitro* studies (Scheme 1).[2] As a

quinone, lawsone can be used to perform Mannich reaction[3] and the resulting Mannich bases show particular interest because of their biological activities.[4]

Candida infections have always posed a heavy burden on public health. The incidence of candidiasis has been increasing, which may be attributable to the growing numbers of immunocompromised patients.[5,6] In addition, vulvovaginal candidiasis (VVC) is a significant problem affecting 75% of all women at least once during their lifetime, and *Candida albicans* is the causative agent in more than 90% of these infections.[7] However, besides

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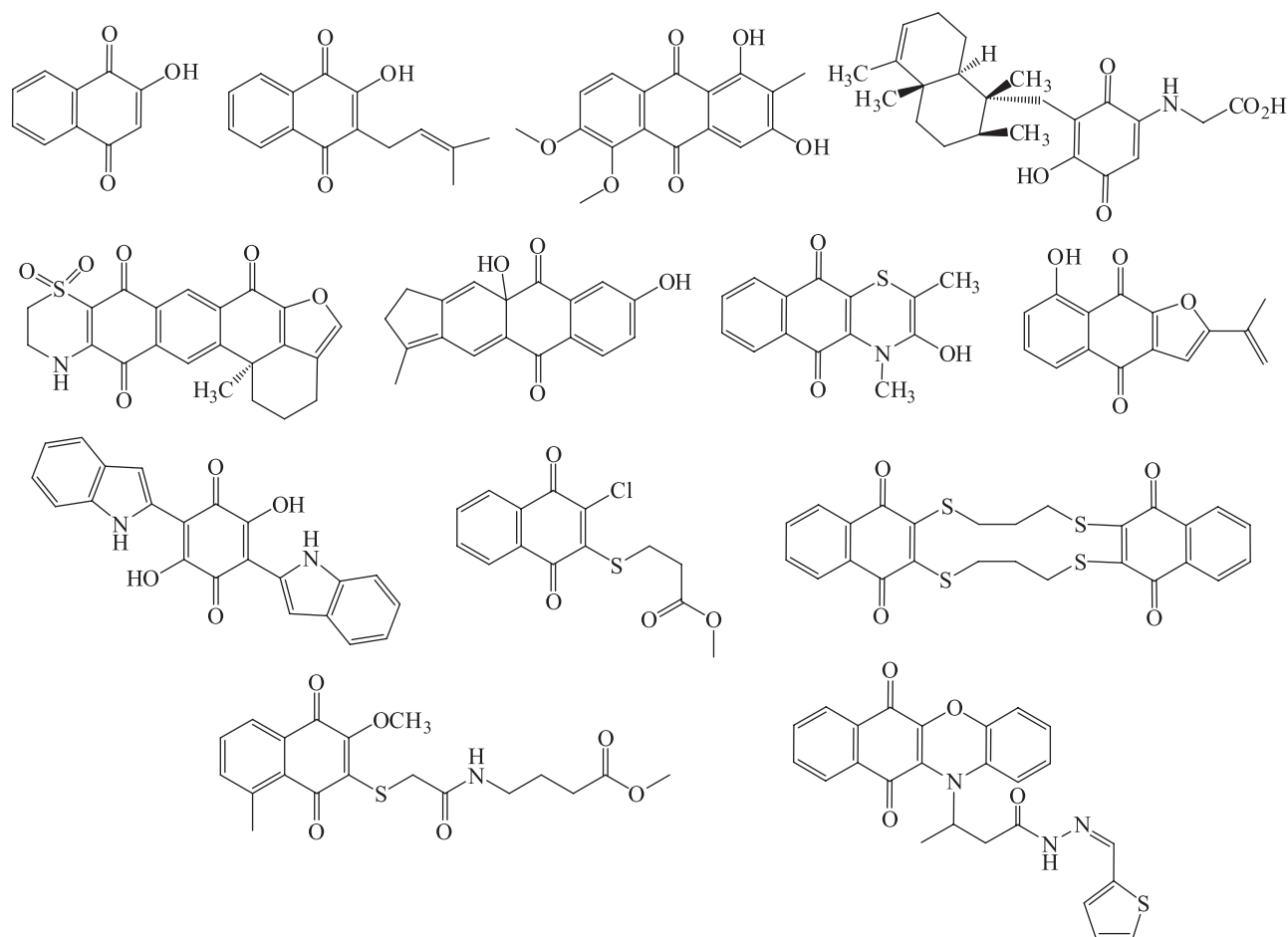
Scheme 1. Naphthoquinones with antifungal activity.

C. albicans species, an increased incidence has been observed of invasive candidiasis caused by non-albicans *Candida* species, such as *Candida glabrata*, *Candida tropicalis*, *Candida krusei* or *Candida parapsilosis*.[8] Many problems remain to be solved for most of the antifungal drugs available. Mechanisms of resistance to azole drugs have been described for *C. albicans*.[9] Thus, it has become necessary to develop new drugs capable of effectively combating these types of infection.

In this work, we evaluated the antifungal activity of synthetic derivative compounds of lawsone, which we synthesized for the first time, and tested these against several clinically important microorganisms. This way, we searched for thirteen new enolamines **4-8**, **10-14**, **16**, **17** and **23**, which contain 2-phenyl-2*H*-1,2,3-triazole or substituted and non-substituted phenyl groups, themselves synthesized using a multicomponent Mannich reaction from lawsone.[3] These compounds were also tested against baby hamster kidney (BHK) cells to study toxicity effects. Moreover, molecular modeling tools were used to highlight properties used in drug design,[10] for example druglikeness. In addition, docking studies[11-13] described the main

molecular interactions between these compounds and the molecular target. These data suggest a lead optimization process to search for a second generation of antifungal lawsone-based drugs.

Experimental

General information

All solvents and reagents were commercially purchased and were used without any purification.

Melting points were determined using Fisatom 430D equipment. Infrared (IR) spectra were recorded on a FTLA 2000-102-ABB BOMEM Fourier transform (FT) IR spectrophotometer with KBr technique and on a Perkin Elmer Spectrum 400 FTIR spectrometer model using attenuated total reflectance (ATR) technique. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Varian VNMRS spectrometer model 400 (400 MHz for ^{1}H NMR) using CDCl$_3$ or dimethyl sulfoxide (DMSO)-*d*$_6$ as solvents and tetramethylsilane (TMS) as the internal standard.

Mass spectra (MS) were recorded on ultra-high resolution and accuracy mass spectrometer (model 9.4 T Solarix, Bruker Daltonics), operated in both ionization modes: positive and negative electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI(+) and ESI(−)-FT-ICR MS, respectively). FT-ICR MS spectra were acquired with resolving power of m / $\Delta m_{50\%}$ ca. 500000, in which $\Delta m_{50\%}$ is the full peak width at half-maximum peak height of m/z 400 and a mass accuracy of less than 1 ppm. It provides an unambiguous molecular formula assignment for singly charged molecular ions such as $[M − H]^+$ or $[M + H]^−$ and double bound equivalents (DBE) values.

Synthesis of lawsone derivatives 4-8, 10-14, 17 and 23 - general procedure

A mixture of the aldehyde (1.2 eq) and amine (1.1 eq) in ethanol was stirred during a few minutes. After this time, lawsone (1.0 eq) was added to the solution and the reaction was stirred for 24 h at room temperature in the dark. The solid formed was filtered under vacuum and washed with cold ethanol. Recrystallization with ethanol or ethyl acetate:hexane 1:3 was performed for the impure compounds. Experimental protocols were initially tested as described in previous reports.[3,14,15]

2-Hydroxy-3-((2-hydroxyphenyl)(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione (4)

Compound **4** was obtained as a red crystalline solid (77%); m.p. decomposition at 146 °C; IR (ATR) ν_{max} / cm^{-1} 3595, 3088, 2977, 2884, 1685, 1579, 1505, 1271; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.95 (br s, 4H, CH$_2$), 3.18 (br s, 4H, NCH$_2$), 5.82 (s, 1H, CH), 6.78 (td, 1H, *J* 8.0, 1.0 Hz, Ar–H), 6.82 (dd, 1H, *J* 8.0, 1.0 Hz, Ar–H), 7.10 (td, 1H, *J* 8.0, 1.0 Hz, Ar–H), 7.60 (td, 1H, *J* 8.0, 1.0 Hz, Ar–H), 7.67 (td, 1H, *J* 7.5, 1.0 Hz, Naph–H*), 7.72 (td, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.83 (dd, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.92 (dd, 1H, *J* 7.5, 1.0 Hz, Naph–H), 10.36 (sl, 2H, O**H**); ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.0, 22.5, 43.8, 52.0, 59.8, 112.5, 115.2, 118.2, 123.4, 123.9, 124.2, 27.9, 128.1, 129.9, 130.2, 132.7, 133.3, 153.8, 169.6, 177.7, 183.0; ESI(+)-FT-ICR-MS calcd. for C$_{21}$H$_{20}$NO$_4^+$ [M + H]$^+$: 350.13868; found: 350.13476 (DBE = 13); [2M + Na]$^+$: 721.2520; found: 721.2528. *Naph–H: naphthoquinone hydrogens.

2-Hydroxy-3-(phenyl(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione (5)

Compound **5** was obtained as a red crystalline solid (32%); m.p. 177-179 °C with decomposition; IR (ATR)

ν_{max} / cm^{-1} 3343, 3064, 2983, 2874, 1680, 1587, 1531, 1272; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.93 (m, 4H, CH$_2$), 3.15 (br s, 4H, NCH$_2$), 5.48 (s, 1H, CH), 7.25 (m, 1H, Ar–H), 7.32 (m, 2H, Ar–H), 7.54 (td, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.67 (m, 3H, 2Ar–H and 1H, Naph–H), 7.78 (dd, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.89 (dd, 1H, *J* 7.5, 1.0 Hz, Naph–H), 10.48 (br s, 1H, O**H**); ^{13}C NMR (101 MHz, DMSO-d_6) δ 23.7, 53.9, 67.6, 113.6, 125.5, 125.7, 128.3, 128.4, 128.8, 131.2, 131.9, 134.2, 135.1, 139.1, 170.6, 178.2, 184.9; ESI(+)-FT-ICR-MS calcd. for C$_{21}$H$_{20}$NO$_3^+$ [M + H]$^+$: 334.14377; found: 334.13985 (DBE = 13); [2M + H]$^+$: 667.2803; found: 667.2790; [2M + Na]$^+$: 689.2622; found: 689.2609.

2-Hydroxy-3-(naphthalen-1-yl(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione (6)

Compound **6** was obtained as an orange solid (38%); m.p. decomposition at 185 °C; IR (ATR) ν_{max} / cm^{-1} 3337, 3042, 2868, 1678, 1583, 1532, 1275; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.92 (m, 4H, CH$_2$), 3.29 (m, 4H, NCH$_2$), 6.37 (s, 1H, CH), 7.51 (m, 4H, 3Ar-H and 1H, Naph–H), 7.66 (td, 1H, *J* 7.4, 1.0 Hz, Naph–H), 7.76 (dd, 1H, *J* 7.4, 1.0 Hz, Naph–H), 7.84 (d, 1H, *J* 8.3 Hz, Ar–H), 7.89 (m, 2H, 1Ar–H and 1H, Naph–H), 8.25 (d, 1H, *J* 7.4 Hz, Ar–H), 8.59 (d, 1H, *J* 8.3 Hz, Ar–H), 10.28 (br s, 1H, O**H**); ^{13}C NMR (101 MHz, DMSO-d_6) δ 23.8, 54.1, 63.3, 112.9, 124.6, 125.5, 125.6, 125.7, 126.1, 126.7, 128.4, 128.9, 131.2, 131.3, 131.9, 133.5, 134.2, 134.5, 135.3, 171.1, 178.4, 184.9; ESI(+)-FT-ICR-MS calcd. for C$_{25}$H$_{23}$NO$_3^+$ [M + H]$^+$: 384.15550; found: 384.15982 (DBE = 16).

2-Hydroxy-3-((4-nitrophenyl)(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione (7)

Compound **7** was obtained as an orange solid (46%); m.p. 186-188 °C; IR (ATR) ν_{max} / cm^{-1} 3059, 2863, 1686, 1586, 1532, 1343, 1275; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.94 (m, 4H, CH$_2$), 3.20 (br s, 4H, NCH$_2$), 5.71 (s, 1H, CH), 7.55 (td, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.68 (td, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.78 (d, 1H, *J* 7.5, 1.0 Hz, Naph–H), 7.90 (d, 3H, *J* 8.7 Hz, 2Ar–H and 1H, Naph–H), 8.20 (d, 2H, *J* 8.7 Hz, Ar–H), 10.46 (br s, 1H, O**H**); ^{13}C NMR (101 MHz, DMSO-d_6) δ 23.8, 54.2, 66.6, 112.4, 124.0, 125.6, 125.9, 129.1, 131.4, 131.9, 134.2, 135.1, 146.0, 146.6, 147.2, 170.8, 178.1, 184.6; ESI(−)-FT-ICR-MS calcd. for C$_{21}$H$_{19}$N$_2$O$_5^-$ [M − H]$^-$: 377.11430; found: 377.11413 (DBE = 14).

2-Hydroxy-3-((4-methoxyphenyl)(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione (8)

Compound **8** was obtained as an orange solid (44%); m.p. 181-183 °C; IR (ATR) ν_{max} / cm^{-1} 3068, 2954, 2835,

1681, 1586, 1538, 1252, 1233; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.92 (br s, 4H, CH$_2$), 3.10 (br s, 4H, NCH$_2$), 3.70 (s, 3H, OCH$_3$), 5.40 (s, 1H, CH), 6.86 (d, 2H, *J* 8.7 Hz, Ar–H), 7.56 (m, 3H, 2Ar–H and 1H, Naph–H), 7.67 (t, 1H, *J* 7.4 Hz, Naph–H), 7.78 (d, 1H, *J* 7.4 Hz, Naph–H), 7.89 (d, 1H, *J* 7.4 Hz, Naph–H), 10.52 (br s, 1H, O$\underline{H}$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 23.6, 53.6, 55.5, 67.2, 113.9, 114.1, 125.5, 125.7, 129.9, 131.2, 131.9, 134.1, 135.1, 159.4, 170.5, 178.2, 184.9; ESI(+)-FT-ICR-MS calcd. for C$_{22}$H$_{22}$NO$_4^+$ [M + H]$^+$: 364.15432; found: 364.15041 (DBE = 13); [2M + Na]$^+$: 749.28347; found: 749.28334 (DBE = 25).

2-Hydroxy-3-((4-nitrophenyl)((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione (10)

Compound **10** was obtained as an yellow solid (82%); m.p. 134-136 °C; IR (KBr) ν$_{max}$ / cm^{-1} 3446, 3328, 3199, 1675, 1593, 1573, 1507, 1344, 1299; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.37 (s, 1H, CH), 6.93 (d, 2H, *J* 8.7 Hz, Ar–H), 7.81 (d, 2H, *J* 8.7 Hz, Ar–H), 7.93 (td, 1H, *J* 7.5, 1.5 Hz, Naph–H), 7.98 (td, 1H, *J* 7.5, 1.5 Hz, Naph–H), 8.10 (m, 4H, 2Ar–H and 2H, Naph–H), 8.31 (d, 2H, *J* 8.7 Hz, Ar–H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 49.5, 111.2, 119.2, 122.2, 123.1, 124.8, 124.9, 125.2, 126.6, 129.0, 129.4, 130.5, 132.3, 133.7, 135.5, 145.3, 147.0, 152.3, 154.5, 155.9, 179.9, 182.1, 191.2; ESI(–)-FT-ICR-MS calcd. for C$_{23}$H$_{14}$N$_3$O$_7^-$ [M – H]$^-$: 444.08372; found: 444.08358 (DBE = 18).

2-Hydroxy-3-(((4-nitrophenyl)amino)(phenyl)methyl)naphthalene-1,4-dione (11)

Compound **11** was obtained as an pumpkin solid (93%); m.p. 187-189 °C with decomposition; IR (ATR) ν$_{max}$ / cm^{-1} 3351, 3339, 1656, 1641, 1598, 1585, 1327, 1264; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.09 (s, 1H, CH), 6.55 (d, 1H, *J* 9.3 Hz, N$\underline{H}$), 6.74 (d, 2H, *J* 8.5 Hz, Ar–H), 7.19 (m, 1H, Ar–H), 7.28 (m, 2H, Ar–H), 7.41 (m, 2H, Ar–H), 7.75 (td, 1H, *J* 7.5, 1.5 Hz, Naph–H), 7.80 (td, 1H, *J* 7.5, 1.5 Hz, Naph–H), 7.93 (m, 4H, 2Ar–H and 2H, Naph–H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 51.4, 112.8, 121.5, 126.3, 126.3, 126.5, 126.8, 127.1, 127.4, 128.7, 129.6, 129.9, 130.5, 132.1, 133.9, 135.2, 136.7, 140.1, 154.0, 156.1, 156.9, 181.5, 183.9, 193.7; ESI(–)-FT-ICR-MS calcd. for C$_{23}$H$_{15}$N$_2$O$_5^-$ [M – H]$^-$: 399.09865; found: 399.09808 (DBE = 20); [M – C$_6$H$_5$N$_2$O$_2$ + C$_{10}$H$_5$O$_3$]$^-$: 435.08741; found: 435.08684 (DBE = 17).

2-Hydroxy-3-((2-hydroxyphenyl)((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione (12)

Compound **12** was obtained as an orange solid (69%); m.p. 237-239 °C with decomposition; IR (ATR) ν$_{max}$ / cm^{-1}

3349, 3062, 1687, 1640, 1592, 1537, 1320, 1240; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.29 (d, 1H, *J* 9.4 Hz, CH), 7.07 (br s, 2H, Ar–H), 7.32 (m, 1H, Ar–H), 7.50 (m, 2H, 1Ar–H and 1H, Naph–H), 7.56 (d, 1H, *J* 7.5 Hz, Naph–H), 7.73 (d, 1H, *J* 9.4 Hz, N$\underline{H}$), 7.94 (m, 2H, 1Ar–H and 1H, Naph–H), 8.06 (m, 1H, Ar–H), 8.09 (d, 2H, *J* 9.0 Hz, 1Ar–H and 1H, Naph–H), 8.16 (m, 1H, Ar–H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.9, 112.0, 117.7, 118.9, 122.4, 126.4, 126.5, 126.7, 129.6, 130.2, 131.0, 131.8, 134.6, 135.4, 136.8, 149.1, 151.4, 152.9, 178.5, 183.3; ESI(–)-FT-ICR-MS calcd. for C$_{23}$H$_{13}$N$_2$O$_5^-$ [M – 2H – OH]$^-$: 397.08300; found: 397.08265 (DBE = 17); [M – H – OH + Cl]$^-$: 433.05967; found: 433.05932 (DBE = 17).

2-Hydroxy-3-(naphthalen-1-yl((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione (13)

Compound **13** was obtained as an orange solid (90%); m.p. 165-167 °C with decomposition; IR (ATR) ν$_{max}$ / cm^{-1} 3641, 3407, 3396, 3362, 1678, 1590, 1503, 1308, 1276; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.81 (d, 1H, *J* 9.3 Hz, CH); 6.67 (dd, 2H, *J* 7.2, 2.0 Hz, Ar–H), 6.94 (d, 1H, *J* 9.3 Hz, N$\underline{H}$), 7.43 (t, 1H, *J* 7.7 Hz, Ar–H), 7.52 (td, 1H, *J* 7.0, 1.0 Hz, Naph–H), 7.56 (td, 1H, *J* 7.0, 1.0 Hz, Naph–H), 7.68 (d, 1H, *J* 7.4 Hz, Ar–H), 7.71 (td, 1H, *J* 7.7, 1.0 Hz, Ar–H), 7.79 (td, 1H, *J* 7.7, 1.0 Hz, Ar–H), 7.83 (d, 1H, *J* 8.3 Hz, Ar–H), 7.89 (dd, 1H, *J* 7.7, 1.0 Hz, Ar–H), 8.06 (dd, 2H, *J* 7.2, 2.0 Hz, Ar–H), 8.09 (dd, 1H, *J* 7.7, 1.0 Hz, Naph–H), 8.12 (dd, 1H, *J* 7.7, 1.0 Hz, Naph–H), 8.20 (d, 1H, *J* 8.3 Hz, Ar–H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 49.5, 111.8, 113.3, 120.6, 123.1, 125.2, 125.3, 125.9, 126.5, 126.5, 126.9, 127.3, 129.1, 129.2, 131.2, 132.5, 133.2, 133.5, 134.0, 135.6, 138.7, 151.9, 153.6, 181.0, 184.2; ESI(–)-FT-ICR-MS calcd. for C$_{27}$H$_{17}$N$_2$O$_5^-$ [M – H]$^-$: 449.11430; found: 449.11367 (DBE = 20); [M – C$_6$H$_5$N$_2$O$_2$ + OH]$^-$: 329.08193; found: 329.08162 (DBE = 18).

2-Hydroxy-3-((4-methoxyphenyl)((4-nitrophenyl)amino)methyl)naphthalene-1,4-dione (14)

Compound **14** was obtained as an yellow solid (68%); m.p. 222-224 °C with decomposition; IR (ATR) ν$_{max}$ / cm^{-1} 3394, 3245, 1666, 1636, 1593, 1362, 1260, 1237; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.65 (s, 3H, OCH$_3$), 5.88 (s, 1H, CH), 6.71 (m, 2H, Ar–H), 7.09 (m, 2H, Ar–H), 7.73 (td, 2H, *J* 7.4, 1.6 Hz, 1Ar–H and 1H, Naph–H), 7.77 (td, 2H, *J* 7.5, 1.6 Hz, 1Ar–H and 1H, Naph–H), 7.87 (m, 2H, 1Ar–H and 1H, Naph–H), 7.93 (m, 2H, 1Ar–H and 1H, Naph–H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 37.4, 55.3, 113.4, 123.9, 125.9, 126.4, 129.6, 130.2, 132.6, 133.0, 133.5, 135.1, 156.6, 157.6, 181.7, 184.0; ESI(–)-FT-ICR-MS calcd. for C$_{28}$H$_{17}$O$_7^-$ [M – C$_6$H$_5$N$_2$O$_2$ + C$_{10}$H$_5$O$_3$]$^-$: 465.09798; found: 465.09757 (DBE = 20).

2-((2-Phenyl-2*H*-1,2,3-triazol-4-yl)(pyrrolidin-1-yl)methyl)-3-((1,3,4-trihydroxynaphthalen-2-yl)oxy)naphthalene-1,4-dione (17)

Compound **17** was obtained as an orange solid after recrystallization from ethyl acetate:hexane 1:3 (36%); m.p. above 200 °C with decomposition; IR (KBr) ν_{max} / cm^{-1} 3494, 3067, 2976, 2777, 1675, 1597, 1571, 1359, 1278; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.73 (t, 4H, *J* 6.3 Hz, CH$_2$), 3.19 (br s, 4H, NCH$_2$), 6.90 (s, 1H, CH), 7.16 (t, 1H, *J* 7.2 Hz, Bt–H*), 7.29 (m, 2H, Bt–H), 7.47 (t, 2H, *J* 7.6 Hz, 1Ar–H and 1H, Naph–H), 7.55 (s, 1H, Bt–H), 7.61 (t, 2H, *J* 7.7 Hz, 1Ar–H and 1H, Naph–H), 7.81 (d, 2H, *J* 7.6 Hz, 1Ar–H and 1H, Naph–H), 7.85 (d, 2H, *J* 8.1 Hz, 1Ar–H and 1H, Naph–H), 8.12 (d, 2H, *J* 7.7 Hz, Bt–H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 24.0, 26.8, 45.8, 118.2, 122.4, 125.5, 126.6, 126.8, 129.0, 130.8, 131.9, 133.3, 134.1, 135.1, 138.8, 139.8, 149.5, 162.8, 183.5, 185.2; ESI(+)-FT-ICR-MS calcd. for C$_{33}$H$_{26}$N$_4$O$_6$ [M]$^+$: 574.1852; found: 574.1847 (DBE = 23); [M + H]$^+$: 575.1886; found 575.1925. *Bt–H: benzotriazole hydrogens.

2-(Morpholino(2-phenyl-2*H*-1,2,3-triazol-4-yl)methyl)-3-((1,3,4-trihydroxynaphthalen-2-yl)oxy)naphthalene-1,4-dione (23)

Compound **23** was obtained as an orange solid after recrystallization from ethyl acetate:hexane 1:3 (56%); m.p. decomposition at 160 °C; IR (ATR) ν_{max} / cm^{-1} 3500, 3066, 2866, 2486, 1675, 1598, 1571, 1361, 1279; ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.26 (m, 4H, NCH$_2$), 3.68 (m, 4H, OCH$_2$), 6.84 (s, 1H, CH), 7.15 (t, 1H, *J* 7.7 Hz, Bt–H), 7.26 (m, 2H, Bt–H), 7.37 (td, 2H, *J* 7.6, 1.0 Hz, 1Ar–H and 1H, Naph–H), 7.48 (td, 2H, *J* 7.5, 1.0 Hz, 1Ar–H and 1H, Naph–H), 7.60 (s, 1H, Bt–H), 7.73 (d, 2H, *J* 7.6 Hz, 1Ar–H and 1H, Naph–H), 7.84 (d, 2H, *J* 7.7 Hz, 1Ar–H and 1H, Naph–H), 7.98 (d, 2H, *J* 7.5 Hz, Bt–H); ^{13}C NMR (101MHz, CDCl$_3$) δ 26.8, 44.1, 63.7, 118.3, 122.1, 125.7, 126.6, 126.7, 129.0, 130.7, 132.0, 133.1, 134.0, 135.1, 139.7, 149.3, 183.7, 184.8; ESI(+)-MS-FT-ICR calcd. for C$_{33}$H$_{26}$N$_4$O$_7$ [M]$^+$: 590.1802; found 590.1796 (DBE = 23); [M + H]$^+$: 591.1835; found 591.1874.

Synthesis of the lawsone derivative 2-hydroxy-3-((2-phenyl-2*H*-1,2,3-triazol-4-yl)(pyrrolidin-1-yl)methyl)naphthalene-1,4-dione (16)

A mixture of 1.2 eq of 2-phenyl-2*H*-1,2,3-triazole-4-carbaldehyde **15** and 1.1 eq of pyrrolidine **3** in ethanol (10 mL) was stirred overnight with at 50 °C. After this time, 1.0 eq of lawsone **1** was added and the mixture was allowed to react for 24 h in the dark. After the completion of the reaction, the solid was isolated by filtration, washed with cold water and cold ethanol and dried under vacuum

to give the Mannich adduct **16** as an orange solid (66%), m.p. 178-180 °C with decomposition. IR (ATR) ν_{max} / cm^{-1} 3353, 3062, 2955, 1684, 1583, 1530, 1326, 1285; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.94 (br s, 4H, CH$_2$), 3.29 (m, 4H, NCH$_2$), 5.90 (s, 1H, CH), 7.40 (t, 1H, *J* 7.4 Hz, Bt–H), 7.56 (m, 3H, 2Bt–H and 2H, Naph–H), 7.71 (t, 1H, *J* 7.1 Hz, Naph–H), 7.83 (d, 1H, *J* 7.1 Hz, Naph–H), 7.96 (m, 3H, 2Bt–H and 1H, Naph–H), 8.11 (s, 1H, Bt–H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 23.6, 53.8, 58.5, 110.7, 118.8, 125.7, 125.9, 128.2, 130.2, 131.4, 132.1, 134.2, 135.2, 136.2, 139.4, 147.5, 171.5, 178.4, 184.7; ESI(–)-FT-ICR-MS calcd. for C$_{23}$H$_{19}$N$_4$O$_3^-$ [M − H]$^-$: 399.1463; found: 399.1462 (DBE = 16); [M − C$_4$H$_8$N + OH]$^-$: 346.0833; found: 346.0833 (DBE = 15).

Antimicrobial assay

Microorganism targets

The antimicrobial activity was evaluated using the following microorganisms from the American Type Culture Collection (ATCC): *C. albicans* ATCC 10231, *C. glabrata* ATCC 2001 and *C. krusei* ATCC 34135. In addition, five *C. albicans* clinical strains given by the Biocentro Laboratory Ltd., in Divinópolis-MG, Brazil, were employed: CA1, CA2, CA3, CA4 and CA5. All the fungal strains were maintained on Sabouraud dextrose agar (SDA) (Oxoid).

The macromorphology and micromorphology identifications of clinical origin strains were previously confirmed: (*i*) growth in broth and SDA; (*ii*) evidence of germ tube; (*iii*) chlamydospores formation test; and (*iv*) biochemical tests.[16]

Culture media and inocula

Sabouraud dextrose broth (SDB) (HiMedia) was prepared according to the Clinical and Laboratory Standards Institute (CLSI) document M27-A2[17] for minimal inhibitory concentration (MIC) fungal assays. The fungal cultures of the *Candida* species were freshly grown at 35 °C and the inoculum suspensions were prepared by the spectrophotometric method according to the CLSI document M27-A3[18] with a final concentration of $1.5 \pm 1.0 \times 10^3$ cells mL^{-1} for susceptibility tests. Yeast cells in the exponential phase were collected aseptically with a sterile loop and resuspended in a tube containing 10 mL of sterile saline; the inoculum of the *Candida* strains used in the experiments was standardized to match 0.5 on the McFarland scale (106 colony forming units (CFU) mL^{-1}) and confirmed by spectrophotometry (optical density (OD) 0.08-0.1 at 625 nm). The suspension was produced by making a 1:50 dilution followed by a 1:20 dilution in SDB. The suspensions were prepared according to the CLSI

document M27-A3 to obtain a final inoculum size suitable for each strain.[18]

Susceptibility test

Broth microdilution testing was performed according the guidelines presented in the CLSI documents M27-A3 for fungi.[18] The susceptibility to antimicrobial agents was determined by the microbroth dilution method and was performed in sterile flat-bottomed 96-well microplates (Difco). The compounds were dissolved in DMSO after the addition of SDB. Subsequently, serial dilutions were prepared using the corresponding media as the diluent, maintaining a constant volume of 0.1 mL in each well. The compounds were tested at ten concentrations ranging from 1.5-1500 μg mL^{-1}.

For growth and sterility control, the media was used without the addition of the test compounds. As a control for the toxicity of the solvent, a culture was inoculated with DMSO. The antifungal ketoconazole (Sigma-Aldrich®; initial concentration 100 μg mL^{-1}) was used as a positive antifungal control.

After the plates were prepared, the inoculate of each strain was added and the plates were incubated at 37 ºC for 48 h. Each test was performed in triplicate. The endpoints were determined visually by comparison of the samples with the drug-free control well. The MIC was detected following the addition of 0.05 mL 2.0% triphenyltetrazolium chloride solution (TTC) (Sigma-Aldrich®). The growth of yeast was visualized by changes to a red color. The MIC was defined as the lowest compound concentration at which the well was optically clear, and this value was expressed in μg mL^{-1}.

The minimal fungicidal concentration (MFC) of compounds that were showing MIC was determined by plating up 10 μL of the MIC by technique spread plate in SDA (Himedia).[19] The plates were incubated at 35 ºC for 48 h. The MFC was considered to be the lowest concentration of the tested agent capable of preventing the growth of any yeast colony in SDA. The experiments were performed in triplicate.

Mechanism of action on exogenous ergosterol

The MICs were determined using *C. albicans* ATCC 10231, *C. glabrata* ATCC 2001 and *C. krusei* ATCC 34135 by the standard broth microdilution procedure described above. Duplicate plates were prepared: one contained the test compounds and exogenous ergosterol (Sigma; 200 μg mL^{-1}) and the other contained the compounds alone. The MICs were determined after 48 h.[20] Nystatin (Sigma; initial concentration 100 μg mL^{-1}) was used as the positive control.

Analysis of cell viability

The BHK cells were cultured in 96-well plates until 90% of confluence was reached. The plates were pre-incubated in a humidified 5% CO$_2$/95% air atmosphere at 37 ºC for 24 h to allow the cells to adapt prior to the addition of the test compounds. All the compounds were dissolved in DMSO prior to dilution. The cytotoxicity was determined over a concentration ranging from 9 to 1080 μg mL^{-1}. All the cell cultures were incubated in a 5% CO$_2$/95% air atmosphere at 37 ºC for 48 h. The cell viability was estimated by measuring the rate of the mitochondrial reduction of a yellow tetrazolium salt, 2-(3,5-diphenyltetrazol-2-ium-2-yl)-4,5-dimethyl-1,3-thiazole bromide (MTT) (Sigma-Aldrich), to insoluble purple formazan crystals.[21] After incubation of the cultures with the test compounds, the MTT solution (0.028 mL at 2 mg mL^{-1}) was added to each well and the plates were incubated for 3 h at 37 ºC. At the end of this incubation period, the supernatant was removed and 0.1 mL of DMSO were added. The plate was then placed under medium agitation for 7 min, the absorbance was read at 540 nm in an enzyme linked immunosorbent assay (ELISA) spectrophotometer (Powder Wave XS2, Biotec) to determine the concentration that killed 50% of cells (CC$_{50}$).[22] The cytotoxicity was calculated after comparing with the control (treated with 0.1% DMSO). The CC$_{50}$ values were obtained by regression analysis of the percentages for the different concentrations of test material. GraphPad Prism 5.0 software[23] was used for non-linear regression calculations.[24] The CC$_{50}$ values represent the average of three independent experiments.

Molecular modeling studies

Initially, the most active compounds determined in the biological assay step (compounds **4**, **11** and **12**) and ketoconazole were built and adjusted for the protonation state at pH 7.4 using MarvinSketch software.[25] These structures were refined by parametric method 7 (PM7)[26] implemented in MOPAC 2012.[27] Next, the ligands and molecular target (under protein data bank code 3OZW[28]) were prepared using the Autodock tools standard protocol and submitted to Autodock Vina.[29] A grid box was generated with 24, 20 and 20 Å for x, y, z respectively, centered on the ligand with –17.68, 36.32, –32.46 Å. The flavin-adenine dinucleotide (FAD) was retained at the binding site. The crystallographic ketoconazole was redocked against 3OZW to validate the methodology, following the docking of ligands. All docking simulations were performed with the 'exhaustiveness' term set to 20 to achieve more accurate results. The results were depicted by Discovery Studio 4.1.[30] In addition, the physicochemical properties (molecular

weight, partition coefficient (log P), hydrogen acceptors and donors groups and druglikeness) were calculated using DataWarrior software.[31]
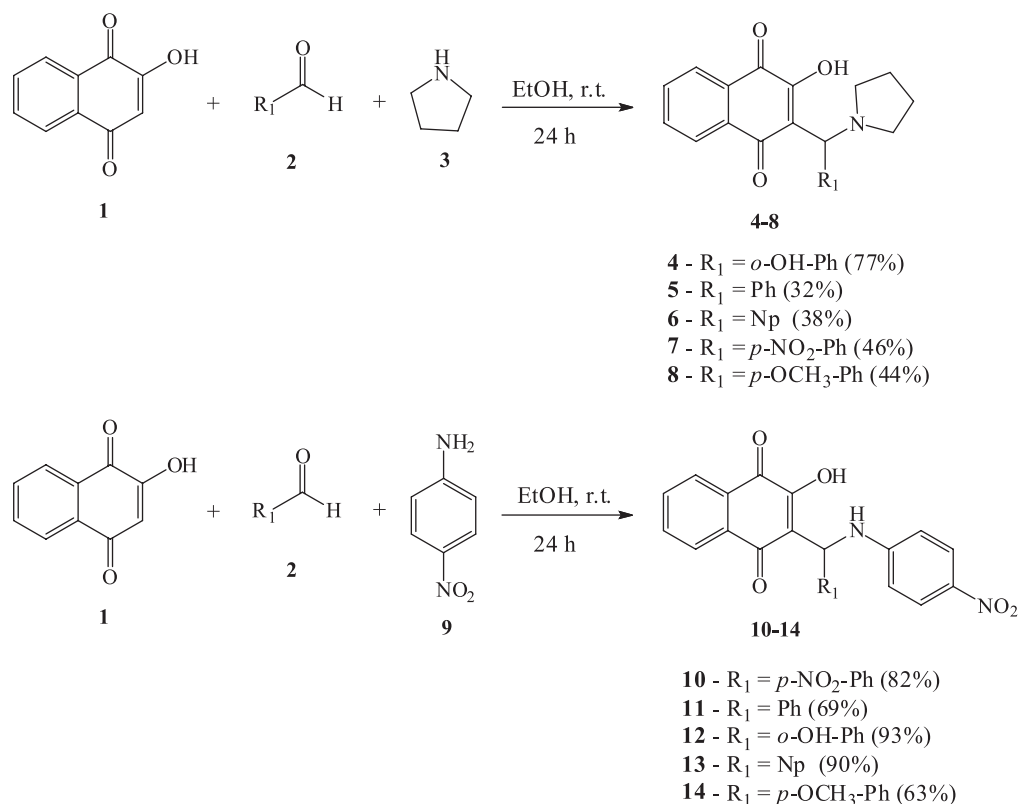
Results and Discussion

Synthesis of lawsone derivatives

In order to develop the Mannich reaction, and especially to broaden its scope, we conducted a series of experiments to optimize the conditions for the reaction between lawsone **1**, the salicylaldehyde **2** and pyrrolidine **3** to form the corresponding Mannich adduct **4** (Scheme 2). The best results were obtained using a slightly modified procedure, which used 10 mol% of excess amine in order to increase the nucleophilic character of lawsone by deprotonating the hydroxyl and an excess of 20 mol% aldehyde to shift the equilibrium towards the incipient imine or iminium intermediates.[3,14,15] Several solvents were tested and the best results were obtained with ethanol to prepare compound **4** in good yields as a pure red solid after filtration. This protocol allowed to reach the Mannich adducts **5-8** and **10-14** in moderate to high yields using pyrrolidine **3** and *p*-nitroaniline **9**, respectively and various aromatic aldehydes. The higher
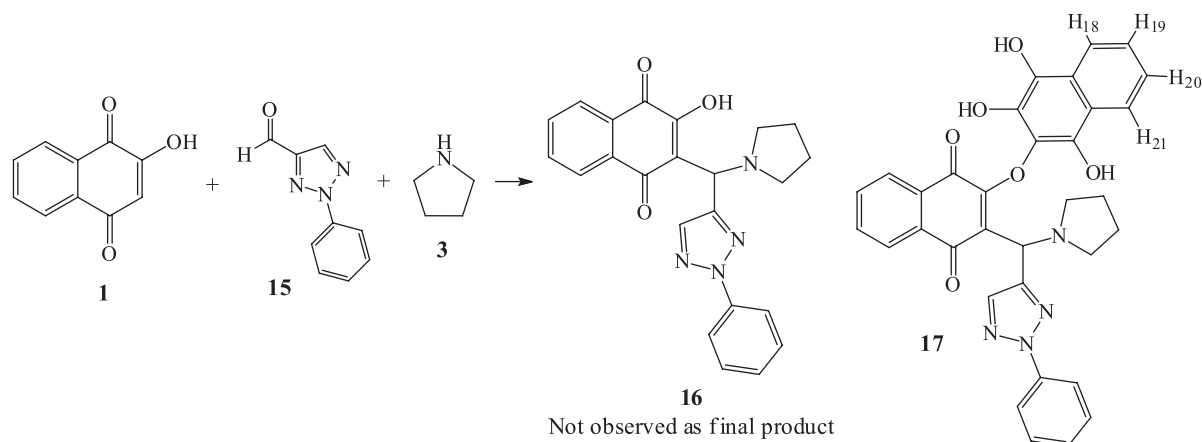
yields were obtained in the reaction with *p*-nitroaniline, probably due to increased stability of the imine formed as intermediate. The increased stability shifts the chemical equilibrium towards its formation thereby increasing the concentration of electrophile in the reaction medium.

Aiming to exploit the profile of 1,2,3-triazoles as antifungals[32] the aldehyde 2-phenyl-2*H*-1,2,3-triazole **15** was prepared in three steps from *D*-(+)-glucose, as previously reported.[33]

To couple the triazole group and naphthoquinone, we then performed a multicomponent Mannich reaction between lawsone **1**, aldehyde **15** and pyrrolidine **3** in ethanol at room temperature. However, rather than obtaining the corresponding Mannich adduct **16**, compound **17** was obtained in this reaction (Scheme 3) as a pure orange solid in 36% yield after recrystallization from ethyl acetate:hexane 1:3. IR of the compound **17** showed a band at 1278 cm^{-1} likely due to the C–O–C ether bond. Additionally, the integration of signals in the ^{1}H NMR spectrum revealed additionally four hydrogen atoms (7.82 ppm, d, 2H, *J* 7.3 Hz, H$_{18}$/H$_{21}$ and 7.48 ppm, t, 2H, *J* 7.3 Hz, H$_{19}$/H$_{20}$) in the aromatic region. The compound was also characterized by ESI(+)-FT-ICR MS, which provided an unambiguous molecular formula of C$_{33}$H$_{26}$N$_4$O$_6$, giving an ion [C$_{33}$H$_{26}$N$_4$O$_6$ + H]$^+$ with *m/z* of 575.1925.

4 - R$_1$ = *o*-OH-Ph (77%)
5 - R$_1$ = Ph (32%)
6 - R$_1$ = Np (38%)
7 - R$_1$ = *p*-NO$_2$-Ph (46%)
8 - R$_1$ = *p*-OCH$_3$-Ph (44%)

10 - R$_1$ = *p*-NO$_2$-Ph (82%)
11 - R$_1$ = Ph (69%)
12 - R$_1$ = *o*-OH-Ph (93%)
13 - R$_1$ = Np (90%)
14 - R$_1$ = *p*-OCH$_3$-Ph (63%)

Scheme 2. Multicomponent Mannich reaction in lawsone.

Scheme 3. Synthesis of compound **17**.

Compound **17** was likely formed via Michael addition between the alkoxide derived **20**, obtained from the deprotonation of the hydroxyl of the Mannich adduct **16** by pyrrolidine and the lawsone **1** remaining in the reaction medium (Scheme 4). Compound **16** was formed in low concentration, probably due to low concentration of the iminium ion **18** in the reaction medium. The formation of **17** from **21** is thermodynamically favored due to the formation of the aromatic ring indicated with arrow in enolization step of the intermediate **21**. This reaction was repeated using *p*-nitroaniline and morpholine but also in this case the compound **23** was obtained in 56% yield as an orange solid after recrystallization from ethyl acetate:hexane 1:3, as a result from the Michael addition between the alkoxide of the Mannich adduct **22** and lawsone (Scheme 4).

For compound **23**, the presence of four aromatic hydrogen atoms in the ^{1}H NMR spectrum was also observed by integrating the signals. Additionally, an ion $[C_{33}H_{26}N_4O_7 + H]^+$ with an *m/z* of 591.18733 was detected in the ESI(+)-FT-ICR mass spectrum.

Thus, the Mannich adduct **16** containing the triazole group was obtained by a slight experimental modification (Scheme 5). For this, the 2-phenyl-2*H*-1,2,3-triazole-4-carbaldehyde **15** and pyrrolidine **3** in ethanolic solution were allowed to react overnight with a constant temperature of 50 °C. Then, lawsone **1** was added to the reaction and after 24 h the Mannich adduct **16** was obtained in 66% yield.

Antimicrobial assay

The synthetic compounds were tested against three *Candida* strains and five clinical isolates. The best antifungal activities were observed against *C. albicans* ATCC 10231 (Table 1). The compounds **4**, **11** and **12** showed the greatest activity, with MICs ranging from 20 to 330 μg mL^{-1} and MFCs ranging from 80 to 330 μg mL^{-1} for the *C. albicans*

strains. Compound **4** (MIC = 20 μg mL^{-1}) showed the highest inhibitory effect growth of *C. albicans* when compared to ketoconazole (MIC = 62.5 μg mL^{-1}), the standard antifungal agent utilized in this assay. The compounds **4**, **11** and **12** showed fungicidal activity against *C. albicans* ATCC 10231, which was not observed for the antifungal ketoconazole. The MIC value of compound **4** obtained against this yeast was lower than the MFC value, which suggests that this compound is fungistatic at a lower concentration and fungicidal at a slightly higher concentration.

It is worth mentioning that *C. glabrata* ATCC 2001 displayed sensitivity towards the compounds **4** and **12** that showed fungistatic activity. In addition, *C. krusei* ATCC 34135 was susceptible to the compound **12** that showed MIC and MFC of 330 μg mL^{-1}. All the yeasts tested were resistant to compounds **5**, **6**, **7**, **8**, **14** and **23**.

Regarding clinical strains, the compound **4** showed the best fungistatic activity against *C. albicans* clinical strains CA1, CA2, CA4 and CA5 with MICs ranging from 40-670 μg mL^{-1}. The compound **10** showed fungistatic activity against CA1 with an MIC of 170 μg mL^{-1}. The other compounds showed less activity.

In this study, the compounds **4**, **11** and **12** had the greatest activity against *C. albicans* strains; therefore, their effect on membrane ergosterol was determined using the exogenous ergosterol assay. Compound **4** showed binding action on membrane ergosterol, since the MIC was enhanced 4 fold against *C. albicans* ATCC 10231, when performed in the presence of exogenous ergosterol (MIC = 80 μg mL^{-1}) indicating their binding to ergosterol (Table 2).

The cytotoxicity assay was performed with the compounds **4**, **11** and **12**, which showed best MIC values against *C. albicans* ATCC 10231. The CC$_{50}$ obtained for those compounds were 52.81, 52.58 and 85.94 μg mL^{-1}, respectively.
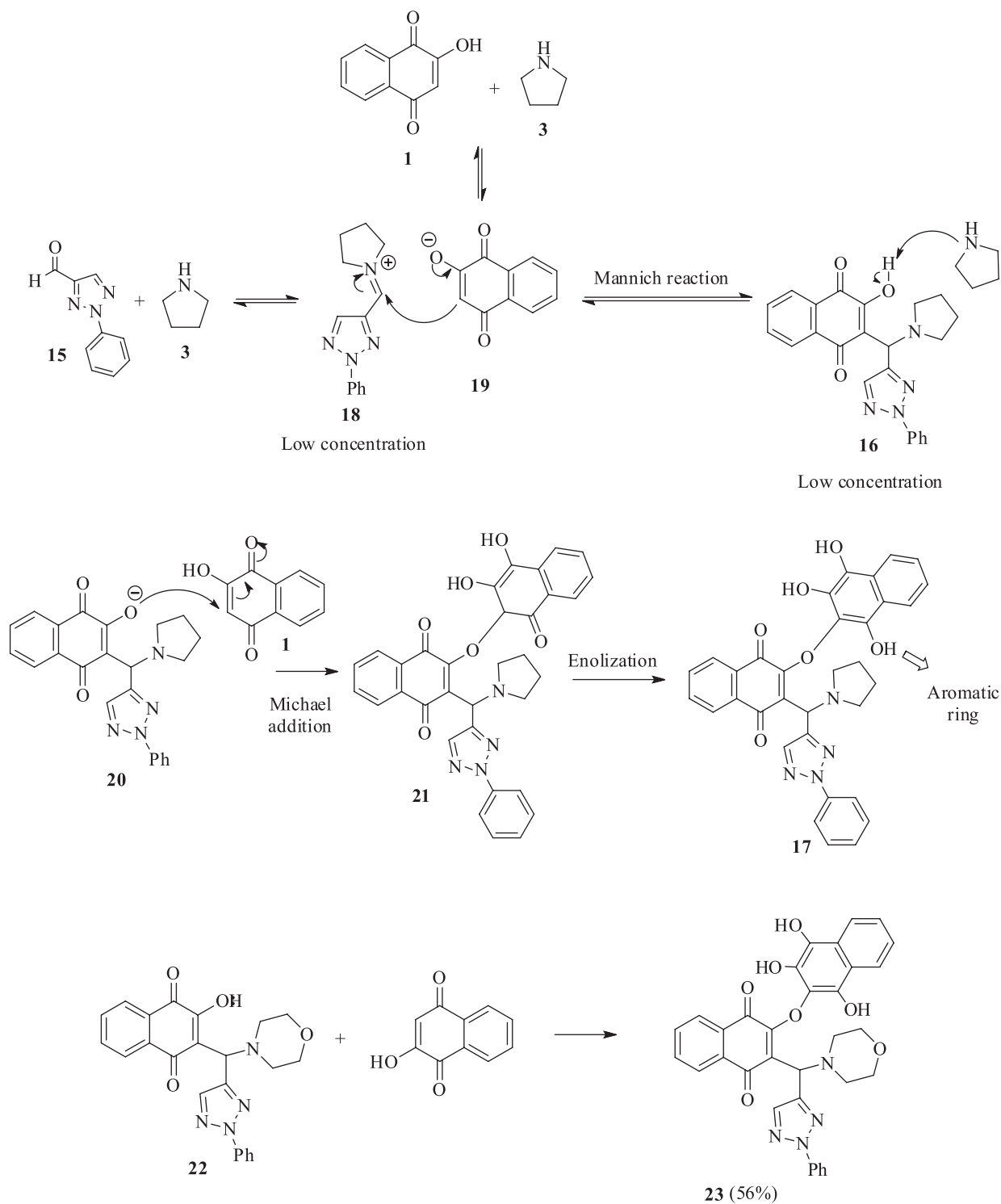
Scheme 4. Proposed mechanism for the formation of compounds **17** and **23**.

Molecular modeling studies

In order to initiate a lead optimization process, docking studies (Figure 1) were performed between the most active lawsone compounds and the molecular target of ketoconazole (3OZW).[28] Initially, the docking methodology

was evaluated by redocking[34] of ketoconazole, which yielded a root mean square deviation (RMSD) of 2.29 Å (Figure 1a). Even though this value deviates 0.29 Å from the ideal value (2.0 Å), as can be seen in Figure 1a, it could keep the moieties in the similar order of crystallographic ligand. Next, the compounds **4**, **11** and **12** were docked into the
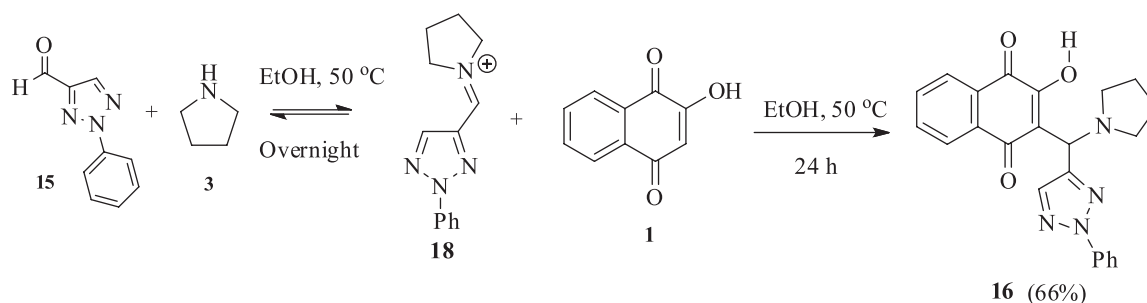
Scheme 5. Synthesis of compound **16**.

Table 1. Antifungal activity (µg mL⁻¹) of 2-hydroxy-1,4-naphthoquinone derivatives against *Candida* spp. strains

	C. albicans ATCC 10231		*C. albicans* ATCC 1417		*C. albicans* ATCC 1083		*C. albicans* ATCC 1203		*C. albicans* ATCC 1252		*C. albicans* ATCC 11763		*C. glabrata* ATCC 2001		*C. krusei* ATCC 34135	
	Concentration / (µg mL⁻¹)															
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
4	20	80	670	–	40	–	670	–	–	–	170	170	170	–	670	–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	170	–	350	–	–	–	–	–	700	–	700	–	–	–
11	330	330	–	–	660	–	660	–	–	–	–	–	–	–	–	–
12	85	85	670	–	330	–	670	–	330	670	–	–	330	–	330	330
13	–	–	350	–	–	–	–	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	730	–	–	–	730	–	730	–	–	–	730	–	730	–	–	–
17	–	–	670	–	–	–	–	–	–	–	–	–	670	670	–	–
23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

MIC: minimum inhibitory concentration; MFC: minimum fungicidal concentration; –: no activity.

Table 2. Mechanism of action on membrane ergosterol against three *Candida* spp. strains

	C. albicans ATCC 10231		*C. glabrata* ATCC 2001		*C. krusei* ATCC 34135	
	MIC with ergosterol / (µg mL⁻¹)	MIC without ergosterol / (µg mL⁻¹)	MIC with ergosterol / (µg mL⁻¹)	MIC without ergosterol / (µg mL⁻¹)	MIC with ergosterol / (µg mL⁻¹)	MIC without ergosterol / (µg mL⁻¹)
4	80	20	170	170	N	N
11	330	330	N	N	N	N
12	85	85	330	330	330	330
Nystatin	31.25	3.9	31.25	3.9	31.25	3.9

MIC: minimum inhibitory concentration; N: not performed.

binding site following a similar conformation (Figure 1b). The intermolecular interactions between the most active compound **4** and target are illustrated by Figures 1c and 1d. As can be observed, the pyrrolidine moiety is able to bind to the heme group by a cation-pi, whereas the naphthoquinone and phenyl moieties perform van der Waals interactions. Further, the binding site cavity was studied by hydrophobic (Figure 1e) and hydrogen bond (Figure 1f) surfaces. In general, the binding site showed a high hydrophobic character (Figure 1e). However, close to the phenyl moiety, the binding site can recognize a hydrogen bond acceptor and donor. In other words, nonpolar moieties and hydrogen acceptor and donor moieties in the phenyl ring can improve the biological activity.
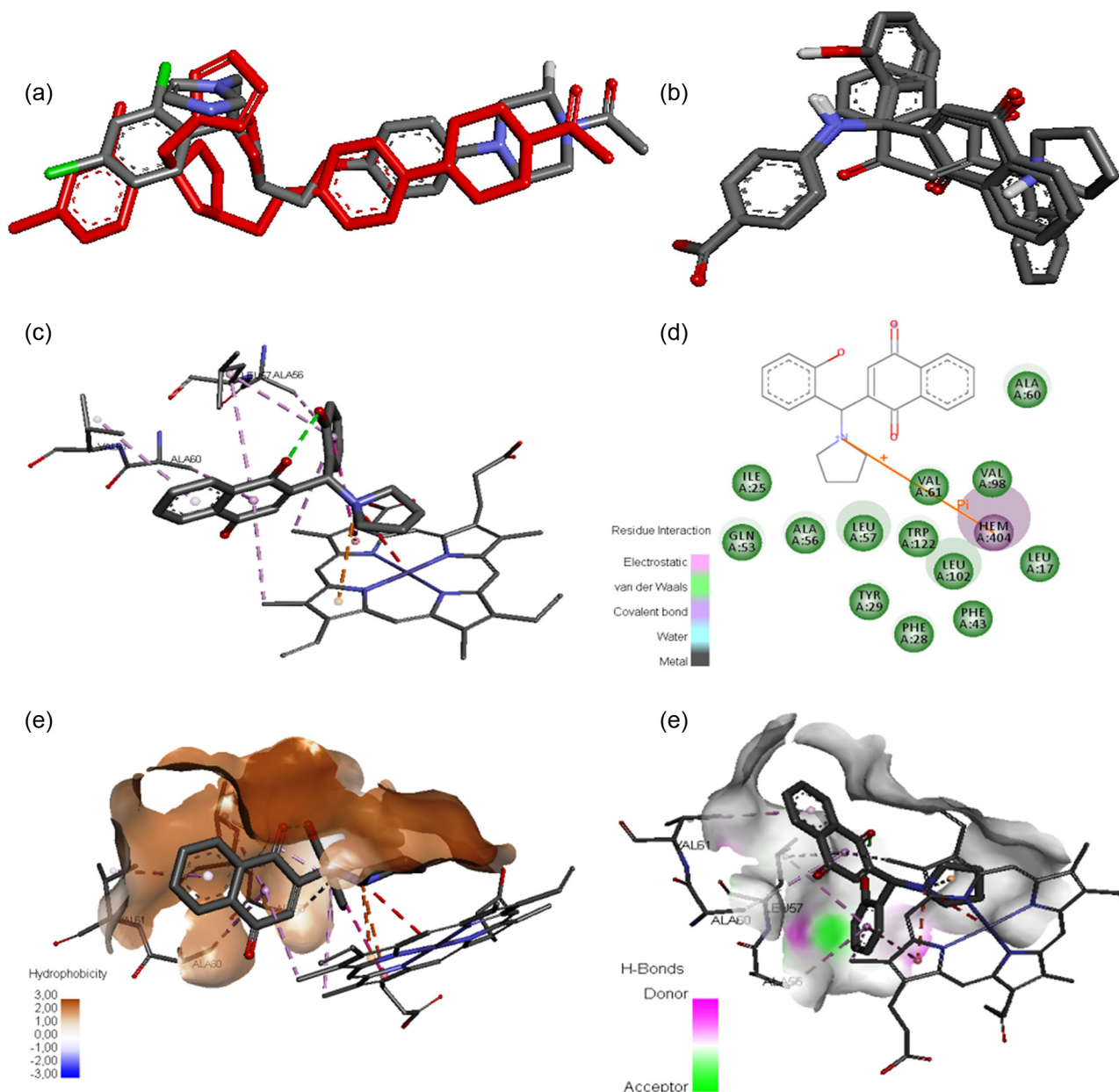
Figure 1. Docking results between lawsone and 3OZW. (a) Redocking of ketoconazole; (b) cluster formed by compounds **4**, **11** and **12**; (c) 3D compound **4**-3OZW intermolecular interactions; (d) 2D intermolecular interactions diagram between compound **4** and 3OZW; (e) hydrophobic; and (f) hydrogen bond surfaces of binding site. The hydrogen atoms were omitted for a better visualization.

Finally, tests on the physicochemical properties were carried out to evaluate the most promising ligand[35] among compounds **4**, **11** and **12** to reach the market (Table 3). According to the rule of 5 (Ro5) proposed by Lipinski *et al.*,[36] the lawsone derivatives **4**, **11** and **12** do not violate any rule. They showed molecular weights less than 500, the number of hydrogen bond donors and acceptors less than 5 and 10, respectively, and log P less than 5. In addition, the binding energy obtained from docking studies is similar to that of ketoconazole. On the other hand, ketoconazole and nystatin violated one and two Ro5 rules,

respectively. Moreover, druglikeness[37] shows that these compounds contain fragments present in commercial drugs, of which 80% have positive druglikeness values close to 3. These findings suggest that the compounds **4**, **11** and **12** are more promising drugs than ketoconazole and nystatin.

Conclusions

In this study, we determined the MICs and MFCs of thirteen new naphthoquinones derivatives **4-8**, **10-14**, **16**, **17** and **23** obtained by multicomponent Mannich reaction

Table 3. Physicochemical properties for the bioactive lawsone derivatives

Compound	Binding energy / (kcal mol^{-1})	Molecular weight / (g mol^{-1})	log P	H-acceptors	H-donors	Druglikeness
4	−10.2	334.39	0.81	4	2	0.00
11	−11.7	383.40	3.40	5	2	1.80
12	−11.6	399.40	3.05	6	3	1.80
Ketoconazole	−10.5	533.45	3.12	8	1	6.58
Nystatin	−	926.10	0.40	18	12	−4.30

log P: partition coefficient.

containing 2-phenyl-2*H*-1,2,3-triazole or substituted and non-substituted phenyl groups against *Candida* strains and clinical isolates. Among them, compounds **4**, **11** and **12** were the most active. These compounds showed fungistatic and fungicidal activity against strains of *C. albicans* ATCC 10231. This finding indicates that they are strong candidates for clinical use, since the complete elimination of the pathogen is the safest option.[38] Moreover, the results indicate that these compounds were more active against *C. albicans* ATCC 10231, which shows greater sensitivity than *C. glabrata* and *C. krusei*. It is noteworthy that *C. albicans* is the causative agent in 90% of fungal infections such as VVC.[39] Besides, *C. albicans* and *C. tropicalis* strains are responsible for a number of major diseases as well as providing recent cases of resistance to the current main antifungals.[40]

In addition, the effect on the membrane ergosterol was determined for compounds **4**, **11** and **12**. The evaluation of this mechanism of action is important due to the ergosterol molecule, or enzymes used in its biosynthesis, being important targets for the action of azole antifungals.[41] Hence, the antifungal activity of the compound **4** against *C. albicans* ATCC 10231 can be attributed to its action on membrane ergosterol. The antifungal activity of the compounds **11** and **12** against *C. albicans* ATCC 10231 can be attributed probably to the reactive oxygen species (ROS) generation, because these compounds do not have the triazole group and because of the presence of naphthoquinone core with recognized pharmacological action via formation of ROS.[1,42]

The MICs for the compounds **4**, **11** and **12** against yeasts indicate that these compounds are excellent choices for the development of novel drugs to treat fungal infections. However, our data highlighted that the compound **4** showed superior antifungal activity against *C. albicans* ATCC 10231 when compared to ketoconazole, the basis for most drugs available for treatment of fungal infections currently on the market. In our study, these compounds presented moderate to low cytotoxicity in BHK cells. These results also indicate that the compounds present low cytotoxicity on the cell strain tested.[43]

The most active compounds showed similar binding energy with ketoconazole, suggesting their mechanism of action. In general, the cavity of the binding site has a hydrophobic character, addressing for lipophilic moieties in phenyl ring. Furthermore, the compounds **4**, **11** and **12** are under Ro5 with a druglikeness score better than that for ketoconazole and nystatin. These findings suggest a therapeutic alternative against the increased resistance of *Candida* spp. to azole-based antifungals.[40]

Supplementary Information

Supplementary information (spectra for IR, ^{1}H and ^{13}C NMR and FT-ICR MS of the synthesized compounds) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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