Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Synthesis and biological evaluation of substituted α- and β-furan naphthoquinones as potent anti-Candida agents

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MATERIALS AND METHODS

Reagents were purchased from Aldrich or Acros Chemical Co. and were used without further purification. Column chromatography was performed with silica gel 60 (Merck 70-230 mesh). Analytical thin-layer chromatography was performed with silica gel plates (Merck, TLC silica gel 60 F254), and the plots were visualized using UV light or aqueous solutions of sodium sulfate. Yields refer to chromatographically and spectroscopically homogeneous materials. Melting points were obtained on a Fischer-Johns apparatus, and are uncorrected. Infrared spectra were measured using KBr pellets on a Perkin-Elmer model 1420 FT-IR Spectrophotometer, calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene. NMR spectra were recorded on a Varian Unity Plus VXR (300 MHz) instrument in DMSO-d_6 and $CDCl_3$ solutions. The chemical shift data were reported in units of δ (ppm) downfield from tetramethylsilane, which was used as an internal standard; coupling constants (J) are reported in Hertz, and refer to apparent peak multiplicities. The CHN elemental analyses were performed on a Perkin–Elmer 2400 CHN elemental analyzer (São Paulo University, USP/Brazil).

General Procedure for preparation of 1a-i and 2a-i

To a round-bottom flask equipped with a magnetic stirring bar a solution of CAN (1.260 g, 2.3 mmol) in dried THF (10 mL) was added dropwise to an ice-cooled solution of 2-hydroxy-1,4-naphthoquinone (0.174 g, 1 mmol) and diene (1 mmol) in dried THF (10 mL). The result mixture was stirred for 3 hours (room temperature). Then the mixture was extracted with ethyl acetate and water. The combined organic extracts washed with water, and dried over anhydrous sodium sulfate, was filtered and concentrated *in vacuo*. The residual crude product was then purified by column chromatography on silica gel using a mixture gradient of hexane and ethyl acetate as eluent.

2,3-Dihydro- 2-phenylnaphtho[1,2-b] furan-4,9-dione (**1a**). Yellow solid, m.p.= 95-97 ^{o}C; **IV ν_{max} (cm^{-1}):** 3431, 2924, 1675,1630, 1590, 1374, 1240, 1195, 954, 750; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.26 (1H, dd, J= 8.5 and 17.3, C$\underline{H}_2$); 3.67 (1H, dd, J= 10.7 and 17.3, C$\underline{H}_2$); 6.00 (1H, dd, J = 8.5 and 10.7, OC$\underline{H}$); 7.36-7.41 (5H, m, H-phenyl); 7.66-7.73 (2H, m, H-quinone); 8.08-8.12 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz): δ** 35.0 (C-3); 86.5 (C-2); 123.6 (C-3a); 125.7 (C-5 and C-8); 125.8 (C-2 and C-6 phenyl); 126.1 (C-4 phenyl); 128.7 (C-3 andC-5 phenyl); 131.3 (C-8a); 132.8 (C-4a); 134.0 (C-6 and C-7); 139.3 (C-1 phenyl); 159.6 (C-9a); 177.4 (C-9); 181.9 (C-4). **Anal. Calcd for** $C_{18}H_{12}O_3$: C, 78.25; H, ..4.38. Found: C, 78.30; H, 4.42.

2,3-Dihydro-2-(p-tolyl)naphtho[2,3-b]furan-4,9-dione (**1b)**. Yellow solid, m.p.= 140-143 ^{o}C**; IV ν_{max} (cm^{-1}):** 3382, 3055, 1645, 1571, 1407, 1224, 1150, 887, 769; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 2.31 (3H, s, CH_3); 3.25 (1H, dd, J= 8.6 and 17.3, C$\underline{H}_2$); 3.64 (1H, dd, J= 10.7 and 17.4, C$\underline{H}_2$); 5.96 (1H, dd, J = 8.6 and 10.7, OC$\underline{H}$); 7.19-7.30 (4H, m, H-phenyl); 7.66-7.76 (2H, m, H-quinone); 8.07-8.12 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** δ 21.0 (CH_3); 34.9 (C-3); 86.7 (C-2); 123.7 (C-3a); 125.8 (C-5 and C-8); 126.1 (C-2 and C-6 phenyl); 137.5 (C-4 phenyl); 128.2 (C-3 andC-5 phenyl); 131.8 (C-8a); 132.9 (C-4a); 134.2 (C-6 and C-7); 140.3 (C-1 phenyl); 159.7 (C-9a); 177.6 (C-9); 180.9 (C-4). **Anal. Calcd for** $C_{19}H_{14}O_3$: C, 78.61; H, 4.86. Found: C, 78.41; H, 4.67.

2-(4-Chlorophenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione (**1c**). Yellow solid, m.p.= 166-168 ^{o}C; **IV ν_{max} (cm^{-1}):** 3431, 2922, 1644, 1647, 1590, 1239, 1195, 974, 713; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.20 (1H, dd, J= 8.5 and 17.4, C$\underline{H}_2$); 3.67 (1H, dd, J= 10.9 and 17.4, C$\underline{H}_2$); 5.97 (1H, dd, J = 8.5 and 10.9, OC$\underline{H}$); 7.32-7.39 (4H, m, H-phenyl); 7.66-7.76 (2H, m, H-quinone); 8.06-8.11 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** δ 35.9 (C-3); 87.7 (C-2); 124.5 (C-3a); 126.8 (C-5 and C-8); 126.9 (C-2 and C-6 phenyl); 133.5 (C-4 phenyl); 129.2 (C-3 andC-5 phenyl); 130.8 (C-8a); 132.5 (C-4a); 135.0 (C-6 and C-7); 137.3 (C-1 phenyl); 160.7 (C-9a); 179.6 (C-9); 184.9 (C-4). **Anal. Calcd for** $C_{18}H_{11}ClO_3$: C, 69.58; H, 3.57. Found: C, 69.74; H, 3.59.

2-(4-fluorophenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione (1d). Yellow solid, m.p.= 165-167 ^{o}C; **IV ν_{max} (cm^{-1}):** 3435, 2921, 1671, 1640, 1239, 1137, 958, 718; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.23 (1H, dd, J= 8.4 and 17.3, C$\underline{H}_2$); 3.66 (1H, dd, J= 10.9 and 17.3, C$\underline{H}_2$); 5.98 (1H, dd, J = 8.4 and 10.9, OC$\underline{H}$); 7.06-7.11 (2H, m, H-3 and H-5 phenyl); 7.36-7.41 (2H, m, H-2 and H-6 phenyl); 7.66-7.76 (2H, m, H-quinone); 8.07-8.11 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 36.7 (C-3); 88.7 (C-2); 124.5 (C-3a); 126.7 (C-5 and C-8); 126.8 (C-2 and C-6 phenyl); 160.5 (C-4 phenyl); 110.2 (C-3 andC-5 phenyl); 130.1 (C-8a); 131.5 (C-4a); 135.1 (C-6 and C-7); 134.3 (C-1 phenyl); 160.2 (C-9a); 179.8 (C-9); 184.3 (C-4). **Anal. Calcd for** $C_{18}H_{11}FO_3$: C, 73.47; H, 3.77. Found: C, 73.31; H, 3.65.

2-(4-bromophenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione (1e). Yellow solid, m.p.= 185-188 ^{o}C**; IV ν_{max} (cm^{-1}):** 3236, 2922, 1675, 1646, 1239, 1195, 976, 826, 713; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.21 (1H, dd, J= 8.5 and 17.3, C$\underline{H}_2$); 3.65 (1H, dd, J= 10.1 and 17.3, C$\underline{H}_2$); 5.95 (1H, dd, J = 8.5 and 10.1, OC$\underline{H}$); 7.35-7.42 (4H, m, H-phenyl); 7.67-7.78 (2H, m, H-quinone); 8.00-8.15 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 36.9 (C-3); 82.7 (C-2); 124.3 (C-3a); 126.3 (C-5 and C-8); 127.8 (C-2 and C-6 phenyl); 122.0 (C-4 phenyl); 131.2 (C-3 andC-5 phenyl); 130.8 (C-8a); 131.7 (C-4a); 135.2 (C-6 and C-7); 137.3 (C-1 phenyl); 160.3 (C-9a); 179.9 (C-9); 184.1 (C-4). **Anal. Calcd for** $C_{18}H_{11}BrO_3$: C, 60.87; H, 3.12. Found: C, 61.01; H, 3.94.

2,3-Dihydro- 2-methyl-2-phenylnaphtho[2,3-b] furan-4,9-dione (1f). Yellow solid, m.p.= 169-170 ^{o}C; **IV ν_{max} (cm^{-1}):** 3434, 2976, 1678, 1640, 1587, 1254, 1206, 956, 712; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 1.87 (3H, s, CH_3); 3.37 (1H, d, J= 17.6, C$\underline{H_2}$); 3.47 (1H, d, J= 17.6, C$\underline{H_2}$); 7.30-7.47 (5H, m, H-phenyl); 7.65-7.77 (2H, m, H-quinone); 8.04-8.13 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz)**: 29.5 (CH_3); 40.9 (C-3); 78.7 (C-2); 125.3 (C-3a); 126.8 (C-5 and C-8); 126.1 (C-2 and C-6 phenyl); 125.9 (C-4 phenyl); 128.2 (C-3 andC-5 phenyl); 130.5 (C-8a); 131.8 (C-4a); 135.0 (C-6 and C-7); 140.3 (C-1 phenyl); 159.3 (C-9a); 179.5 (C-9); 184.6 (C-4). **Anal. Calcd for** $C_{19}H_{14}O_3$: C, 78.61; H, 4.86. Found: C, 78.73; H, 4.99.

2-(2,4-dimethylphenyl)-2,3-dihydronaphtho[2,3-b]furan-4,9-dione (1g). Yellow solid, m.p.= 110-111 ^{o}C; **IV ν_{max} (cm^{-1}):** 3350, 2920, 1681, 1648, 1392, 1240, 1196, 973, 712; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 2.31 (3H, s, CH_3); 2.34 (3H, s, CH_3); 3.11 (1H, dd, J= 8.5 and 17.3, CH2); 3.65 (1H, dd, J= 10.1 and 17.3, CH2); 6.14 (1H, dd, J = 8.5 and 10.1, OCH); 7.01-7.03 (2H, m, H-5 and H-6 phenyl); 7.24-7.27 (1H, m, H-3 phenyl); 7.66-7.76 (2H, m, H-quinone); 8.07-8.13 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 19.7 (CH_3); 21.5 (CH_3); 37.9 (C-3); 79.7 (C-2); 124.3 (C-3a); 126.8 (C-5 and C-8); 134.1 (C-2phenyl); 126.9 (C-6 phenyl); 137.9 (C-4 phenyl); 131.2 (C-3 phenyl); 126.2 (C-5 phenyl); 130.6 (C-8a); 131.8 (C-4a); 135.0 (C-6 and C-7); 136.3 (C-1 phenyl); 160.3 (C-9a); 179.8 (C-9); 184.2 (C-4). **Anal. Calcd for** $C_{20}H_{16}O_3$: C, 78.93; H, 5.30. Found: C, 78.99; H, 6.01.

2,3-Dihydro- 2,2-dimethylnaphtho[2,3-b] furan-4,9-dione (1h). Yellow solid, m.p.= 110-112 ^{o}C**; IV ν_{max} (cm^{-1}):** 3350, 2922, 1678, 1649, 1350, 1240, 1198, 984, 715**; ^{1}H NMR (DMSO-d$_6$, 300 MHz):** 1.87 (6H, s, CH_3); 3.35 (1H, d, J= 17.5, C$\underline{H_2}$); 3.45 (1H, d, J= 17.5, C$\underline{H_2}$); 7.66-7.77 (2H, m, H-quinone); 8.04-8.11 (2H, m, H-quinone);**^{13}C NMR (DMSO-d$_6$, 75 MHz):** 28.5 (2CH_3); 48.9 (C-3); 90.7 (C-2); 125.3 (C-3a); 126.8 (C-5 and C-8); 130.8 (C-8a); 131.7 (C-4a); 135.4 (C-6 and C-7); 159.3 (C-9a); 179.9 (C-9); 185.2 (C-4). **Anal. Calcd for** $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.60; H, 5.28.

3*a*,10*b*-dihydro-1*H*-naphtho[*b*]cyclopenta[*d*]furan-5,10-dione (1i). Yellow solid, m.p.= 191-193 ^{o}C; **IV ν_{max} (cm^{-1}):** 3283, 1677, 1638, 1584, 1452, 1384, 1360, 1287, 1196; **^{1}H NMR (CDCl$_3$, 300 MHz):** 2.75 (1H, dquint, *J* = 17.9 and 2.2 Hz, H-1' or H-1''); 2.94 (1H, dddt, *J* = 17.9, 8.2, 2.2 and 1.0 Hz, H-1' or H-1''); 4.14 (1H, dt, *J* = 8.2 and 2.2 Hz, H-3a); 5,94 (1H; ddd; *J* = 5.7, 4.5 and 2.2 Hz, H-3); 6,06 (1H. dsept; 8,9 e 1,0 Hz; H-10b); 6.17 (1H, ddt, 57; 4,5 e 2,2 Hz; H-2); 7.69 (2H, ddt, *J* = 16.5, 7.5 and 1.7 Hz, H-7 and H-8); 8.07-8.06 (2H, m, H-6 and H-9); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 38.1 (C-1); 42.0 (C-10b); 95.9 (C-3a); 125.9 (C-6 or C-8); 126.1 (C-6 or C-8); 126.9 (C-10a); 127.7 (C-2); 131.5 (C-5a or C-9a); 132.8 (C-7 or C-9); 133.1 (C-5a or C-9a); 134.1 (C-7 or C-9); 137.5 (C-3); 178.5 (C-10); 158.5 (C-4a); 182.3 (C-5).

2,3-Dihydro-2-phenylnaphtho[1,2-b]furan-4,5-dione (2a). Orange solid, m.p.= 80-83 ^{o}C; **IV ν_{max} (cm^{-1}):** 3375, 2925, 1694, 1648, 1406, 1238, 1148, 1079, 881, 765, 698; **^{1}H NMR (DMSO-d$_6$, 300 MHz)**: 3.20 (1H, dd, J= 8.4 and 17.2, C$\underline{H}_2$); 3.61 (1H, dd, J= 10.7 and 17.2, C$\underline{H}_2$); 6.06 (1H, dd, J = 8.4 and 10.7, OC$\underline{H}$); 7.39-7.4-41 (5H, m, H-phenyl); 7.57-7.60 (1H, m, H-quinone); 7.65-7.72 (2H, m, H-quinone); 8.10-8.13 (1H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 36.9 (C-8); 78.7 (C-9); 101.8 (C-7a); 123.5 (C-2); 126.1 (C-5); 128.4.3 (C-1b); 135.3 (C-3); 138.8 (C-1 phenyl); 127.0 (C-4 phenyl); 172.6 (C-1a); 181.5 (C-6); 180.3 (C-7). **Anal. Calcd for** $C_{18}H_{12}O_3$: C, 78.25; H, 4.38. Found: C, 78.15; H, 4.31.

2,3-Dihydro-2-(p-tolyl)naphtho[1,2-b]furan-4,5-dione (2b). Orange solid, m.p.= 125-127 ^{o}C; **IV ν_{max} (cm^{-1}):** 3382, 2920, 1699, 1645, 1571, 1407, 1224, 1150, 887, 763; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 2.38 (3H, s, CH_3); 3.19 (1H, dd, J= 8.4 and 17.3, C$\underline{H}_2$); 3.58 (1H, dd, J= 10.3and 17.4, C$\underline{H}_2$); 6.02 (1H, dd, J = 8.4 and 10.3, OC$\underline{H}$); 7.21-7.31 (4H, m, H-phenyl); 7.56-7.60 (1H, m, H-quinone); 7.63-7.69 (2H, m, H-quinone); 8.09-8.12 (1H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** δ 36.4 (C-8); 79.2 (C-9); 101.8 (C-7a); 123.5 (C-2); 126.1 (C-5); 129.2 (C-3 and C-5 phenyl); 125.2 (C-2 and C-6 phenyl); 128.3 (C-4); 130.0 (C-5a); 133.4 (C-1b); 134.2 (C-3); 135.6 (C-1 phenyl); 137.0 (C-4 phenyl); 171.5 (C-1a); 180.6 (C-6); 179.9 (C-7). **Anal. Calcd for** $C_{19}H_{14}O_3$: C, 78.61; H, 4.86. Found: C, 77.83; H, 4.89.

2-(4-Chlorophenyl)-2,3-Dihydronaphtho[1,2-b]furan-4,5-dione (2c). Orange solid, m.p.= 160-162 ^{o}C; **IV ν_{max} (cm^{-1}):** 3382, 2929, 1656, 1623, 1345, 1240, 1084, 887, 776; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.14 (1H, dd, J= 8.1 and 17.4, C$\underline{H}_2$); 3.60 (1H, dd, J= 10.7 and 17.4, C$\underline{H}_2$); 6.03 (1H, dd, J = 8.1 and 10.7, OC$\underline{H}$); 7.33-7.42 (4H, m, H-phenyl); 7.58-7.63 (1H, m, H-quinone); 7.66-7.68 (2H, m, H-quinone); 8.09-8.12 (1H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 36.5 (C-8); 78.2 (C-9); 101.6 (C-7a); 123.3 (C-2); 126.0 (C-5); 129.0 (C-3 and C-5 phenyl); 126.2 (C-2 and C-6 phenyl); 128.1 (C-4); 130.3 (C-5a); 132.3 (C-1b); 134.3 (C-3); 136.9 (C-1 phenyl); 133.0 (C-4 phenyl); 171.6 (C-1a); 180.5 (C-6); 179.3 (C-7). **Anal. Calcd for** $C_{18}H_{11}ClO_3$: C, 69.58; H, 3.57. Found: C, 69.57; H,4.18.

2-(4-fluorophenyl)-2,3-Dihydronaphtho[1,2-b]furan-4,5-dione (2d). Orange solid, m.p.= 155-157 ^{o}C; **IV ν_{max} (cm^{-1}):** 3437, 2926, 1657, 1624, 1512, 1222, 875, 776; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.15 (1H, dd, J= 8.1 and 17.0, C$\underline{H}_2$); 3.65 (1H, dd, J= 10.9 and 17.0, C$\underline{H}_2$); 5.95 (1H, dd, J = 8.1 and 10.9, OC$\underline{H}$); 7.10-7.21 (2H, m, H-3 and H-5 phenyl); 7.38-7.42 (2H, m, H-2 and H-6 phenyl); 7.60-7.65 (1H, m, H-quinone); 7.66-7.76 (2H, m, H-quinone); 8.00-8.09 (2H, m, H-quinone);**^{13}C NMR (DMSO-d$_6$, 75 MHz):** 36.7 (C-8); 79.2 (C-9); 101.6 (C-7a); 122.9 (C-2); 126.1 (C-5); 115.4 (C-3 and C-5 phenyl); 127.0 (C-2 and C-6 phenyl); 128.6 (C-4); 130.2 (C-5a); 132.3 (C-1b); 134.4 (C-3); 134.9 (C-1 phenyl); 161.0 (C-4 phenyl); 171.6 (C-1a); 179.6 (C-6); 178.1 (C-7). **Anal. Calcd for** $C_{18}H_{11}FO_3$: C, 73.47; H, 3.77. Found: C, 73.53; H, 3.95.

2-(4-bromophenyl)-2,3-Dihydronaphtho[1,2-b]furan-4,5-dione (2e). Orange solid, m.p.= 150-153 ^{o}C; **IV ν_{max} (cm^{-1}):** 3381, 2927, 1655, 1622, 1239, 1076, 876, 775; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 3.20 (1H, dd, J= 8.1 and 17.4, C$\underline{H}_2$); 3.60 (1H, dd, J= 10.1 and 17.4, C$\underline{H}_2$); 6.50 (1H, dd, J = 8.1 and 10.1, OC$\underline{H}$); 7.38-7.45 (4H, m, H-phenyl); 7.67-7.70 (1H, m, H-quinone); 7.71-7.78 (2H, m, H-quinone); 8.05-8.10 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 36.8 (C-8); 76.2 (C-9); 101.3 (C-7a); 123.7 (C-2); 126.2 (C-5); 131.4 (C-3 and C-5 phenyl); 127.2 (C-2 and C-6 phenyl); 128.4 (C-4); 130.1 (C-5a); 132.5 (C-1b); 134.5 (C-3); 137.9 (C-1 phenyl); 122.0 (C-4 phenyl); 171.8 (C-1a); 179.5 (C-6); 178.3 (C-7). **Anal. Calcd for** $C_{18}H_{11}BrO_3$: C, 60.87; H, 3.12. Found: C, 60.77; H, 3.10.

2,3-Dihydro-2-methyl-2-phenylnaphtho[1,2-b]furan-4,5-dione (2f). Orange solid, m.p.= 130-133 ^{o}C**; IV ν_{max} (cm^{-1}):** 3380, 2980, 1654, 1624, 1352, 1254, 1050, 876, 767; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 1.90 (3H, s, CH_3); 3.31 (1H, d, J= 17.0, C$\underline{H_2}$); 3.41 (1H, d, J= 17.0, C$\underline{H_2}$); 7.36-7.46 (5H, m, H-phenyl); 7.58-7.64 (1H, m, H-quinone); 7.67-7.72 (1H, m, H-quinone); 7.78-7.80 (1H, m, H-quinone); 8.09-8.12 (1H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz)**: 29.9 ($\underline{C}H_3$); 40.8 (C-8); 76.0 (C-9); 102.3 (C-7a); 123.0 (C-2); 126.1 (C-5); 128.4 (C-3 and C-5 phenyl); 126.2 (C-2 and C-6 phenyl); 128.3 (C-4); 130.0 (C-5a); 132.2 (C-1b); 134.3 (C-3); 140.9 (C-1 phenyl); 125.2 (C-4 phenyl); 171.3 (C-1a); 179.6 (C-6); 178.6 (C-7). **Anal. Calcd for** $C_{19}H_{14}O_3$: C, 78.61; H, 4.86. Found: C, 78.29; H, 4.90.

2-(2,4-dimethylphenyl)-2,3-Dihydronaphtho[1,2-b]furan-4,5-dione (2g). Orange solid, m.p.= 150-153 ^{o}C; **IV ν_{max} (cm^{-1}):** 3379, 2922, 1651, 1617, 1406, 1242, 982, 814; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 2.33 (3H, s, CH_3); 2.37 (3H, s, CH_3); 3.07 (1H, dd, J= 8.4 and 17.3, CH2); 3.58 (1H, dd, J= 10.5 and 17.3, CH2); 6.22 (1H, dd, J = 8.4 and 10.5, OCH); 7.03-7.07 (2H, m, H-5 and H-6 phenyl); 7.21-7.23 (1H, m, H-3 phenyl); 7.57-7.64 (1H, m, H-quinone); 7.66-7.72 (2H, m, H-quinone); 8.10-8.13 (2H, m, H-quinone); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 19.8 ($\underline{C}H_3$); 21.6 ($\underline{C}H_3$); 37.8 (C-8); 89.0 (C-9); 101.3 (C-7a); 120.0 (C-2); 126.3 (C-5); 126.4 (C-5 phenyl); 126.8 (C-6 phenyl); 128.3 (C-4); 130.9 (C-5a); 131.9 (C-3 phenyl); 132.0 (C-1b); 134.0 (C-3); 134.4 (C-2 phenyl); 136.2 (C-1 phenyl); 137.2 (C-4 phenyl); 170.3 (C-1a); 179.2 (C-6); 179.6 (C-7). **Anal. Calcd for** $C_{20}H_{16}O_3$: C, 78.93; H, 5.30. Found: C, 78.87; H, 5.29.

2,3-Dihydro-2-dimethylnaphtho[1,2-b]furan-4,5-dione (2h). Orange solid, m.p.= 132-133 ^{o}C; **IV ν_{max} (cm^{-1}):** 3380, 2931, 1654, 1636, 1306, 1201, 982, 814, 714; **^{1}H NMR (DMSO-d$_6$, 300 MHz)**: 1.89 (6H, s, CH_3); 3.30 (1H, d, J= 17.0, C$\underline{H_2}$); 3.40 (1H, d, J= 17.0, C$\underline{H_2}$); 7.50-7.55 (1H, m, H-quinone); 7.59-7.61 (2H, m, H-quinone); 8.00-8.05 (2H, m, H-quinone);**^{13}C NMR (DMSO-d$_6$, 75 MHz):** 28.8 ($2CH_3$); 48.6 (C-8); 97.5 (C-9); 102.4 (C-7a); 123.3 (C-2); 126.3 (C-5); 128.8 (C-4); 130.7 (C-5a); 132.4 (C-1b); 134.3 (C-3); 171.9 (C-1a); 179.0 (C-6); 179.5 (C-7). **Anal. Calcd for** $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.62; H, 5.32.

3*a*, 10*b*-dihydro-1*H*-naphtho[*b*]cyclopenta[*d*]furan-9,10-dione (2i). Orange solid, m.p.= 141-143 ^{o}C; **IV ν_{max} (cm^{-1}):** 3127, 1695, 1643, 1610, 1569, 1467, 1440, 1403, 1354, 1285, 1219, 1146, 1040; **^{1}H NMR (DMSO-d$_6$, 300 MHz):** 2.89 (1H, dddt, *J* = 18.1, 8.1, 2.4 and 0.9 Hz, H-1' or H-1''); 2.70 (1H, dquint, *J* = 18.1 and 2.4 Hz, H-1' or H-1''); 4.08 (12H, ddd, *J* = 10.5, 8.1 and 2.2 Hz, H-3a); 5.94 (1H, m, H-10b); 6.20-6.11 (2H, m, H-2 and H-3); 7.59-7.53 (1H, m, H-5); 7.64 (2H, dd, *J* = 5.0 and 1.1 Hz, H-6 and H-7); 8.06 (1H, td, *J* = 7.4 and 1.1 Hz, H-8); **^{13}C NMR (DMSO-d$_6$, 75 MHz):** 38.1 (C-1); 41.1 (C-10b); 97.6 (C-3a); 119.3 (C-4b); 124.7 (C-6 or C-8); 127.2 (C-6 or C-8); 127.8 (C-10a); 129.2 (C-2); 130.7 (C-8a); 131.8 (C-5 or C-7); 134.4 (C-5 or C-7); 138.3 (C-3); 158.2 C-4a; 175.4 (C-10); 181.3 (C-9).

2. Potencial hemolytic of compounds tested

Hemolytic potential of the compounds were tested using mice (*Mus musculus* Swiss) total blood. First, were collected (via intracardiac) 200 μL of blood from mice in heparin tube for the preparation of the solution of 2% erythrocytes using a saline solution (NaCl 0.85% + 10 mM CaCl). This volume was placed in Falcon tube of 15 mL containing 2 mL of saline solution and centrifuged at 1500 rpm for 5 min., discarding the supernatant. This procedure was repeated twice. At the end of the last centrifugation, the pellet was resuspended in 10 mL of saline solution. Ten microliters of test substances and DMSO (negative control) were plated in 96-well plate with 90 μL of saline solution. For positive control we used Triton X-100 (40 μL in 60 μL of saline solution). Were added 100 μL of 2% erythrocytes solution in each well and incubated the plate at room temperature for 1 hour under constant agitation. To remove the influence of the color of the testes substances, they were plated and added 100 μL of saline solution (solution without receiving erythrocytes). After the incubation period the plate was centrifuged at 1500 rpm for 10 min. At the end of centrifugation the supernatant was transferred to another plate, which was read (by absorbance) in plate spectrophotometer at 450 nm.

Table 1. Potencial hemolytic of compounds tested

Compound	% hemolysis	SD
1a	**-1.4379**	**0.4118**
1b	**-0.7863**	**0.7394**
1c	**1.1608**	**0.5145**
1d	**-1.9546**	**0.1839**
1e	**2.5313**	**0.6171**
1f	**4.5383**	**0.9046**
1g	**-0.2172**	**1.1121**
1h	**2.1044**	**2.8491**
1i	**5.9163**	**0.1911**
2a	**1.9771**	**0.4124**
2b	**5.2797**	**1.5606**
2c	**-1.5053**	**2.0445**
2d	**-0.2022**	**0.9721**
2e	**4.5907**	**0.7305**
2f	**-1.1982**	**0.6933**
2g	**4.3735**	**0.4883**
2h	**-2.4040**	**3.0406**
2i	**-1.6326**	**1.2598**

3. Toxicity test in NIH3T3 Cell Culture

Toxicity in NIH 3T3 cells was performed according[1] to were cells was grown in Dulbeco`s Modified Eagle`s medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 50U/mL penicillin and 50µg/mL streptomycin, and maintained at 37 ˚C under 5% CO_2 atmosphere. The naftoquinones were dissolved in dimethilsulfoxide (DMSO) to a concentration of 12,5 µg/mL. The concentration of DMSO in culture never exceeded 1,25% and exhibited 100% cell viability when used as a control. For measurement of cell viability, $1x10^4$ cells were seeded per well on 96 well plate and incubated at 37 ˚C under 5% CO_2 atmosphere. After 24 attachment period they were treated with naftoquinones (12,5µg/mL) in quadruplicate. Following 71 h incubation, 10µL of Alamar Blue were added to each well. After 1 h the fluorescence was readed at excitation: 540 and emission: 585. The viability was calculated as following, where F_2: flurescence of treatment, F_1: fluorescence of blank (naftoquinones + DMEM). (% viability = F_2 - F_1 x 100 / $F_{control}$)

Table 2. Toxicity of naftoquinones in NIH 3T3 fibroblasts p15, accessed by the Alamar blue method after 72h of treatment.

Substances (12µg/mL)	Dead cells (%)
1a	0
1b	0
1c	0
1d	0
1e	0
1f	0
1g	0
1h	20
1i	9
2a	0
2b	0
2c	0
2d	0
2e	0
2f	0
2g	17
2h	11
2i	8
Doxorrubicine	36
Control (-)	0

4. Antifungal test

4.1 Fungal Strains

C. albicans, *C. krusei*, *C. parapsilosis*, *C. kefyr*, *C. tropicalis and C. dubliniensis*, isolated from the oral cavity of patients with removable dentures were used. An ATCC reference strain of *C. albicans* (90028) was also assessed to ensure result reproducibility These strains were identified by biochemical tests, where was used the kit of identification Candifast® (International Microbio), through morphological testing on corn meal agar with Tween 80 (cultured) and molecular biology, being used the kit of molecular biology that is in the process of patenting, developed by the group coordinated by Dr. Adriana Sotero Martinez – FIOCRUZ.

4.2 Diffusion test from the hole in the culture medium (*hole plate*)

The susceptibility of synthetic naphthoquinones were tested initially by using the simple diffusion from the hole in the culture medium.[2]

Preparation of inocula of yeasts

For inoculum preparations were used Candida strains mentioned above. The fungi were transferred into sterile tubes with Sabourau dextrose agar, running passages to ensure purity and viability. The incubation temperature was 35 ° C for 24 hours. After incubation, were transferred to a sterile tube containing 5 ml of sterile saline solution 0.145 mol / L (8.5 g / L NaCl, 0.85% saline) five colonies of about 1mm in diameter. This suspension was placed to stir for 15 seconds on a vortex shaker, and cell density measured by a spectrophotometer obtaining the transmittance equivalent of a standard solution of 0.5 McFarland, at a wavelength of 530 nm. Thus the procedure provided a standard yeast suspension containing 1 x 10^6 - 5 x 10^6 cells per mL.

Inoculation of test plates

We used a sterile cotton swab and it was tuned in suspension, until 15 minutes after adjusting the turbidity of the inoculum. The swab was rotated several times and pressed firmly against the inner wall of the tube above the liquid level. The dry surface of the agar plate was inoculated by rubbing the swab over the surface. This procedure was repeated three more times, rotating the plate approximately 60° each time, aiming to ensure a homogeneous distribution of the inoculum. As a final step, it moved the swab

on the agar plate margin. Soon after, seeded plates were used where there have been four holes of 6 mm in diameter with the aid of sterile Pasteur pipette. In three holes were placed 20 μL of substances with a concentration of 250 μg/mL, and another hole was placed 20 μL of DMSO. The plates were incubated at 37 ° C for 24 hours. After incubation was performed with a caliper or reading a millimeter ruler, measuring the inhibition zone of fungal growth. We used standard substances, such as Fluconazole and Itraconazole, for comparison of antifungal activity.

4.3 Broth Microdilution Testing

Preparation of inocula

The standard yeast suspension was shaken for 15 seconds in a vortex, diluted to 1:50 and then 1:20 with RPMI culture medium, to obtain the twice concentrated inoculum used in the test (1 x 10^3 - 5 x 10^3 CFU / mL). The concentrated inoculum was diluted to 1:1 when the wells were inoculated, reaching the final concentration (0,5 x 10^3a 2,5 x 10^3 CFU/mL).

Microdilution Tests

The microdilution test was performed in sterile microdilution plates with 96 wells U-shaped. Concentrations twice concentrated drug were dispensed in the wells of rows 1-10 of microdilution plates in 100μL, with a multichannel pipette. Row 1 contained a higher concentration of the drug (800 or 16μg/mL) and row 10 to lower drug concentration (1.562 or 0.03 μg/mL). Each well of the microdilution plate was inoculated with 100μL of concentrated suspension of the corresponding inoculum. The growth control wells had 100μL of sterile, free of drugs, and were inoculated with 100μL of concentrated suspensions of inocula. The row 12 of the microdilution plate was used to effect control of sterility with only medium, containing no drugs. The microdilution plates were incubated at 35 ° C for 24 hours.

Determination of endpoint of MIC[3]

Microdilution wells were assigned a score, the growth in each well was compared with the pit growth control (no drug) with the help of a reading mirror. Each microdilution well of the plate received a numerical value, using the following scale: 0 = optically clear, 1 = indeterminate growth, 2 = prominent reduction of growth, 3 = slight growth

reduction and 4 = no reduction in growth. The value of MIC of compounds was defined as the lowest concentration that can be observed score 2 (prominent growth reduction).

[1] G. R. Nakayama, M. C. Caton, M. P. Nova, Z. Parandoosh. *J. Immun. Meth.*, **1997**, 204, 205-208.

[2] M. C. C. Ayres, M. S. Brandão, G. M. Vieira Jr, J. C. A. S. Menor, H. B. Silva, M. J. S. Soares, M. H. Chaves. *Revista brasileira de farmacognosia*, **2008,** 18, 90-97.

[3] The National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, M27-A2. **2002**, 22, 1-30.