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Original article

Synthesis using microwave irradiation and antibacterial evaluation of new *N*,*O*-acetals and *N*,*S*-acetals derived from 2-amino-1,4-naphthoquinones

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ABSTRACT

This paper describes a novel series of *N*,*O*-acetals and *N*,*S*-acetals (**7a**–**o**) derived from 2-amino-1,4-naphthoquinones that were synthesized and evaluated as potential antimicrobial agents. These compounds were obtained in good yields using microwave irradiation, and several of them showed promising antibacterial profiles. Three of our biologically active 2-amino-1,4-naphthoquinone *N*,*O*-acetals and *N*,*S*-acetals tested against hospital bacterial strains were identified as potential lead compounds. Characterization of all compounds was performed using one-dimensional NMR techniques (^{1}H, ^{13}C-APT), IR spectra, elemental analyses and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS).

1. Introduction

Naphthoquinones are important natural substances that are commonly found throughout different families of plants, fungi and certain animals [1]. This class of compounds has been studied extensively for their antitumor [2], molluscicidal [3], antiparasitic [4], leischmanicidal [5], anti-inflammatory [6], antifungal [7], antimicrobial [8] and trypanocidal [9] properties. Reports indicate that the biological activity of these molecules is derived from their *ortho*- or *para*-quinonoid moieties, which accept one or two electrons (redox cycling) to form the corresponding radical anion or dianion species *in situ* [10]. The resultant semi-quinone radicals accelerate intracellular hypoxic conditions by producing superoxide anions [11,12]. By this mechanism, quinones may be cytotoxic in mammalian cells by affecting such enzymes as topoisomerases, a group of enzymes that are critical for DNA replication [13].

By introducing amino substituents onto the quinone moiety, its redox properties can be tuned to induce oxidative stress in cells and to alkylate DNA. Such aminonaphthoquinones are found in important bioactive compounds, such as antitumor (**1**) [14], antimalarial (**2**) [15] and antibacterial agents (**3**) [16]. Recently, our research group described the synthesis of diethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-hydrazono]-malonate (**4**), which shows promising antibacterial activity [17] (Fig. 1).

2. Results and discussion

2.1. Synthesis

The aim of this work was to synthesize a new series of *N*,*O*-acetals and *N*,*S*-acetals derived from 2-amino-1,4-naphthoquinones and to evaluate their antimicrobial properties. *N*,*O*-Acetals reported by Barluenga and co-workers [18] were unstable compounds at room temperature and needed to be stored at −18 °C to avoid decomposition. In this study, we describe a methodology that employs microwave irradiation to obtain *N*,*O*-acetals and *N*,*S*-acetals in moderate to good yields.

In the first step, 1,4-naphthoquinone (**5a**) or 2-methyl-1,4-naphthoquinone (**5b**) was reacted with sodium azide in acetic acid to produce 2-amino-1,4-naphthoquinone (**6a**) [19] and 2-amino-3-methyl-1,4-naphthoquinone (**6b**) [20], respectively, according to a previous report.

Compounds **6a** and **6b** were converted readily into their corresponding aminonaphthoquinone derivatives (**7a**–**o**). Treatment

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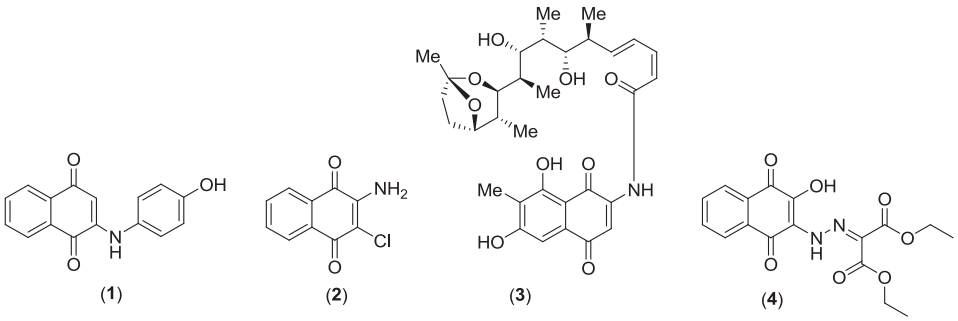


Fig. 1. Examples of bioactive aminonaphthoquinones.

with paraformaldehyde in the presence of selected alcohols or thiols under microwave irradiation afforded the *N*,*O*-acetals and *N*,*S*-acetals in moderate yields (Scheme 1). The aim of the use of heat microwave is that solvents can be applied at higher temperatures than usual since an increased pressure limit can be achieved and also low-absorbing solvents can be used efficiently in microwave protocols without any problems.

N,*O*-Acetals **7a**–**g** were prepared by the condensation of compound **6a** with paraformaldehyde using the corresponding alcohols simultaneously as both solvent and reactant (route I). Sulfur derivatives *N*,*S*-acetals **7h**–**o** were obtained by the condensation of compounds **6a**, **b** with paraformaldehyde and various thiols in chloroform (route II).

By utilizing this synthetic route, it was possible to prepare 2-amino-1,4-naphthoquinone *N*,*O*-acetals and *N*,*S*-acetals **7a**–**o** in moderate to good yields. The reactions were performed in a sealed glass vessel. These compounds were characterized using spectroscopic methods, such as ^{1}H and ^{13}C NMR.

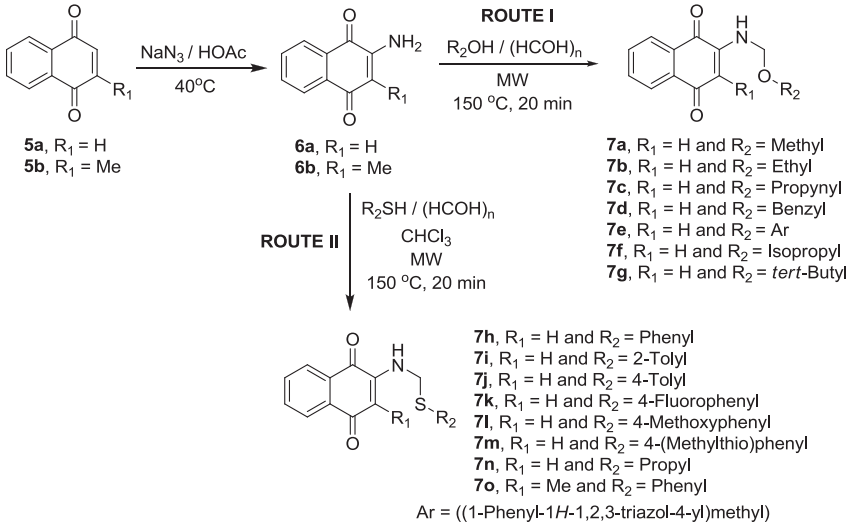
These synthetic compounds are the first examples of stable *N*,*O*-acetals and *N*,*S*-acetals derived from naphthoquinones and are notably promising for biological activity screenings.

2.2. Antibacterial activity

Due to the increasing antibiotic resistance of various bacterial strains, primarily in hospital environments, new alternatives to conventional antibiotics are needed [21,22]. To that end, we evaluated our new 2-amino-1,4-naphthoquinone *N*,*O*-acetal and *N*,*S*-acetals series against several bacterial strains, including a control strain with susceptibility to several known antibiotics (*Staphylococcus ATCC* 25923) and seven hospital strains [five gram-positive strains (*Staphylococcus aureus*, methicillin-resistant *S. aureus* – MRSA, oxacillin-resistant *S. aureus* – ORSA, and oxacillin-resistant staphylococci – *Staphylococcus epidermidis* ORS and *Staphylococcus haemolyticus* ORS) and two gram-negative strains (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*)] (Tables 1 and 2).

Initially, we screened our *N*,*O*-acetal and *N*,*S*-acetals series against all bacterial strains with the disc diffusion method. Interestingly, three compounds (**7a**, **7b** and **7c**) showed an active profile against almost all of the strains tested (Table 1). This antibacterial spectrum is advantageous as it reveals biological activity against multi-resistant strains of medical importance [23]. Importantly, none of 2-amino-1,4-naphthoquinone-*N*,*S*-acetals, **7**(**h**–**o**) showed antibacterial activity.

According to our results, only *N*,*O*-acetals **7**(**a**–**g**) were effective against the bacteria evaluated herein including gram-positive (staphylococci) and gram-negative strains. The *S. aureus* strains was the most susceptible to the new derivatives, whereas the *K. pneumoniae* was the least affected. This in accord to the current literature that reports more antibacterial lead compounds against gram-positive than against gram-negative bacteria. This phenomenon is generally due to the cellular organization of gram-negative bacteria that includes an outer lipid bilayer membrane. Known antibiotics, such as aminoglycosides, tetracyclines and chloramphenicol, and several newer ones are prevented from entering into these bacteria due to the presence of enzyme-containing



Scheme 1. Synthesis of 2-amino-1,4-naphthoquinone *N*,*O*-acetals and *N*,*S*-acetals **7a**–**o**.

Table 1
Antibacterial profile of the active compounds from 2-amino-1,4-naphthoquinone *N,O*-acetals and *N,S*-acetals series against hospital multi-resistant bacterial strains on disc diffusion assays.

Bacterial strain	Inhibition growth zone (Halo = mm)[a]		
	7a	**7b**	**7c**
Staphylococcus aureus ATCC 25923	17	15	16
Staphylococcus aureus	24	18	20
Staphylococcus aureus MRSA	16	14	16
Staphylococcus aureus ORSA	18	17	12
Staphylococcus epidermidis ORS	14	10	10
Staphylococcus haemolyticus ORS	20	16	18
Klebsiella pneumoniae	17	0	0
Pseudomonas aeruginosa	0	14	18

[a] Ciprofloxacin (Halo = 22 mm) and vancomycin (16–19 mm) were used as positive controls for gram-negative and gram-positive bacteria, respectively.

periplasmic space, the peptide glycans layer and the polysaccharides in the membrane surface mainly associate with the efflux pump resistance mechanism [24]. These features increase bacterial resistance to antibiotics and reiterate the need for finding new molecules that are active against gram-negative strains.

The inhibitory results from some of the biologically active derivatives (24 mm) were greater than those observed for the positive control, which was vancomycin (16–19 mm). The biological profiles of the three active derivatives (**7a**–**c**) correlate with the nature of their R_2 substituents (**7a**, R_2 = methyl, **7b**, R_2 = ethyl and **7c**, R_2 = propynyl), especially when contrasted with the inactive derivatives (**7d**–**g**, route I and **7h**–**n**, route II).

Because the disk diffusion test is a qualitative assay [25,26], these derivatives were also submitted to a minimal inhibitory concentration (MIC) determination assay (Table 2). Notably, the MIC values for the active compounds ranged from 4 to 64 μg/mL against the gram-positive bacteria, which are close to the reported values of some commercially available antibiotics.

According to the literature, the emergence of resistant *Staphylococcus* species has increased continually, including the methicillin-resistant and oxacillin-resistant *S. aureus* (MRSA and ORSA, respectively) and oxacillin-resistant staphylococci (ORS). These bacterial strains cause several diseases ranging from soft tissue infections to life-threatening diseases such as toxic shock syndrome, endocarditis and necrotizing pneumonia [27].

The quantitative assay data confirmed that active derivatives **7a**–**c** significantly affect these *Staphylococcus* resistant strains with substituent-dependent biological profiles. Derivative **7a** (R_2 = methyl) presented similar antibacterial effects against all staphylococci strains studied (MIC = 16 μg/mL), independent of the bacteria resistance profile. The only exception was for *S. epidermidis*, in which compound **7a** presented a more active profile (MIC = 8 μg/mL, Table 2). By changing R_2 = methyl (**7a**) to R_2 = ethyl (**7b**), the activity against non- or less-resistant strains was increased, leading to the lowest observed MIC values (4–8 μg/mL, Table 2). However, this substitution negatively affected the compound's activity against the multi-resistant bacterial strains (Table 2).

Finally, changing R_2 = methyl (**7a**) to R_2 = propynyl (**7c**) led to the highest observed *S. aureus* MIC values (64 μg/mL), suggesting adverse steric effects resulting from the large propynyl substituent. Despite this result, this compound is still effective against *P. aeruginosa*.

Importantly, **7a** and **7b** antimicrobial profile is biologically significant ($p \geq 0.005$) and suggests that these derivatives should be further explored for targeting a future use against *S. aureus*-related infections. This feasible use may allow other effective antibiotics, such as vancomycin, to be saved for using only against exceptional dangerous multi-resistant strains.

Our qualitative antibacterial data also revealed an active profile for compounds **7a**, **7b** and **7c** against *K. pneumoniae* or *P. aeruginosa*, two gram-negative bacteria. However the MIC assay revealed that compound **7c** is more active against *P. aeruginosa* (MIC = 16 μg/mL) than **7b** (MIC = 128 μg/mL) whereas **7a** showed a lower active profile against *K. pneumoniae* (MIC = 256 μg/mL) (Table 2). Due to the importance of these bacteria worldwide and the current need for more treatment options, further structural modification in **7c** may allow exploring and/or increasing its antimicrobial properties for future treatment purpose.

Many studies have shown that some antimicrobials are able to affect genes involved in the bacterial oxidative stress response by inducing the production of reactive oxygen species (ROS) [28,29]. The presence of the quinone group in certain derivatives is described as essential for the inhibitory activity on enzymes involved in ROS formation. For future perspective, additional studies to investigate the antibacterial effects of 2-amino-1,4-naphthoquinone *N,O*-acetals and their relationship with ROS formation [30] will be performed.

Table 2
Comparison of the minimal inhibitory concentration values of the active 2-amino-1,4-naphthoquinone *N,O*-acetals against various bacterial strains.

Strain	MIC (μg/mL)		
	7a	**7b**	**7c**
Staphylococcus aureus ATCC 25923	16	8	32
Staphylococcus aureus	16	4	32
Staphylococcus aureus MRSA	16	32	64
Staphylococcus aureus ORSA	16	32	256
Staphylococcus haemolyticus ORS	16	64	32
Staphylococcus epidermidis ORS	8	16	16
Klebsiella pneumoniae	256	≥256	≥256
Pseudomonas aeruginosa	≥256	128	16

3. Conclusion

Since their discovery in the twentieth century, antimicrobial agents have been responsible for significant reduction of worldwide bacterial infection rates [31,32]. Currently, the urgent worldwide need for new bacterial outbreak treatment options warrants the need for further exploration of the new derivatives described herein. Three of our biologically active 2-amino-1,4-naphthoquinone *N,O*-acetals tested against hospital bacterial strains were identified as potential lead compounds that can be explored to generate new and more active antibiotics. At the same time, it is necessary to evaluate these compounds' biological activity against additional bacterial strains to assess their ability to treat other threatening bacteria. Further development of the biological profiles of these compounds may help to emphasize their potential as lead compounds for treating a large variety of bacterial infections.

4. Experimental section

Melting points were determined with a Fisher-Johns instrument and are uncorrected. Infrared (IR) spectra were recorded on an ABB FTLA2000-100 spectrophotometer using KBr pellets. NMR spectra, unless otherwise stated, were obtained in Me_2SO-d_6 or $CDCl_3$ with a Varian Unity Plus 300 MHz spectrometer. Chemical shifts (δ) are expressed in ppm and the coupling constants (J) in Hertz. Flash column chromatography purification was performed on silica gel from Acros. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel pre-coated F_{254} plates from Merck. Microanalyses were performed on a Perkin–Elmer Model 2400 instrument, and all values were within ±0.4% of the

calculated composition values. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed in positive ion mode on a Waters-Micromass Q-Tof Micro instrument. Compounds **7a**–**o** were prepared using an Anton Paar Monowave 300 microwave reactor.

4.1. Chemistry

4.1.1. General procedures for the preparation of amino-1,4-naphthoquinone acetals *7a–g*

A 10 mL microwave tube was loaded with **6a** (1.4 mmol), paraformaldehyde (4 mmol), the appropriate alcohol (4.5 mmol) and were irradiated for 20 min. The internal temperature reached 150 °C. The alcohol excess was evaporated under reduced pressure. The residual solid product was purified by column chromatography on silica gel and eluted with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1). For the product **7e** was used 5 mL of chloroform as solvent.

*4.1.1.1. 2-(Methoxymethyl)-amino-1,4-naphthoquinone (**7a**).* Obtained as a yellow solid (232 mg, 74%); mp: 115–116 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.35$ (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 6.09 (s, 1H, H-6), 7.61–7.67 (m, 1H, H-1 or H-4), 7.70–7.76 (m, 1H, H-1 or H-4), 8.05–8.11 (m, 2H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 55.4$ (CH_3), 74.5 (CH_2), 104.5 (C-6), 126.2 (C-1 and C-4), 130.4 (C-4a), 132.3 (C-2 and C-3), 133.0 (C-8b), 134.7 (C-2 and C-3), 147.3 (C-7), 181.7 (C=O), 183.5 (C=O) ppm; IR (KBr): $\tilde{\nu}$ 3341, 1681, 1611, 1571 cm^{-1}; HRMS (ESI) $[M + H]^+$. Found: 218.0807. Calc. for $C_{12}H_{12}NO_3^+$: 218.0812. Anal. Calcd. for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.04; H, 5.50; N, 6.31.

*4.1.1.2. 2-(Ethoxymethyl)-amino-1,4-naphthoquinone **7b**.* Obtained as a yellow solid (157 mg, 47%); mp: 134–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.22$ (t, 3H, CH_3, $J = 14.1$), 3.54 (q, 2H, $J = 14.1$, CH_2), 4.73 (s, 1H, CH_2), 6.09 (s, 1H, H-6), 7.60–7.66 (m, 1H, H-1 or H-4), 7.70–7.76 (m, 1H, H-1 or H-4), 8.04–8.10 (m, 2H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.9$ (CH_3), 63.4 (CH_2), 72.9 (CH_2), 104.2 (C-6), 126.2 (C-1 and C-4), 130.4 (C-4a), 132.3 (C-2 and C-3), 133.0 (C-8b), 134.6 (C-2 and C-3), 147.3 (C-7), 181.7 (C=O), 183.5 (C=O) ppm; IR (KBr): $\tilde{\nu}$ 3273, 1679, 1625, 1542 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 232.0967. Calc. for $C_{13}H_{14}NO_3^+$: 232.0968. Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.58; H, 6.04; N, 5.76.

*4.1.1.3. 2-((Prop-2-ynyloxy)methyl)-amino-1,4-naphthoquinone **7c**.* Obtained as a yellow solid (73 mg, 21%; mp: 126–127 °C); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.50$ (s, 1H, CH), 4.19 (s, 2H, CH_2), 4.87 (s, 1H, CH_2), 6.14 (s, 1H, H-6), 7.61–7.66 (m, 1H, H-1 or H-4), 7.70–7.76 (m, 1H, H-1 or H-4), 8.04–8.10 (m, 2H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 54.4$ (O–CH_2–C), 71.4 (CH_2), 73.6 (C≡CH), 75.5 (C≡CH), 104.9 (C-6), 126.2 (C-1 and C-4), 130.4 (C-4a), 132.3 (C-2 or C-3), 132.9 (C-8b), 134.7 (C-2 or C-3), 147.1 (C-7), 181.6 (C=O), 183.5 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3365, 1677, 1608, 1515 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 242.0812. Calc. for $C_{14}H_{12}NO_3^+$: 242.0812. Anal. Calcd. for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81. Found: C, 69.47; H, 4.49; N, 5.77.

*4.1.1.4. 2-(Benzyloxymethyl)-amino-1,4-naphthoquinone **7d**.* Obtained as a yellow solid (191 mg, 45%); mp: 149–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.57$ (s, 2H), 4.80 (s, 2H), 6.13 (s, 1H, H-6), 7.31–7.36 (m, 5H, H-2′, H-3′, H-4′, H-5′ and H-6′), 7.62–7.67 (m, 1H, H-1 or H-4), 7.71–7.77 (m, 1H, H-1 or H-4), 8.05–8.11 (m, 2H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 69.7$ (CH_2), 72.4 (CH_2), 104.6 (C-6), 126.2 (C-1 and C-4), 127.8 (C-2′ and C-6′), 128.0 (C-4′), 128.5 (C-3′ and C-5′), 130.4 (C-4a), 132.3 (C-2 and C-3), 133.0 (C-8b), 134.6 (C-2 and C-3), 137.0 (C-1′), 147.2 (C-7), 181.7 (C=O), 183.5 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3340, 1681, 1597, 1570 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 294.1128. Calc. for $C_{18}H_{16}NO_3^+$: 294.1125. Anal. Calcd. for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.33; H, 5.47; N, 4.45.

*4.1.1.5. 2-(((1-Phenyl-1H-1,2,3-triazol-4-yl)methoxy)methyl)-amino-1,4-naphthoquinone **7e**.* Obtained as a yellow solid (114 mg, 22%); mp: 145–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.33$ (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 5.96 (s, 1H, H-6), 7.45–7.50 (m, 1H, H-4′), 7.55–7.61 (m, 2H, H-3′ and H-5′), 7.73–7.77 (m, 1H, H-1 or H-4), 7.81–7.87 (m, 3H, H-2′, H-6′, H-1 or H-4), 7.98–8.01 (m, 2H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 59.9$ (CH_2), 71.9 (CH_2), 103.2 (C-6), 119.9 (C-10), 125.2 (C-2′ and C-6′), 125.8 (C-1 and C-4), 128.5 (C-4′), 129.7 (C-3′ and C-5′), 130.2 (C-4a), 132.4 (C-8b), 134.6 (C-2 and C-3), 136.4 (C-1′), 144.7 (C-9), 148.1 (C-7), 181.3 (C=O), 182.2 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3340, 1681, 1597, 1570 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 361.1297. Calc. for $C_{20}H_{17}N_4O_3^+$: 361.1295. Anal. Calcd. for $C_{20}H_{16}N_4O_3$: C, 66.66; H, 4.48; N, 15.55. Found: C, 66.69; H, 4.64; N, 15.24.

*4.1.1.6. 2-(Isopropoxymethyl)-amino-1,4-naphthoquinone **7f**.* Obtained as a yellow solid (223 mg, 63%); mp: 125–126 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.20$ (d, 6H, $J = 6.4$, CH_3), 3.77 (hep, 1H, $J = 6.4$, CH), 4.75 (s, 2H, CH_2), 6.07 (s, 1H, H-6), 7.61–7.65 (m, 2H, H-1 or H-4), 7.72–7.74 (m, 2H, H-1 or H-4), 8.04–8.10 (m, 2H, H-2 and H-3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 22.1$ (CH_3), 69.2 (CH), 71.1 (CH_2), 104.1 (C-6), 126.2 (C-1 and C-4), 130.4 (C-4a), 133.1 (C-8b), 134.6 (C-2 and C-3), 147.3 (C-7), 181.7 (C=O), 183.5 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3249, 1677, 1604, 1573 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 246.1129. Calc. for $C_{14}H_{16}NO_3^+$: 246.1125. Anal. Calcd. for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.32; H, 6.49; N, 5.39.

*4.1.1.7. 2-(tert-Butoxymethyl)-amino-1,4-naphthoquinone **7g**.* Obtained as a yellow solid (113 mg, 30%); mp: 104–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.28$ (s, 9H), 4.75 (s, 2H, CH_2), 6.07 (s, 1H, H-6), 7.61–7.64 (m, 2H, H-1 or H-4), 7.70–7.73 (m, 2H, H-1 or H-4), 8.03–8.10 (m, 2H, H-2 and H-3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 27.9$ (CH_3), 67.1 (C), 74.1 (CH_2), 103.5 (C-6), 126.2 (C-1 and C-4), 130.4 (C-4a), 133.2 (C-8b), 134.5 (C-2 and C-3), 147.2 (C-7), 181.7 (C=O), 183.4 (C=O) ppm. IR (KBr) $\tilde{\nu}$ 3361, 1673, 1603, 1572 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 260.1280. Calc. for $C_{15}H_{18}NO_3^+$: 260.1281. Anal. Calcd. for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.13; H, 6.44; N, 5.57.

4.1.2. General procedures for the preparation of amino-1,4-naphthoquinone acetals *7h–o*

A 10 mL microwave tube was loaded with **6a** or **6b** (1.4 mmol), paraformaldehyde (4 mmol), the appropriate thiol (1.5 mmol) and chloroform (5 mL) and were irradiated for 20 min. The internal temperature reached 150 °C. The solvent was evaporated under reduced pressure. The residual solid product was purified by column chromatography on silica gel and eluted with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1).

*4.1.2.1. 2-(Phenylthiomethyl)-amino-1,4-naphthoquinone **7h**.* Obtained as a yellow solid (404 mg, 95%); mp: 196–197 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.62$ (s, 2H, CH_2), 5.88 (s, 1H, H-6), 7.32–7.33 (m, 3H, H-3′, H-4′ and H-5′), 7.44–7.46 (m, 2H, H-2′ and H-6′), 7.62–7.65 (m, 1H, H-1 or H-4), 7.73–7.76 (m, 1H, H-1 or H-4), 8.03–8.04 (m, 1H, H-2 or H-3), 8.09–8.11 (m, 1H, H-2 or H-3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 48.4$ (CH_2), 104.1 (C-6), 126.2 (C-1 and C-4), 128.3 (C-4′), 129.3 (C-3′ and C-5′), 130.4 (C-4a), 132.9 (C-2′ and C-6′), 133.2 (C-8b), 134.7 (C-2 and C-3), 146.1 (C-7), 181.5 (C=O), 183.0 (C=O) ppm. IR (KBr) $\tilde{\nu}$ 3340, 1677, 1624, 1571 cm^{-1}; HMRS (ESI) $[M + H]^+$. Found: 296.0738. Calc. for $C_{17}H_{14}NO_2S^+$: 296.0740. Anal. Calcd. for

$C_{17}H_{13}NO_2S$: C, 69.13; H, 4.44; N, 4.74. Found: C, 69.58; H, 4.84; N, 4.45.

4.1.2.2. 2-(2-Tolylthiomethyl)-amino-1,4-naphthoquinone ***7i***. Obtained as a yellow solid (195 mg, 46%); mp: 179–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.43 (s, 3H, C$\underline{H}_3$), 4.58 (s, 2H, C$\underline{H}_2$), 5.89 (s, 1H, H-6), 7.15–7.17 (m, 1H, H-5′), 7.20–7.23 (m, 2H, H-4′ and H-6′), 7.39–7.41 (m, 1H, H-3′), 7.62–7.65 (m, 1H, H-1 or H-4), 7.72–7.76 (m, 1H, H-1 or H-4), 8.02–8.04 (m, 1H, H-2 or H-3), 8.10–8.12 (m, 1H, H-2 or H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.8 (C$\underline{H}_3$), 47.6 (C$\underline{H}_2$), 104.0 (C-6), 126.2 (C-4′), 126.8 (C-5′), 126.8 (C-1 and C-4), 128.5 (C-6′), 130.4 (C-4a), 130.7 (C-3′), 132.2 (C-1′), 133.4 (C-8b), 134.7 (C-2 and C-3), 140.7 (C-2′), 146.2 (C-7), 181.5 (C=O), 183.0 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3351, 1681, 1620, 1569 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 310.0911. Calc. for $C_{18}H_{16}NO_2S^+$: 310.0896. Anal. Calcd. for $C_{18}H_{15}NO_2S$: C, 69.88; H, 4.89; N, 4.53. Found: C, 69.89; H, 5.11; N, 4.17.

4.1.2.3. 2-(4-Tolylthiomethyl)-amino-1,4-naphthoquinone ***7j***. Obtained as a yellow solid (165 mg, 39%); mp: 185–186 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.32 (s, 3H, C$\underline{H}_3$), 4.56 (s, 2H, C$\underline{H}_2$), 5.87 (s, 1H, H-6), 7.10–7.14 (m, 1H, H-3′ and H-5′), 7.33–7.37 (m, 1H, H-2′ and H-6′), 7.62–7.65 (m, 1H, H-1 or H-4), 7.72–7.76 (m, 1H, H-1 or H-4), 8.03–8.11 (m, 2H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.1 (C$\underline{H}_3$), 48.8 (C$\underline{H}_2$), 104.0 (C-6), 129.2 (C-1′), 129.8 (C-1 and C-4), 130.4 (C-4a), 130.7 (C-2′ and C-6′), 132.2 (C-3′ and C-5′), 133.2 (C-8b), 134.7 (C-2 and C-3), 138.7 (C-4′), 146.1 (C-7), 181.5 (C=O), 182.9 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3413, 1680, 1624, 1572 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 310.0911. Calc. for $C_{18}H_{16}NO_2S^+$: 310.0896. Anal. Calcd. for $C_{18}H_{15}NO_2S$: C, 69.88; H, 4.89; N, 4.53. Found: C, 69.53; H, 5.11; N, 4.18.

4.1.2.4. 2-((4-Fluorophenylthio)methyl)-amino-1,4-naphthoquinone ***7k***. Obtained as a yellow solid (247 mg, 61%); mp: 199–200 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.56 (s, 2H, C$\underline{H}_2$), 5.88 (s, 1H, H-6), 7.01–7.05 (m, 2H, H-3′ and H-5′), 7.43–7.47 (m, 2H, H-2′ and H-6′), 7.63–7.66 (m, 1H, H-1 or H-4), 7.73–7.76 (m, 1H, H-1 or H-4), 8.03–8.05 (m, 1H, H-2 or H-3), 8.10–8.12 (m, 1H, H-2 or H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 49.1 (C$\underline{H}_2$), 104.3 (C-6), 116.6 (C-3′ and C-5′, J = 21.5), 126.3 (C-1 and C-4), 127.9 (C-1′), 130.4 (C-4a), 133.2 (C-8b), 134.9 (C-2 and C-3), 135.9 (C-2′ and C-6′, J = 8.8), 146.0 (C-7), 163.1 (C-4′, J = 249.4), 181.5 (C=O), 182.9 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3253, 1680, 1623, 1572 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 314.0647. Calc. for $C_{17}H_{13}FNO_2S^+$: 314.0646. Anal. Calcd. for $C_{17}H_{12}FNO_2S^+$: C, 65.16; H, 3.86; N, 4.47. Found: C, 65.51; H, 3.37; N, 4.48.

4.1.2.5. 2-((4-Methoxyphenylthio)methyl)-amino-1,4-naphthoquinone ***7l***. Obtained as a yellow solid (412 mg, 89%); mp: 140–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 3.78 (s, 3H, C$\underline{H}_3$), 4.50 (s, 2H, C$\underline{H}_2$), 5.86 (s, 1H, H-6), 6.84 (d, 2H, J = 5.4, H-2′ and H-6′), 7.39 (d, 2H, J = 5.4, H-3′ and H-5′), 7.61–7.65 (m, 1H, H-1 or H-4), 7.72–7.75 (m, 1H, H-1 or H-4), 8.02–8.04 (m, 1H, H-2 or H-3), 8.09–8.11 (m, 1H, H-2 or H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.4 (C$\underline{H}_2$), 55.3 (C$\underline{H}_3$), 103.4 (C-6), 114.9 (C-3′ and C-5′), 123.1 (C-1′), 126.2 (C-1 and C-4), 130.4 (C-4a), 132.1 (C-2′ and C-6′), 133.2 (C-8b), 134.7 (C-2 and C-3), 146.1 (C-7), 160.3 (C-4′), 181.5 (C=O), 182.9 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3258, 1680, 1624, 1571 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 326.0853. Calc. for $C_{18}H_{16}NO_3S^+$: 326.0845. Anal. Calcd. for $C_{18}H_{15}NO_3S$: C, 66.44; H, 4.65; N, 4.30. Found: C, 66.61; H, 4.82; N, 3.93.

4.1.2.6. 2-((4-(Methylthio)phenylthio)methyl)-amino-1,4-naphthoquinone ***7m***. Obtained as a yellow solid (380 mg, 78%); mp: 176–177 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.46 (s, 3H, C$\underline{H}_3$), 4.57 (s, 2H, C$\underline{H}_2$), 5.87 (s, 1H, H-6), 7.19 (d, 2H, J = 8.4, H-2′ and H-6′ or H-3′ and H-5′), 7.36 (d, 2H, J = 8.4, H-2′ and H-6′ or H-3′ and H-5′), 7.60–7.66 (m, 1H, H-1 or H-4), 7.71–7.76 (m, 1H, H-1 or H-4), 8.03–8.06 (m, 1H, H-2 or H-3), 8.10–8.12 (m, 1H, H-2 or H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4 (C$\underline{H}_3$), 48.9 (C$\underline{H}_2$), 104.1 (C-6), 126.2 (C-1 and C-4), 126.9 (C-2′ and C-6′ or C-3′ and C-5′), 128.7 (C-2′ and C-6′ or C-3′ and C-5′), 130.4 (C-4a), 132.2 (C-4′), 133.2 (C-8b), 134.7 (C-2 and C-3), 140.0 (C-1′), 146.0 (C-7), 181.5 (C=O), 182.9 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3252, 1679, 1624, 1571 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 342.0619. Calc. for $C_{18}H_{16}NO_2S_2^+$: 342.0617. Anal. Calcd. for $C_{18}H_{15}NO_2S_2$: C, 63.32; H, 4.43; N, 4.10. Found: C, 63.32; H, 4.51; N, 3.75.

4.1.2.7. 2-(Propylthiomethyl)-amino-1,4-naphthoquinone ***7n***. Obtained as a yellow solid (262 mg, 70%); mp: 140–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.98–1.01 (m, 3H, C$\underline{H}_3$), 1.63–1.66 (m, 2H, H-10), 2.58–2.61 (m, 2H, H-9), 4.33 (s, 2H, C$\underline{H}_2$), 5.87 (s, 1H, H-6), 7.62–7.64 (m, 1H, H-1 or H-4), 7.72–7.75 (m, 1H, H-1 or H-4), 8.05–8.11 (m, 2H, H-2 and H-3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.4 (C$\underline{H}_3$), 22.9 (C-10), 33.5 (C-9), 44.7 (C$\underline{H}_2$), 103.2 (C-6), 126.2 (C-1 and C-4), 130.4 (C-4a), 133.3 (C-8b), 134.7 (C-2 and C-3), 146.6 (C-7), 181.6 (C=O), 182.9 (C=O) ppm; IR (KBr) $\tilde{\nu}$ 3278, 1678, 1622, 1571 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 262.0901. Calc. for $C_{14}H_{16}NO_2S^+$: 262.0896. Anal. Calcd. for $C_{14}H_{15}NO_2S$: C, 64.34; H, 5.79; N, 5.36. Found: C, 64.57; H, 5.89; N, 5.25.

4.1.2.8. 2-(Phenylthiomethyl)-amino-3-methyl-1,4-naphthoquinone ***7o***. Obtained as a yellow solid (124 mg, 30%); mp: 104–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.19 (s, 3H, C$\underline{H}_3$), 4.94 (s, 2H, C$\underline{H}_2$), 7.26–7.28 (m, 3H, H-3′, H-4′ and H-5′), 7.42–7.44 (m, 2H, H-1 or H-4), 7.61–7.64 (m, 1H, H-1 and H-4), 7.69–7.72 (m, 1H, H-1 and H-4), 7.99–8.01 (m, 1H, H-2 and H-3), 8.09–8.10 (m, 1H, H-2 and H-3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.2 (C$\underline{H}_3$), 51.9 (C$\underline{H}_2$), 117.1 (C-6), 126.2 (C-1 and C-4), 128.3 (C-4′), 129.2 (C-3′ and C-5′), 130.3 (C-4a), 132.8 (C-1′), 132.9 (C-8b), 133.4 (C-2′ and C-6′), 134.1 (C-2 and C-3), 144.7 (C-7), 181.9 (C=O), 183.5 (C=O). IR (KBr) $\tilde{\nu}$ 3312, 1662, 1624, 1574 cm^{-1}; HMRS (ESI) [M + H]$^+$. Found: 310.0885. Calc. for $C_{18}H_{16}NO_2S^+$: 310.0896. Anal. Calcd. for $C_{18}H_{15}NO_2S$: C, 69.88; H, 4.89; N, 4.53. Found: C, 69.84; H, 4.79; N, 4.27.

4.2. Microbiological assays

The assays were performed according to the *Clinical and Laboratory Standards Institute* (CLSI) [33]. The antimicrobial disc diffusion assays included gram-positive (*Enterococcus faecalis*, *S. aureus*, *S. aureus* ORSA, *S. aureus* MRSA, *S. aureus* ATCC 25923, *S. epidermidis* ORS and *S. haemolyticus* ORS) and gram-negative (*K. pneumoniae* and *P. aeruginosa*) bacteria. All strains used in this study were from clinical isolates from the University Antônio Pedro Hospital.

The strains were grown briefly at 37 °C in Müeller–Hinton media, and 3 μL of a stock solution (5 mg/mL) of each derivative in dimethyl sulfoxide (DMSO) was placed in Whatman disks. The inocula used in the growth method were those with turbidity that was equal to a 0.5 McFarland standard. Filter paper disks, 5 mm in diameter, were placed on top of the plate containing exponentially growing plated cultures diluted to 1.0×10^7 colony-forming units (CFU/mL). These cultures were subsequently incubated for 18–24 h at 37 °C. Ciprofloxacin and vancomycin were used as positive controls, and DMSO was used as a negative control. The results were verified by measuring the inhibitory zones surrounding the disk.

Minimum inhibitory concentration (MIC) assays were performed using the broth macrodilution method. After 5 h of bacterial growth, the culture was diluted to obtain a concentration of 1.0×10^5 CFU/mL. Next, each compound was added to reach a final concentration ranging from 0.5 to 1024 μg/mL. The mixture was incubated at 37 °C for 18–24 h. The MIC was defined as the lowest compound concentration preventing visible bacterial growth. All

strains were tested at least three times, and ciprofloxacin (MIC = 1 μg/mL) and vancomycin (MIC = 2 μg/mL) were used as positive controls.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.01.010.

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