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Article

Synthesis and Antimicrobial Evaluation of 3-Hydrazino-Naphthoquinones as Analogs of Lapachol

Claudia G. T. Oliveira[a,c], Frederico F. Miranda[a], Vitor F. Ferreira[a]*, Cícero C. Freitas[b], Renata F. Rabello[b], Jupira M. Carballido[d] and Luiz C. D. Corrêa[b]

[a]*Universidade Federal Fluminense, Instituto de Química, Departamento de Química Orgânica, CEG, Campus do Valonguinho, 24210-150, Niterói - RJ, Brazil*

[b]*Universidade Federal Fluminense, Instituto de Biologia, CEG, Campus do Valonguinho, 24210-150, Niterói - RJ, Brazil*

[c] *Núcleo de Pesquisa de Produtos Naturais, Universidade Federal do Rio de Janeiro, 21941-540, Rio de Janeiro - RJ, Brazil*

[d]*Universidade Federal Fluminense, Hospital Universitário Antônio Pedro, 24030-210, Niterói - RJ, Brazil*

Vários derivados de 1,4-naftoquinonas contendo um grupo hidrazino como cadeia lateral foram sintetizados a partir do 3-diazo-naftaleno-1,2,4-triona e foram avaliados como potenciais agentes antimicrobianos. Os derivados naftoquinônicos 2-[N'-(1-acetil-2-oxo-propilideno)-hidrazino]-3-hidroxi-[1,4]naftoquinona, 2-[(3-hidroxi-1,4-dioxo-1,4-diidro-naftaleno-2-il)-hidrazono]-3-oxo-butirato de etila, 2-[(3-hidroxi-1,4-dioxo-1,4-diidro-naftaleno-2-il)-hidrazono]-3-oxo-butirato de *t*-butila, 3-hidroxi-2-[(di-O-isopropilideno-malonato)-hidrazino]-1,4-naftoquinona e 2-[(3-hidroxi-1,4-dioxo-1,4-diidro-naftaleno-2-il)-hidrazono]-malonato de etila mostraram maior atividade antibacteriana, ao nível de teste preliminar em disco, que o lapachol (**1**), uma 1,4-naftoquinona muito conhecida pelas suas variadas atividades biológicas. Estudo sobre a concentração mínima inibitória (MIC) para o *Staphylococcus aureus* mostrou que 2-[(3-hidroxi-1,4-dioxo-1,4-diidro-naftaleno-2-il)-hidrazono]-malonato de etila tem uma atividade duas vezes maior que **1**. Da mesma forma, o estudo da densidade ótica em cultura de *S. aureus* com esta substância mostrou uma atividade similar à da vancomicina na concentração de 2xMIC.

Several 1,4-naphthoquinone derivatives having a hydrazino side chain were synthesized from 3-diazo-naphthalene-1,2,4-trione and tested as potential antimicrobial agents. These naphthoquinone derivatives 2-[N'-(1-acetyl-2-oxo-propylidene)-hydrazino]-3-hydroxy-[1,4]naphthoquinone, ethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-oxo-butyrate, *t*-butyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-oxo-butyrate, 3-hydroxy-2-[(di-O-isopropylidene-malonate)-hydrazino]-1,4-naphthoquinone, and diethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-malonate showed greater antibacterial activity at the level of the preliminary susceptibility testing in disk than lapachol (**1**), a well known 1,4-naphthoquinone which has several biological activities. Studies on the minimal inhibitory concentration (MIC) for *Staphylococcus aureus* showed that diethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-malonate has an activity twofold greater than **1**. On the other hand, optical density measurement for *S. aureus* indicated that this compound has similar activity compared with vancomycin at 2xMIC.

Keywords: naphthoquinone, hydrazino-naphthoquinone, antimicrobial activity, lapachol

Introduction

Quinones have been the subject of much interest for a number of years due to their various biological activities. In 1946 Wendel[1] showed that certain 2-hydroxy-3-alkyl-naphthoquinones inhibited the growth of *Plasmodium*. Further studies proved that the toxicity of naphthoquinones to *Plasmodium sp*. is due to interaction with the mitochondrial respiratory chain[2]. This observation led Fieser and collaborators to start an extensive search for new quinones[3] aiming to discover new drugs for malaria chemotherapy. These resulted in the discovery of 3-(8-

* e-mail: cegvito@vm.uff.br

cyclohexyl-octyl-2-hydroxy-1,4-naphthoquinone (menoctone), a potent inhibitor of NADH and succinate-cytochrome reductases of *Plasmodium lophurae*[4]. The antibacterial and antiprotozoan activities of 2-hydroxy-3-alkyl-1,4-naphthoquinones have been summarized by several authors[5,6]. Besides these biological activities, various other quinones posses activities against several types of cancer cells[7] (*i.e.* mitomycins, anthracyclines, *etc.*), virus[8,9] and fungi[10].

Among the naphthoquinones, lapachol (**1**) and many heterocyclic derivatives have been investigated during the past years, mainly due to their antibacterial[11], antifungal[12] and anticancer[13] activities. More recently, β-lapachone, an isomer of lapachol, was intensely investigated for clinical use in cancer chemotherapy[14].

It is well known that there is a relationship between the side chain attached to 2-hydroxy-1,4-naphthoquinone and its toxic effects on several microorganisms[15,16]. Fieser and Richardson showed that, as the isoalkyl side chain of hydrolapachol is lengthened by the insertion of more methylene groups the activity against *P. lophurae* in the duck increased, reached a maximum with a C9-side chain, and then fell off[17]. Stern *et al.* found that the hydrogen peroxide formation of several 1,4-naphthoquinone derivatives on blood cell metabolism decreased with the increasing alkyl chain length[18]. As the alkyl side chain is an important factor for the activity of 2-hydroxy-1,4-naphthoquinone, it seems appropriate to study the antimicrobial activity of naphthoquinone derivatives having new side chains linked to the quinone moiety.

The present work reports the synthesis and antibacterial activities of 3-hydroxy-2-hydrazino-1,4-naphthoquinone derivatives **2** (Figure 1) compared with **1** and other clinical antibiotics.

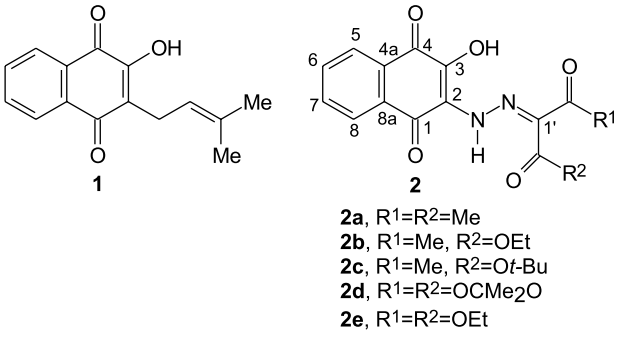
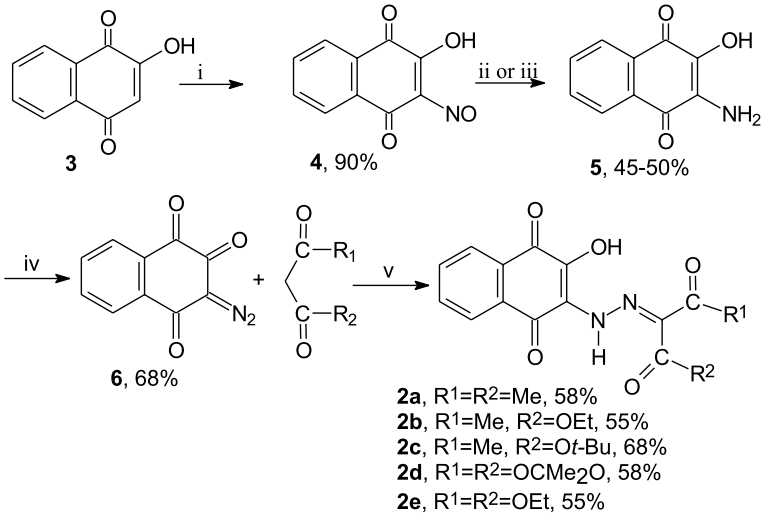


Figure 1. Lapachol (**1**) and hydrazino-1,4-naphthoquinone derivatives **2**.

Results and Discussion

The synthesis of 3-hydroxy-2-hydrazino-1,4-naphthoquinone derivatives **2a-e** was performed by a modified process previously reported (Scheme 1)[19]. Our synthesis of **2a-e** started with lawsone **3** (3-hydroxy-1,4-naphthoquinone) which was converted into nitroso-quinone **4** by nitrosation. Compound **4** was reduced with sodium dithionate in aqueous medium giving a moderate yield of 2-amino-3-hydroxy-1,4-naphthoquinone **5**[20]. Reduction of **4** using sodium borohydride in methanol produced **5** in similar yield. Diazotisation of the latter compound was



Scheme 1. Synthetic route used for preparing hydrazino-naphthoquinone derivatives **2a-e**.

effected using sodium nitrite in hydrochloric acid leading to 3-diazo-naphthalene-1,2,4-trione (**6**). Addition of 1,3-dicarbonyl enolates to the diazo function of **6** gave the hydrazino-1,4-naphthoquinones **2a-e**. These compounds were synthesized from lawsone **3** in 4 steps with overall yields varying from 16 to 20%. Each step is operationally convenient and reproducible and the structures of **2a-e** were supported by NMR data based on HMBC and HMQC experiments.

Table 1 reports the inhibition zones (mm) of **2a**, **2b**, **2e**, **1** and DMSO (solvent) determined for three species of Gram-positive and six Gram-negative bacteria, *S. aureus*, *Staphylococcus epidermidis* and the nonfermentable Gram-negative bacillus showed inhibition zones ranging from 9 to 24 mm, where significant response requires ≥ 12 mm. Other Gram-negatives and *Enterococcus faecalis* had no significant result (halo < 12 mm). Therefore, our derivatives were active against four Gram-positive strains, but only against one Gram-negative strain. On the other hand, while **1** exhibited no positive response (halo < 12 mm), DMSO was completely inactive (halo = 0).

Table 1. Antimicrobial activity of **2a**, **2b**, **2e** and **1** as determined by diffusion techniques.

Microorganism	Gram	Inhibition zone (mm)				
		2a	**2b**	**2e**	Lapachol (**1**)	DMSO
Nonfermentable Gram-negative bacillus		12	12	16	10	0
Enterobacter sp	-	0	0	0	0	0
Enterococcus faecalis	-	0	0	9	0	0
Escherichia coli	+	0	0	0	0	0
Klebsiella sp	-	0	0	0	10	0
Proteus mirabilis	-	0	0	0	11	0
Pseudomonas aeruginosa	-	0	0	0	0	0
Staphylococcus aureus 001	+	14	15	24	0	0
S. aureus 003	+	0	10	9	0	0
S. aureus 004	+	13	12	17	0	0
S. aureus ATCC 29.213[a]	+	10	9	12	0	0
Staphylococcus epidermidis	+	11	15	16	0	0

[a]*S. aureus* ATCC 29.213: oxacillin = 45 mm and vancomycin = 24 mm.

The determinations of the minimal inhibitory concentrations (MIC) in clinical *S. aureus* strains are reported in Table 2. These results indicated that **2e** has a greater activity than **1**, but it is less active than oxacillin and vancomycin, which are currently used as clinical antibiotics.

Table 2. Minimal inhibitory concentration (MIC) for **2e** and **1**.

Microorganism (*Staphylococcus)*	**2e** (μg mL^{-1})	Lapachol (**1**) (μg mL^{-1})
S. aureus 001*	64	256
S. aureus 002	128	256
S. aureus 003	128	256
S. aureus 004*	64	128
S. aureus 005*	128	256
S. aureus 006	128	256
S. aureus 007*	128	256
S. aureus 009*	128	256
S. aureus 010	128	256
S. aureus 012	128	256
S. aureus 013	128	256
S. aureus 015	128	256
S. aureus 033*	128	256
S. aureus 047*	128	256
S. aureus 048*	128	256
S. aureus ATCC 25.923	128	256
S. aureus ATCC 29.213	128	128

*MRSA – Methicillin-resistant *S. aureus*. MICs for *S. aureus ATCC 29.213*: oxacillin = 0.25 mg mL^{-1} and vancomycin = 2 mg mL^{-1}.

Effects on the culture's optical density

The effects on the culture's optical density at 560 nm for *S aureus* strains, were also studied. Cultures in the logarithimic growthphase were exposed to compound **2e** and vancomycin, both at 2xMIC, during a 6 h treatment, with measurements being made at each hour. As is shown in Figure 2, **2e** and vancomycin exhibited similar responses, despite the lower MIC of vancomycin.

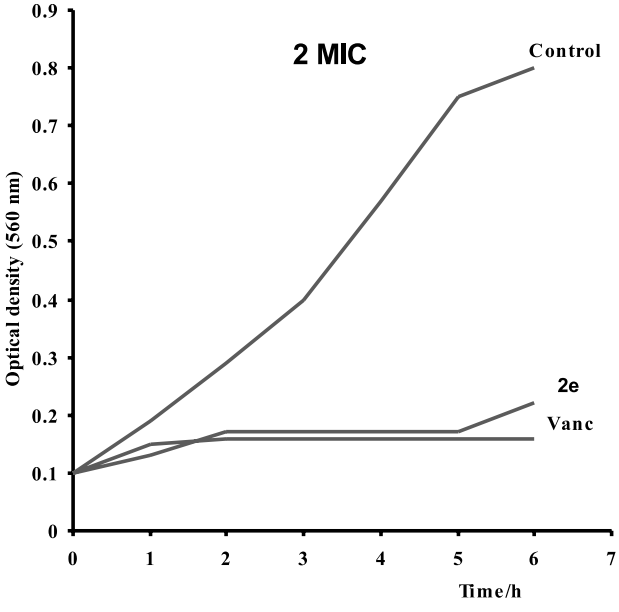


Figure 2. Effects of **2e** and vancomycin on optical density of *S. aureus* cultures in exponential growth at 37 °C (MIC for **2e** = 64 μg mL^{-1}).

Conclusion

Our study revealed a new class of naphthoquinone derivatives with high antibacterial activity. The antibacterial activity of **2a**, **2b** and **2e** compared with **1** allow the conclusion that, at the level of the preliminary susceptibility testing in disk, they were active mainly against Gram-positive bacteria. The minimal inhibitory concentration (MIC) determination on *S. aureus* for **2e** showed an activity twofold greater than **1**. However, **2e** and **1** had MICs much greater than some clinical antibiotics. The optical density measurement for *S. aureus* indicated that **2e** at 2xMIC had almost the same activity as vancomycin despite their different MICs.

Experimental

General

NMR and HRMS confirmed the structures of the substances. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity Plus VXR spectrometer operating at 300 and 75 MHz respectively, with tetramethylsilane as internal standard. Low resolution electron-impact mass spectra (12 eV) were obtained using a Hewlett Packard 5985 instrument and high resolution fast atom bombardment mass spectra (HRFABMS) were recorded in a 3-NBA matrix in the positive ion mode on a VG ZAB-E mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer 783 spectrophotometer. Melting points were observed on a Reichert micro hotstage and are uncorrected. The solvents used were of analytical grade. Column chromatography was performed with silica gel 60 (Merck 70-230 mesh). Merck silica gel F254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with a 25% aqueous solution of ammonium sulfate, followed by heating. Solvents were dried using the appropriate drying agents and then distilled directly before use[21]. Freshly purified samples were used for measurement of physical constants and spectral data. 2-hydroxy-1,4-naphthoquinone was purchased from Aldrich Chemical Co and used without purification.

*2-Hydroxy-3-nitroso-1,4-naphthoquinone (**3**)*

A mixture of lawsone **3** (1 g, 5.75 mmol) in HCl 5% (v/v, 7.5 mL) under stirring was dissolved in dioxane (20 mL). The solution was cooled externally with ice and solid sodium nitrite (1.16 g, 16.8 mmol) was slowly added keeping the temperature below 5 ºC. The reaction was monitored by TLC until complete consumption of **3**. The reaction was allowed to warm to room temperature and extracted with dichloromethane (3 x 20 mL). The combined organic phase was extracted with cold water (3 x 15 mL) and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure yielding **4** (1.16 g, 90%) as a yellow crystalline solid; mp 169 ºC (CH_2Cl_2); IR ν_{max}/cm^{-1} 3310 (OH), 1690, 1665 (C=O), 1530 (C-N=O) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (1H, dd, *J* 7.6 and 1.1 Hz, H_5), 8.20 (1H, dd, *J* 7.6 and 1.1 Hz, H_8), 7.93 (1H, dt, *J* 7.6 and 1.4 Hz, H_6), 7.84 (1H, dt, *J* 7.6 and 1.4 Hz, H_7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1 (C1), 159.2 (C2), 136.0 (C3), 183.0 (C4), 132.3 (C4a), 126.3 (C5), 135.0 (C6), 132.8 (C7), 126.1 (C8), 130.6 (C8a); MS *m/z* (relative abundance %) $M^{+\cdot}$ 203 (20), 172 (72), 104 (100), 76 (48).

*2-Hydroxy-3-amino-1,4-naphthoquinone (**5**)*

A stirred solution of **4** (1 g, 4.90 mmol) in ethanol (10 mL) was warmed to 50 ºC then a freshly prepared solution of $Na_2S_2O_4$ 10% (w/v, 15 mL) was added slowly in three portions of 5 mL. The mixture changed from yellow to deep purple and after 15 min a solid started to form. The reaction was kept undisturbed at room temperature for 24 h. The solid material was collected by vacuum filtration and air dried giving **5** (465 mg, 50%) as a purple solid; mp 132 ºC (EtOH) (lit. 130-140 ºC)[20]; IR ν_{max}/cm^{-1} 3480 (OH), 3390 (NH_2), 1660,1650 (C=O) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (1H, dd, *J* 6.9 and 3.3 Hz, H_5), 8.05 (1H, dd, *J* 6.9 and 3.3 Hz, H_8), 7.62 (1H, dt, *J* 6.9 and 2.4 Hz, H_6), 7.72 (1H, dt, *J* 6.9 and 2.4 Hz, H_7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0 (C1), 135.9 (C2), 133.2 (C3), 181.4 (C4), 130.3 (C4a), 125.1 (C5), 132.5 (C6), 133.8 (C7), 124.9 (C8), 131.0 (C8a).

*3-Diazo-naphthalene-1,2,4-trione (**6**)*

To a solution of **5** (200 mg, 1.05 mmol) in HCl 5% (V/V, 4.0 mL) and dioxane (10 mL) was slowly added solid sodium nitrite (220 mg, 3.19 mmol) at 0 °C during 1 h. The mixture was stirred for an additional 30 min and extracted with dichloromethane (2 x 20 mL). The combined organic phase was washed with an aqueous solution of $NaHCO_3$ 5% (3 x 5 mL), dried over Na_2SO_4 and concentrated under reduced pressure producing a yellow solid which was crystallized from carbon tetrachloride yielding **6** (143 mg, 68 %); mp 121-122 °C (CCl_4) (lit.123 °C)[22]; IR ν_{max}/cm^{-1} 2140 (N=N), 1690, 1670, 1630 (C=O) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (1H, dd, *J* 6.0 and 2.4 Hz, H_5), 8.25 (1H, dd, *J* 6.0 and 2.4 Hz, H_8), 7.88

(1H, dt, *J* 6.6 and 1.5 Hz, H_7), 7.81 (1H, dt, *J* 6.6 and 1.5 Hz, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.2 (C1), 172.1 (C2), 148.6 (C3), 176.5 (C4), 132.6 (C4a), 126.2 (C5), 135.0 (C6), 134.5 (C7), 128.1 (C8), 133.1 (C8a); MS *m/z* (relative abundance %) $M^{+\circ}$ 200 (5), 172 (90), 104 (100), 76 (75).

*General procedure for preparing **2a-e***

A mixture of the appropriate dicarbonyl compound (0.38 mmol), anhydrous K_2CO_3 (57 mg, 0.4 mmol) in dry acetone (15 mL) was stirred for 15 min under a nitrogen atmosphere. To this mixture was added slowly through a syringe a solution of **6** (76.7 mg, 0.38 mmol) in dry acetone (5 mL) during 15 min. After stirring for 2 h the reaction was acidified with 5% (V/V) HCl (40 mL), the solid material was collected by filtration and crystallized from ethanol.

*2-[N'-(1-acetyl-2-oxo-propylidene)-hydrazino]-3-hydroxy-[1,4]naphthoquinone (**2a**)*

(66.7 mg, 58%); mp 195 °C (H_2O) (lit 206 °C)[19]; IR ν_{max}/cm^{-1} 1675, 1665, 1640, 1630 (C=O), 1500 (C=N) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16-8.12 (2H, m, H_5 and H_8), 7.78-7.75 (2H, m, H_6 and H_7), 2.45 (3H, s, $H_{3'}$), 2.66 (3H, s, H_3), 10.13 (1H, s, exchangeable with D_2O, OH), 14.50 (1H, s, exchangeable with D_2O, NH); ^{13}C NMR (75 MHz, DMSO) δ 179.3 (C1), 146.1 (C2), 122.5 (C3), 180.5 (C4), 130.1 (C4a), 125.9 (C5), 134.6 (C6), 134.8 (C7), 125.9 (C8), 130.2 (C8a), 134.1 (C=N), 196.5 (MeC=O), 26.3 (MeC=O), 196.7 (MeC=O), 31.4 (MeC=O); MS *m/z* (relative intensity, %) $M^{+\circ}$ 300 (35), 258 (100), 189 (65), 105 (25), 76 (20); HRFABMS found: 301.0832. Calcd for $[M+H]^+$ $C_{15}H_{13}O_5N_2$: 301.0824.

*Ethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-oxo-butyrate (**2b**)*

(69.3 mg, 55%); mp 163 °C (H_2O) (lit. 160-161 °C)[19]; IR ν_{max}/cm^{-1} 1720, 1675, 1665, 1645 (C=O), 1500 (C=N) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15-8.08 (2H, m, H_5 and H_8), 7.75-7.72 (2H, m, H_6 and H_7), 2.69 (3H, s, H_3), 4.34 (2H, q, *J* 7.2 Hz, H_3), 1.40 (3H, t, *J* 7.2 Hz, H_4), 14.00 (1H, s, exchangeable with D_2O, NH); ^{13}C RMN (75 MHz, $CDCl_3$) δ 179.1 (C1), 145.7 (C2), 122.3 (C3), 178.4 (C4), 129.5 (C4a), 126.1 (C5), 134.0 (C6), 134.1 (C_7), 126.6 (C8), 130.8 (C8a), 124.9 (C=N), 196.9 (MeC=O), 30.9 (MeC=O), 162.2 (COOE), 61.6 (OCH$_2$Me), 14.1 (OCH$_2$Me); MS *m/z* (relative intensity, %) $M^{+\circ}$ 330 (55), 288 (100), 188 (70), 104 (50), 76 (30); HRFABMS Found: 331.093. Calcd for $[M+H]^+$ $C_{16}H_{15}O_6N_2$: 331.0930.

*t-Butyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-oxo-butyrate (**2c**)*

(93.8 mg, 68%); mp 162 °C; IR ν_{max}/cm^{-1} 1740, 1680, 1690, 1640 (C=O), 1520 (C=N) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15-8.08 (2H, m, H_5 and H_8), 7.75-7.72 (2H, m, H_6 and H_7), 2.67 (3H, s, H_3), 1.59 (9H, s, H_4), 12.48 (1H, s, exchangeable with D_2O, OH), 14.20 (1H, s, exchangeable with D_2O, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1 (C1), 145.7 (C2), 122.5 (C3), 178.5 (C4), 129.5 (C4a), 126.1 (C5), 133.9 (C6), 134.1 (C7), 126.6 (C8), 130.8 (C8a), 123.0 (C=N), 197.1 (MeC=O), 31.1 (MeC=O), 161.4 (t-BuOC=O), 82.9 (Me$_3$C), 28.0 (Me$_3$C); MS *m/z* (relative intensity, %) 360 (10), 303 (45), 190 (55), 152 (60), 135 (98), 119 (100); HRFABMS Found: 359.1247. Calcd for $[M+H]^+$ $C_{18}H_{16}O_6N_2$: 359.1243.

*5-[(3-Hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-2,2-dimethyl[1,3]dioxane-4,6-dione (**2d**)*

(66.7 mg, 58%); mp 205 °C; IR ν_{max}/cm^{-1} 1740, 1690, 1670, 1650 (C=O), 1540 (C=N) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17-8.11 (2H, m, H_5 and H_8), 7.98-7.93 (2H, m, H_6 and H_7), 1.86 (3H, s, H_4); ^{13}C NMR (75 MHz, DMSO) δ 180.6 (C1), 148.3 (C2), 121.7 (C3), 178.7 (C4), 130.3 (C4a), 126.0 (C5), 134.1 (C6), 134.8 (C7), 126.1 (C8), 130.4 (C8a), 114.5 (C=N), 173.8 (C=O), 105.6 (OC(Me)$_2$O), 27.1 (C(Me)$_2$); HRFABMS Found: 345.0722. Calcd. for $[M+H]^+$ $C_{16}H_{13}O_7N_2$: 345.0722.

*Diethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-malonate (**2e**)*

(76 mg, 55%); mp 184 °C (lit. 186 °C)[19]; IR ν_{max}/cm^{-1} 1740, 1670, 1650 (C=O), 1540 (C=N) (KBr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15-8.06 (2H, m, H_5 and H_8), 7.77-7.70 (2H, m, H_6 and H_7), 4.46 (2H, q, *J* 7.2 Hz, H_3), 4.34 (2H, q, *J* 7.2 Hz, H_3), 1.44 (3H, t, *J* 7.2 Hz, H_4), 1.38 (3H, t, *J* 7.2 Hz, H_4), 11.15 (1H, s, OH), 12.86 (1H, s, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1 (C1), 143.9 (C2), 120.4 (C3), 178.6 (C4), 130.6 (C4a), 125.9 (C5), 134.0 (C6), 133.7 (C7), 126.4 (C8), 129.3 (C8a), 122.2 (C=N), 160.2 (C=O), 61.6 (OCH$_2$Me), 13.7 (OCH$_2$Me); MS *m/z* (relative intensity, %) $M^{+\cdot}$ 360 (55), 268 (100), 104 (80), 76 (45); HRFABMS Found: 361.1040. Calcd for $[M+H]^+$ $C_{17}H_{17}O_7N_2$: 361.1035.

Microbial cultures growth conditions

Tested microorganisms included the following Gram-positive bacteria: *Enterococcus faecalis*, *Staphylococcus*

aureus and *Staphylococcus epidermidis*, and for Gram-negative: *Enterobacter*, *Escherichia coli*, *Klebsiella* sp, nonfermentable bacillus, *Proteus mirabilis* and, *Pseudomonas aeruginosa*. All bacteria used in this study were isolated from patients at the University Hospital Antônio Pedro/UFF-RJ and grown (at 37 °C) in medium with peptone, yeast extract, sodium chloride and, dibasic-sodium phosphate. Lorian disks (7 mm diameter) were soaked in 5 mg mL^{-1} of substances (**2a-e**) as solutions in dimethylsulfoxide (DMSO). Disks were put on an exponentially growing plated culture with appropriate dilution to $1.0x10^7$ colony forming unit (CFU mL^{-1}). The plates were then incubated for 24 h at 37 °C. The results were recorded by measuring the zones surrounding the disk. Control disks containing DMSO, ATCC 29.213 of *S. aureus* and the antibiotics oxacillin and vancomycin were used as controls in the assay. Significant results: halo ≥ 12 mm.

Minimal inhibitory concentration (MIC)

The hydrazino-quinone **2e** was tested by using the two fold serial dilution in agar. 1 mL of DMSO/compound solution was added to 19 mL of the medium at 55-60 °C in order to reach a final concentration from 512 to 1 μg mL^{-1}. These solutions were plated and after medium solidification, a drop of *S. aureus* in the logarithmic phase (10^5 CFU mL^{-1}) was added. The plates were incubated at 37 °C for 24 h, before being read. MIC was defined as the lowest compound concentration preventing visible bacterial growth. All strains were tested at least in duplicate in four separate experiments. MICs of the reference compound used in this study were similar to those found in other reports[23,24].

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