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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antibacterial and antifungal activities of naphthoquinone derivatives: a structure–activity relationship study

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Abstract The synthesis of 1,4-naphthoquinone derivatives is of great interest since these compounds exhibit strong activity as antimalarial, antibacterial, antifungal and anticancer agents. A series of 50 naphthoquinone derivatives was synthesized and evaluated for antibacterial and antifungal activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida krusei*, *Candida parapsilosis* and *Cryptococcus neoformans* using the broth microdilution method. The *Candida* species were the most susceptible microorganisms. Halogen derivatives of 1,4-naphthoquinone presented strong activity, e.g., 2-bromo-5-hydroxy-1,4-naphthoquinone, which exhibited inhibition at an MIC of 16 μg/mL in *S. aureus*, and 2-chloro-5,8-dihydroxy-1,4-naphthoquinone, with an MIC of 2 μg/mL in *C. krusei*. These compounds showed higher activity against fungi, but the antibacterial activities were very low. The study of structure–activity relationships is very important in the search for new antimicrobial drugs due to the limited therapeutic arsenal.

Keywords Naphthoquinone · Antifungal · Antibacterial · Minimum inhibitory concentration · Structure–activity relationship

Introduction

Naphthoquinones are natural pigments that are widely distributed in plants, fungi and some animals (Premalatha *et al*., 2012; Zhou *et al*., 2012; Yang *et al*., 2013). Naphthoquinones are structurally characterized by the presence of two carbonyl groups at the 1,4-positions and, less frequently, at the 1,2- or 1,3-positions of the naphthalene,

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which has ring (A) and ring (B) (Fig. 1). These compounds, when they occur naturally, have hydroxyl and/or methyl groups as substituents (Glazunov and Berdyshev, 2012). They are privileged structures in medicinal chemistry due to their characteristics, structural properties and biological activities on prokaryotic and eukaryotic cells. In most cases, the biological activity of naphthoquinones is related to their redox and acid–base properties, both of which can be modulated synthetically.

The synthesis of novel derivatives of 1,4-naphthoquinone is of particular interest since these compounds exhibit strong action as antimalarial, antibacterial, antifungal and anticancer agents (Koyama, 2010).

The mechanism of action of naphthoquinones could be due to their properties as oxidizing or dehydrogenation agents, in a similar way to hydrogen peroxide and superoxide radicals (Tran *et al*., 2004b; Pinto and de Castro, 2009). This behavior is related to the ability of quinones to accept one or two electrons to form highly reactive radical anion intermediates, which are responsible for the oxidative stress observed in the cells (Valderrama *et al*., 2008). However, several other mechanisms have been attributed to quinonoid compounds, including DNA intercalation, alkylation, induction of DNA strand breaks or the inhibition of special proteins or enzymes such as topoisomerases (Plyta *et al*., 1998).

Some naphthoquinones, such as juglone (5-hydroxy-1,4-naphthoquinone), lawsone (2-hydroxy-1,4-naphthoquinone) and plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), are present in plants, and their antibacterial effects on various species of aerobic and anaerobic organisms have been demonstrated (Lim *et al*., 2006; Sakunphueak and Panichayupakaranant, 2012; Bhattacharya *et al*., 2013).

Other naphthoquinones, such as toxins derived from naphthazarin (5,8-dihydroxy-1,4-naphthoquinone), which are produced by *Fusarium solani*, can attack plants, fungi and bacteria (Rohnert *et al*., 1998). Alkannin, its enantiomer shikonin and its derivatives are active against Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis/faecium*, *Escherichia coli* and *Pseudomonas aeruginosa* (Shen *et al*., 2002; Al-Mussawi, 2010). Some compounds show strong activity against fungi such as *Candida* sp. and filamentous fungi (Pawar *et al*., 2014). These activities depend on the position(s) of the substituents in the naphthoquinone ring (Figs. 1, 2).

In most studies, antibacterial and antifungal activities have been studied with not standardized methods. Best methods to achieve these activities are based by broth dilution antibacterial and antifungal susceptibility testing of bacteria that grow aerobically and yeasts. These methods are used in all microbiology laboratories around of the world. For this, the aim of our study was to test the antibacterial and antifungal activities of a number of naphthoquinone derivatives by these methods. Some compounds were commercially obtained, and other ones were synthesized in our laboratory to perform a structure–activity relationship study. The compounds are numbered in order to facilitate the discussion of their bioactivity rather than by their chemical nature.

Results and discussion

Fifty compounds were screened for activity against bacteria and yeast; 45 of these generated by parallel synthesis, and 5 purchased for comparison purposes.

Chemistry

Most of the naphthoquinones were prepared from **47**, which can easily be obtained from **38** by reduction with tin(II)chloride (Guerrero-Vásquez *et al*., 2013). Compounds **39**, **40** and **41** were also prepared from **38** by acetylation and methylation. Similarly, **37** was prepared using chlorohydroquinone and maleic anhydride rather than dimethoxybenzene and dichloromaleic anhydride (Bekaert *et al*., 1986). Compound **25** was obtained when the same reaction was carried out using 2-bromo-1,4-dimethoxybenzene (Scheme 1).

Oxidation of **47** with potassium superoxide produced compound **48** in good yield (92 %) (Lewis and Paul, 1977), whereas air oxidation in the presence of a solution of sodium hydroxide yielded **1** (93 %) (Lewis and Paul, 1977). Compound **1** was brominated with Br_2 in acetic acid to produce **43** and **44**, and acetylation of **43** afforded **46** (Horowska *et al*., 1988; Takeya *et al*., 1999; Tandon *et al*., 2005). Compound **42** was prepared by following the same strategy as for the preparation of **43** (Horowska *et al*., 1988; Takeya *et al*., 1999; Tandon *et al*., 2005).

Permethylation of **47** with dimethyl sulfate and potassium hydride gave **33** (Kawasaki *et al*., 1988). This compound was used to produce **32** by bromination with NBS (Bloomer and Zheng, 1998), **34** by formylation with $POCl_3$ in chloroform and **3** by oxidation with ceric ammonium nitrate (CAN) (Scheme 2) (Terada *et al*., 1987; Kawasaki *et al*., 1988). Bromination of **3** produced **45** (Huot and Brassard, 1974). Compounds **16** and **17** were obtained by similar strategies employing bromochloromethane and potassium carbonate for the formation of the methylenedioxy derivatives and NBS for the bromination (Dallaeker *et al*., 1983).

Compounds **20**, **34**, **35** and **36** were obtained by the methodology described for the synthesis of the potent phytotoxic agent naphthotectone (Guerrero-Vásquez *et al*.,

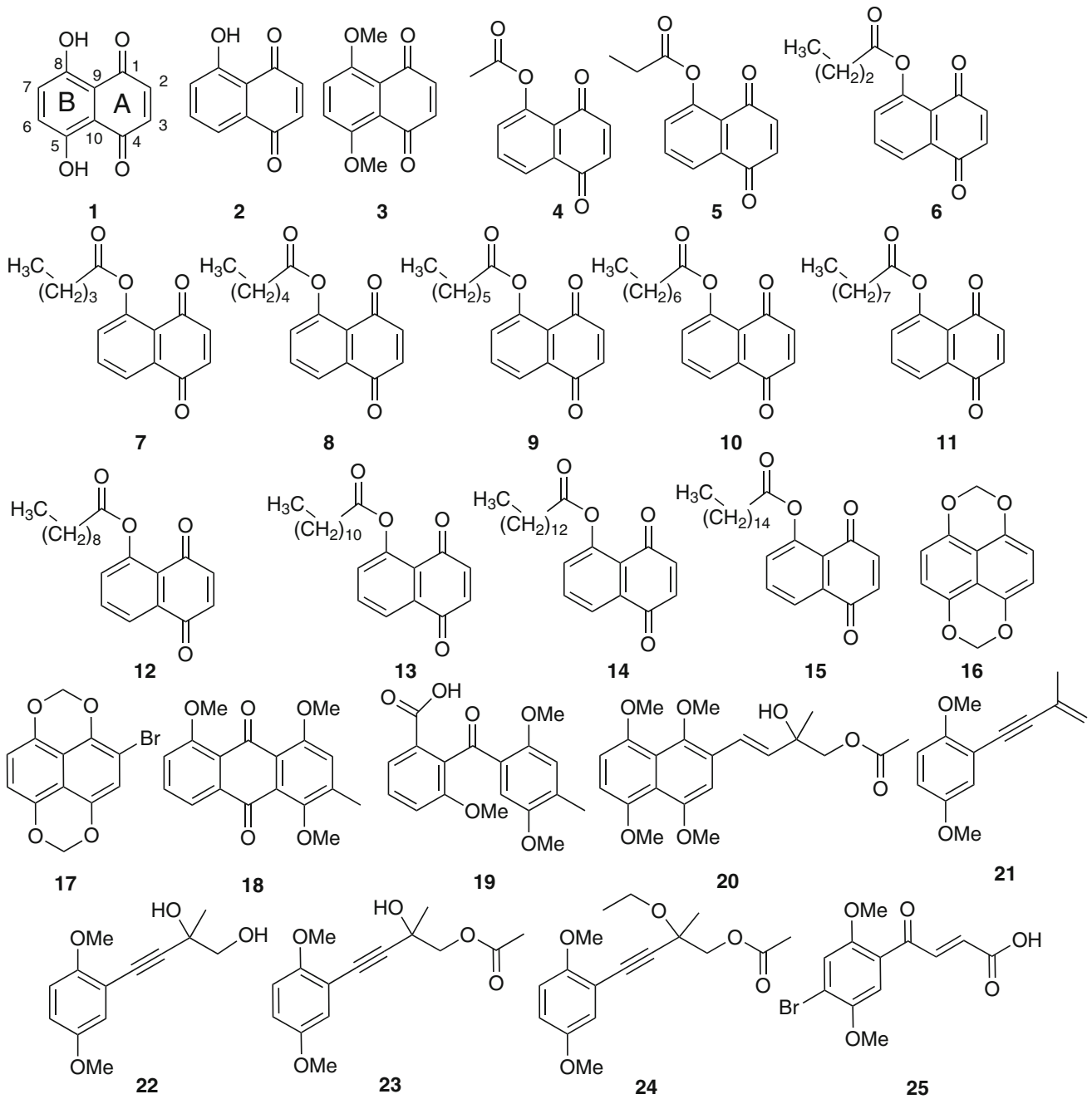


Fig. 1 Natural and synthetic naphthoquinones and derivatives tested (**1**–**25**)

2013). Compounds **21**–**24** were obtained as described for the synthesis of speciosins G and P (Guerrero-Vásquez *et al*., 2014). Acyl derivatives of juglone were obtained using standard conditions; acetylation was carried out using acetic anhydride and pyridine, and the other 5-acetyl-juglone derivatives **5**–**15** were prepared with 4-dimethylaminopyridine (DMAP) as base in reactions with the corresponding acid chloride.

Compounds 2, 29, 39, 49 and 50 were obtained from Sigma-Aldrich

All derivatives were characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR), infrared spectroscopy and high-resolution mass spectrometry (HRMS). The spectroscopic data for the compounds are consistent with the assigned structures (see "Experimental" section).

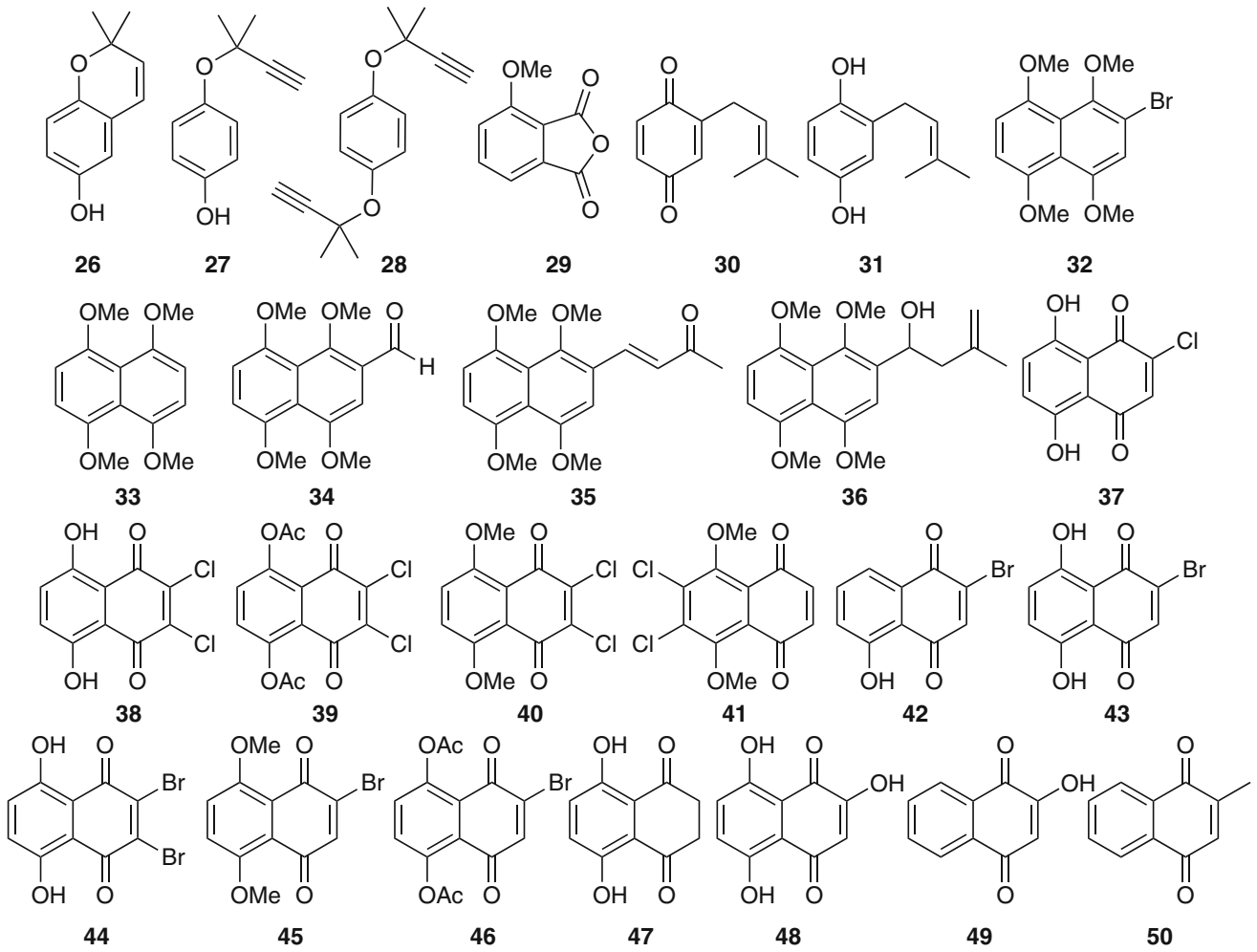
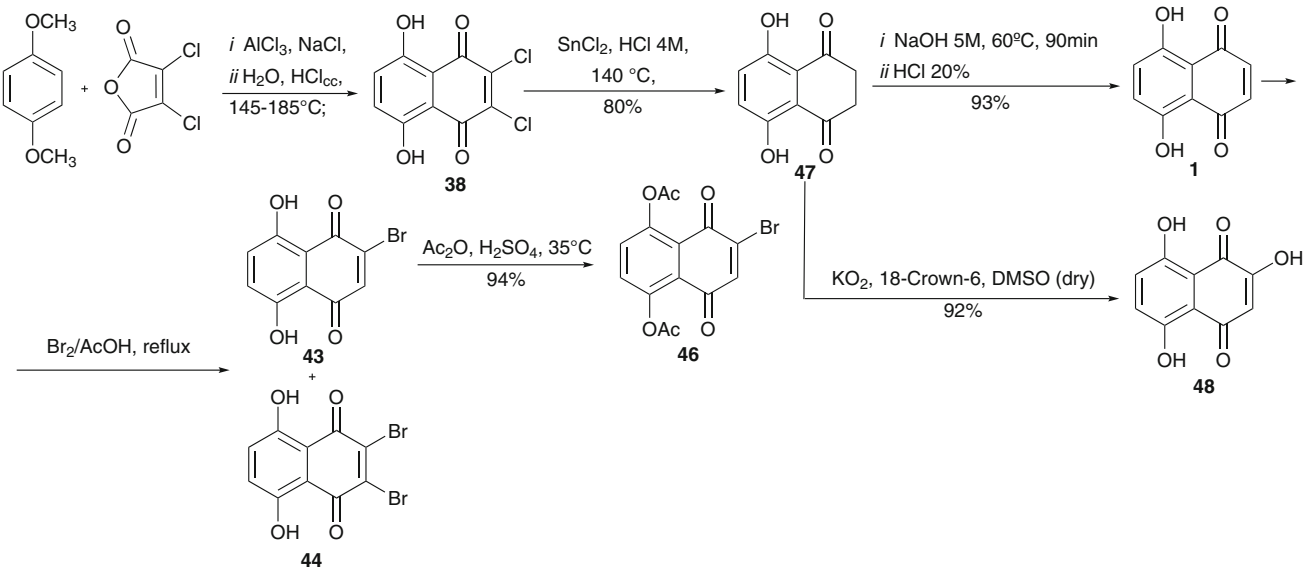
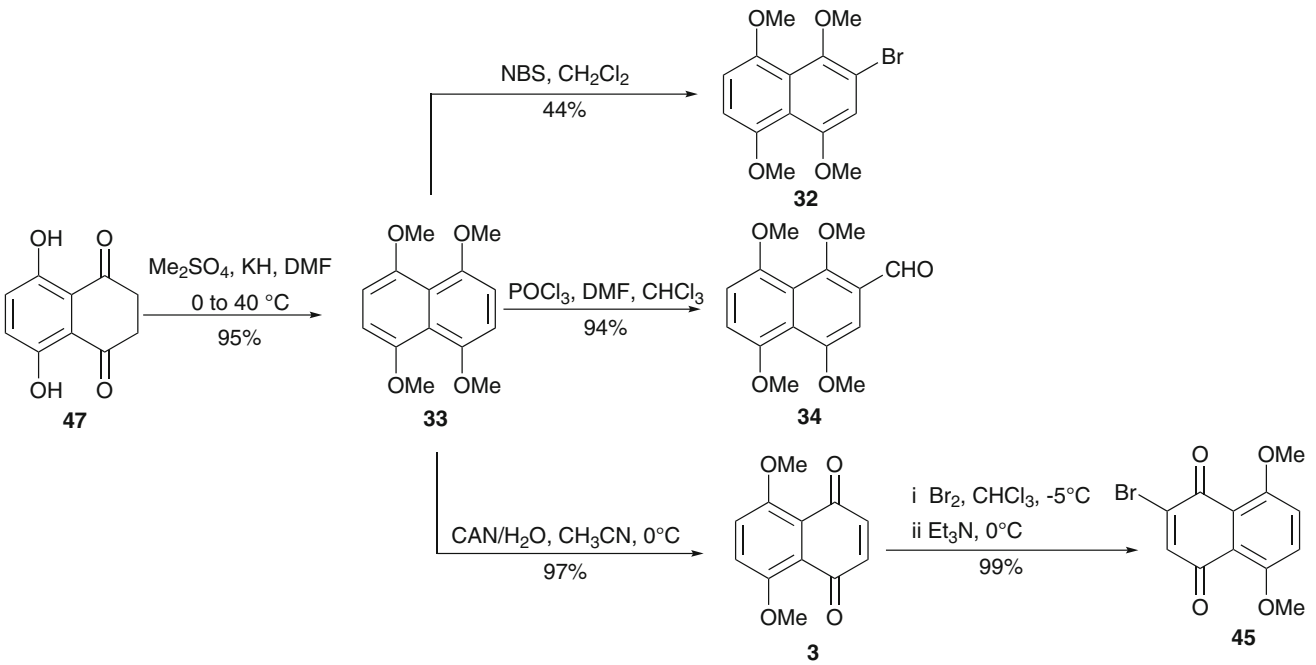


Fig. 2 Naphthoquinone derivatives tested (**26**–**50**)



Scheme 1 General procedure for the preparation of naphthoquinone derivatives



Scheme 2 Procedure for the preparation of methoxylated derivatives of naphthoquinones

Biological evaluation

All of the compounds were screened by the broth microdilution method for their antibacterial activity against two Gram-positive bacteria, *E. faecalis* and *S. aureus*, and two Gram-negative bacteria, *E. coli* and *P. aeruginosa*. The compounds were also evaluated for their in vitro antifungal activity against *Candida krusei*, *Candida parapsilosis* and *Cryptococcus neoformans*. We did not test *Candida albicans* because this one has an antifungal susceptibility profile very similar to *C. parapsilosis*. The lowest concentrations of the compounds that prevented visible growth are listed in Table 1.

It was determined that the solvent did not have antibacterial or antifungal activities against any of the test microorganisms. Gentamicin, doxycycline and amphotericin B were used as standard drugs, and these were also tested under similar conditions for comparison as positive controls. The minimum inhibitory concentrations (MIC) of the synthesized compounds against highly inhibited organisms are listed in Table 1.

Some of the tested compounds were very active against *Candida* sp., showed moderate antifungal activity against *C. neoformans* and were inactive or less active against all other test organisms. *P. aeruginosa* was the least susceptible microorganism as only 6 compounds (**4**, **6**, **7**, **37**, **38** and **42**) exhibited activity at ≥MIC 64 μg/mL. Only compound **42** showed moderate antibacterial activity against *S. aureus* at MIC 16 μg/mL. *C. krusei* was the most susceptible fungus in the study, with an MIC value of 2 μg/mL. This behavior is significant because *C. krusei* possesses an intrinsic resistance to many triazole antifungal drugs, especially fluconazole, which is the main drug used in antifungal therapy (Yadav *et al*., 2012).

SAR for naphthoquinone derivatives

In order to carry out a structure–activity relationship study, a number of naphthoquinone derivatives and related compounds were prepared and tested. These compounds comprise a variety of functionalization and structural modifications that allowed us to establish structural requirements for activity.

Influence of hydroxyl and methoxy groups at the C5 and/or C8 positions

Several authors have reported that the presence of hydroxyl groups in the B ring has a significant effect on activity, particularly in compounds with hydroxyl groups at the C5 and C8 positions. In these compounds, the antimicrobial and antifungal activities were twice as high as in those compounds with only one hydroxyl group (Tran *et al*., 2004b; Yakubovskaya *et al*., 2009a; Hughes *et al*., 2011). In our study, naphthazarin (**1**) showed antimicrobial activity against *E. coli*, *E. faecalis* and *S. aureus* with MIC values between 64 and 128 μg/mL. In yeasts, the MIC values were 32–128 μg/mL. Nevertheless, juglone (**2**)

Table 1 Antimicrobial activity (MIC profiles) of the synthesized compounds

Compounds	Antibacterial activity				Antifungal activity		
	E. coli	*P. aeruginosa*	*E. faecalis*	*S. aureus*	*C. krusei*	*C. parapsilosis*	*C. neoformans*
Minimum inhibitory concentration (MIC) (μg/mL)							
1	64	–	128	128	32	64	128
2	–	–	–	–	32	128	–
3	–	–	–	–	4	8	16
4	128	128	256	64	16	16	32
5	–	–	–	–	8	16	32
6	64	64	128	32	4	8	16
7	128	256	–	256	16	64	64
8	256	–	–	–	64	128	256
9	–	–	–	–	256	256	512
10	–	–	–	–	64	128	256
11	–	–	–	–	128	128	–
12	–	–	–	–	256	256	–
37	64	128	128	32	2	4	8
38	64	128	64	128	8	8	4
39	64	–	128	32	4	8	16
40	–	–	256	256	8	8	64
41	–	–	128	64	256	–	–
42	128	128	128	16	8	8	8
43	64	–	64	64	8	8	16
44	64	–	–	64	4	8	8
45	256	–	256	256	64	128	128
46	–	–	–	–	8	8	16
47	–	–	–	–	4	16	8
48	–	–	64	64	–	–	–
50	128		128	64	16	64	16
Gentamicin	0,5	1	–	0,5	–	–	–
Doxycycline	–	–	4	–	–	–	–
Amphotericin B	–	–	–	–	2	2	4
Control	–	–	–	–	–	–	–

showed antifungal activity against *C. krusei* and *C. parapsilosis* with MICs of 32 and 128 μg/mL, respectively, while it was inactive against *C. neoformans* and all bacteria tested. Antifungal and antibacterial activities have been reported previously in these compounds. For example, naphthazarin was active against *E. coli* and *S. aureus* (Yakubovskaya *et al*., 2009b) and other fungi such as *Saccharomyces carlsbergensis* (Yakubovskaya *et al*., 2009b), while Tandon *et al*. (2004) did not find in vitro antifungal activity against *C. albicans*, *C. neoformans*, *Sporothrix schenckii*, *Trichophyton mentagrophytes*, *Microsporum canis* and *Aspergillus fumigatus*. Juglone is one of the most widely studied naphthoquinones. In some studies, this compound did not demonstrate good antimicrobial activity and it was also inactive against the bacteria *P. aeruginosa*, *S. aureus* and the fungus *C. albicans* (Pawar *et al*., 2012; Sreelatha *et al*., 2014a). However, juglone did exhibit very good activity against the filamentous fungi *Aspergillus niger*, *Paecilomyces variotii*, *Trametes versicolor* and *Gloeophyllum trabeum* (Yang *et al*., 2009). These results are consistent with ours, although the activity against filamentous fungi was not tested in this work.

Methoxylation at the C5 and/or C8 positions converted **1** to 5,8-dimethoxy-1,4-naphthoquinone (**3**), which was inactive against bacteria but very active against yeasts. In these microorganisms, compound **3** had an MIC $\leq$ 16 μg/mL, i.e., eight times lower than that of **1**. This structural change is very important, since it demonstrates that the presence of hydroxyl groups in the naphthoquinone ring improves the cytotoxicity on normal cells, whereas the permethylated products of naphthazarin and its derivatives showed slightly lower cytotoxicity in this respect (Zhou

et al., 2011). This compound was more active than compounds **1** and **2** against the yeasts tested.

Hydrophilicity versus lipophilicity

The level of activity may be influenced by excessive hydrophilicity or lipophilicity, as in compounds that contain substituents with 10 or more carbon atoms (Riffel *et al*., 2002). Among the series of compounds tested, from 5-acetyl-juglone (**4**) to 5-palmitoyl-juglone (**15**), the structures progressively changed by an increase by 1 carbon atom at the C5 position. This increase in the number of carbon atoms resulted in the loss of antimicrobial activity. In bacteria, 5-heptanoyl-juglone (**9**), with 7 carbons at the C5 position, was the first compound that was inactive. In yeast, 5-lauroyl-juglone (**13**) was inactive and this compound has 12 carbons in the same position. 5-Butanoyl-juglone (**6**) was the most active against bacteria and fungi. The activity of these compounds could be influenced by the partition coefficient or log *P*, which is a ratio of concentrations of non-ionized compounds between two solutions. The log *P* value is used in the study of quantitative structure–property relationships as a measure of lipophilicity (Leo *et al*., 1971). Log *P* values for the series of compounds tested were between 1.51 (**4**) and 7.16 (**15**). MIC values in the range 4–32 mg/L for yeasts correspond to log *P* values between 1.51 (**4**) and 2.26 (**6**). Log *P* values >3 were associated with inactive compounds and low activity compounds such as **7**–**15**. This trend is consistent with other compounds in this study, where log *P* values of <1, e.g., compound **49** (0.99), and close to or >3, e.g., compounds **32**–**36**, were inactive against all microorganisms tested.

Number of rings

Number of rings can affect antibacterial and antifungal activities. So, structures with more than two rings did not show any antimicrobial activity, including methylenedioxy-naphthalene (**16**), 2-bromo-methylenedioxy-naphthalene (**17**) and 1,4,8-trimethoxy-3-methyl-anthraquinone (**18**). This finding is supported by the results reported by Tran *et al*. (2004a) who found that addition of other rings led to a marked loss of activity. They also showed that compounds without a quinone structure were less active, as is the case with our compounds (from **19** to **31**), where structures differed from the 1,4-naphthoquinone core.

Influence of carbonyl groups at the C1 and C4 positions

Previous studies showed that the biological activity of 1,4-naphthoquinone and its derivatives are affected and/or modulated by the presence of 1,4-substituents (Tran *et al*., 2004a). The presence of two carbonyl groups at the C1 and C4 positions is essential for antimicrobial activity, since the loss of both groups makes the molecule inactive (Verma and Hansch, 2004). Moreover, the presence of two free keto groups at the C1 and C4 positions leads to a greater inhibitory activity than at the C1 and C2 positions (Tran *et al*., 2004a). In our study, it was found that all compounds that lack the carbonyl groups at these positions were inactive on the seven microorganisms tested, i.e., compounds **32**, **33**, **34**, **35** and **36**. The addition or removal of other substituents in these inactive compounds did not improve their antimicrobial activity.

Halogen-substituted 1,4-naphthoquinones

The addition of halogen groups in the structure of naphthoquinone can produce a marked improvement in the antimicrobial and antifungal activity. The activity of these compounds has been explained as a short circuiting of the cell electron transfer normally executed by quinones (Holmes *et al*., 1964). The compounds with halogen groups in the nucleus had the lowest MIC values of all compounds in our study.

As far as the chloro-1,4-naphthoquinone derivatives are concerned, comparison of compound **1**, which does not have substituents, with 2-chloro-5, 8-dihydroxy-1,4-naphthoquinone (**37**) showed increase in antimicrobial and antifungal activity by a factor of four against *S. aureus* and by a factor of sixteen against all yeasts tested. However, the presence in 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (**38**) of two chloro-substituents in the A ring led to a decrease in the antimicrobial and antifungal activity, except on *C. neoformans*, which gave the lowest MIC value (≤4 μg/mL) in our study for this microorganism. These compounds also showed activity against *P. aeruginosa*, albeit very low in this case (MIC 128 μg/mL).

Methoxylation at C5 and C8, as in the change from 2,3-dichloro-5,8-dimethoxy-1,4-naphthoquinone (**40**) to compound **38**, did not improve the activity. In general, methoxylation led to lower antimicrobial activity, although the activity against *Candida* remained the same. Chlorination at positions C6 and C7, as in 6,7-dichloro-5,8-dimethoxy-1,4-naphthoquinone (**41**), led to the loss of activity.

Ethoxylation at the C5 and C8 positions, as in 2,3-dichloro-5,8-diacethoxy-1,4-naphthoquinone (**39**), improved the activity against *S. aureus* and *C. krusei*, but the activity against *C. neoformans* was four times lower. This finding demonstrates that ethoxylation improved the activities more markedly than methoxylation, although the most active compound against *Candida* sp. in the series tested was the monochloro derivative with hydroxyl groups at C5 and C8 (**37**) (MIC 2 μg/mL).

Regarding bromo 1,4-naphthoquinone derivatives, the presence of a bromo substituent at the C2 position, as in 2-bromo-5,8-dihydroxy-1,4-naphthoquinone (**43**), improved the activity against the Gram-positive bacteria and yeasts tested, albeit to a lesser extent than that observed for the monochloro derivative. The removal of the hydroxyl group at C8, as in 2-bromo-5-hydroxy-1,4-naphthoquinone (**42**), improved the activity against *P. aeruginosa*, *S. aureus* and *C. neoformans*, although the activities against other microorganisms assessed were similar. This compound showed the best antimicrobial activity against *S. aureus* of all compounds studied (MIC 16 μg/mL).

Methoxylation of monobromo derivatives at the C5 and C8 positions, as in 2-bromo-5,8-dimethoxy-1,4-naphthoquinone (**45**), markedly reduced the activity against all microorganisms studied and gave rise to considerably higher MIC values (64 μg/mL) compared to compound **43**. However, ethoxylation of this compound gave 2-bromo-5,8-diacethoxy-1,4-naphthoquinone (**46**), which was inactive against bacteria but showed the same activity against yeast.

Dihalogenation at C2 and C3, as in 2,3-dibromo-5,8-dihydroxy-1,4-naphthoquinone (**44**), led to a slight increase in the antifungal activity but not in the antimicrobial activity.

In general, halogenated derivatives showed activity against all microorganisms assessed and they were more active against *Candida* sp., especially against *C. krusei*, with MIC values between 2 and 8 μg/mL (Ambrogi *et al*., 1970).

Ambrogi *et al*. observed that chloro derivatives showed stronger activity than bromo derivatives. Similar behavior was found in our study, and comparison of compounds **37** and **38** with **42** and **43** shows that the chloro derivatives are slightly more active against most of the microorganisms assessed. It has been demonstrated in several studies that the presence of a Cl group in ring A is essential for antifungal activity (Tandon *et al*., 2009; Tran *et al*., 2009).

In summary, chloro derivatives were more active than bromo derivatives, followed by compounds with methyl and hydroxyl groups, respectively.

Other modifications in the ring of 1,4-naphthoquinone

Finally, we decided to study other modifications in structure of 1,4-naphthoquinone. First, for comparison, the lack of the double bond in the A ring, as compound **47**, enhanced antifungal activity with MIC $\leq$ 16 μg/mL, compared to **1**, although **47** was inactive against all bacteria tested.

Addition of a hydroxyl group at C2 of the 1,4-naphthoquinone moiety, as in 2,5,8-trihydroxy-1,4-naphthoquinone (**48**), compared to **1**, led to a compound that was inactive against Gram-negative bacteria and yeasts but more active against Gram-positive bacteria. This activity was very poor in general. These results are consistent with those obtained in a study carried out by Yakubovskaya *et al*. (2009b) who found significantly higher MIC values (>100 μg/mL) in *E. coli* and *S. aureus*. This behavior could occur because this hydroxyl derivative exhibited low cytotoxicity. The low activity of hydroxyl derivatives could be connected with deprotonation of this group under the conditions of the biological experiment or when it acts in cells (Pelageev *et al*., 2014).

The absence of hydroxyl groups at the C5 and C8 positions in lawsone (**49**), compared to **48**, make this compound inactive against all microorganisms tested. Several authors have found similar results on using different methods for the evaluation of antibacterial and antifungal activities of lawsone, namely the disk diffusion (Tekin *et al*., 2015) and broth microdilution methods (Sreelatha *et al*., 2014b). This behavior could be attributed to the chemical structure of lawsone, which is a hydroxyquinone that can exist as a 1,2-diketone or a 1,4-quinone. Structural modification of the phenolic hydroxyl group could result in a reduction in activity, as observed in our study (Sreelatha *et al*., 2014b).

Replacement of the hydroxyl group at the C2 position of **49** by a methyl group gave 2-methyl-1,4-naphthalenedione (or menadione, **50**), which has a low activity against bacteria but moderate activity against yeast. Compound **50** showed antimicrobial activity, whereas compound **49** was completely inactive. This is because menadione inhibited the growth of *Candida* species by stimulating the production of radical oxygen species (Ueno *et al*., 2008).

Of these compounds, only **47** had a moderate activity against yeasts. The antibacterial activity was very low.

Experimental

General

All reagents (obtained from Aldrich Chemical Co.) and solvents (HPLC grade) were used without further purification. NMR spectra were recorded at room temperature on Agilent Inova 500- and 400-MHz spectrometers. The ^{1}H and ^{13}C chemical shifts are referenced to the $CDCl_3$ solvent at δ_H 7.25 and δ_C 77.0 ppm. Melting points were taken on a Kofler hot stage apparatus and are uncorrected. General IR spectra (KBr) were recorded on a PerkinElmer FTIR Spectrum 1000 spectrophotometer. High-resolution mass spectra (HRMS) were obtained on a WATERS SYNAPT G2 mass spectrometer (70 eV). Reactions were monitored by thin-layer chromatography (TLC) on silica gel (F245 Merck plates).

Procedure for the synthesis of compound 4

Compound **4** was prepared by acetylation of juglone according to a literature procedure with modifications (Greco *et al*., 2010). Juglone (50 mg, 1 eq.) was dissolved in the minimum amount of pyridine. Acetic anhydride (271 μL, 10 eq.) was added dropwise to the solution of juglone, and the reaction mixture was stirred at room temperature and monitored by TLC. Saturated aqueous copper sulfate was added, and liquid–liquid extraction was carried out to remove the pyridine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography using 10 % AcOEt/hexane as eluent to give 5-acetyl-1,4-naphthoquinone (40 % yield).

Procedure for the synthesis of compounds 5–15

These derivatives were obtained according to the methodology previously described by Mathew *et al*. (2010) with some modifications. These acylation reactions were performed in dichloromethane with juglone (50 mg, 1 eq.) as the starting material. 4-Dimethylaminopyridine (68.4 μL, 3 eq.) and the corresponding acyl chloride (125 μL, 5 eq.) were added at 0 °C. The reaction mixture was stirred at room temperature and monitored by TLC. After completion of the reaction, the organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The resulting mixture was purified by column chromatography using 100 % chloroform as eluent to obtain compounds **5**–**15**. New compounds are characterized.

5-O-Butanoyloxy-1,4-naphthoquinone ***(6)*** This compound was obtained as a yellow solid in 78 % yield; mp 72–75 °C; IR (KBr) ν_{max} 3078, 2963, 1753, 1664, 1594, 1138 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.02$ (1H, dd, $J = 1.2$, 7.8 Hz, H-8), 7.74 (1H, dd, $J = 8.0$, 7.8 Hz, H-7), 7.36 (1H, dd, $J = 1.2$, 8.0 Hz, H-6), 6.92 (1H, d, $J = 10.3$ Hz, H-3), 6.82 (1H, d, $J = 10.3$ Hz, H-2), 2.71 (2H, t, J = 7.5 Hz, H-2′), 1.84 (2H, qt, J = 7.5, 7.4 Hz, H-3′), 1.08 (3H, t, $J = 7.4$ Hz, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.0$ (C, C-4), 183.4 (C, C-1), 171.7 (C, C-1′), 149.4 (C, C-5), 139.7 (CH, C-2), 137.1 (CH, C-3), 134.6 (CH, C-7), 133.3 (C, C-8a), 129.6 (CH, C-6), 124.7 (CH, C-8), 123.2 (C, C-4a), 35.8 (CH_2, C-2′), 17.8 (CH_2, C-3′), 13.5 (CH_3, C-4′); HRESIMS *m/z* (pos) 245.0788 $C_{14}H_{13}O_4$ $[M + H]^+$ (calcd.: 245.0814).

5-O-Pentanoyloxy-1,4-naphthoquinone ***(7)*** This compound was obtained as a brown oil in 90 % yield; IR (KBr) ν_{max} 3079, 2957, 1762, 1669, 1594, 1138 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.02$ (1H, dd, $J = 1.2$, 7.8 Hz, H-8), 7.73 (1H, dd, $J = 8.0$, 7.8 Hz, H-7), 7.36 (1H, dd, $J = 1.2$, 8.0 Hz, H-6), 6.91 (1H, d, $J = 10.3$ Hz, H-3), 6.82 (1H, d, $J = 10.3$ Hz, H-2), 2.73 (2H, t, J = 7.5 Hz, H-2′), 1.80 (2H, tt, J = 7.5, 7.5 Hz, H-3′), 1.48 (2H, tt, J = 7.5, 7.4 Hz, H-4′), 0.98 (3H, t, $J = 7.4$ Hz, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.3$ (C, C-4), 183.7 (C, C-1), 172.2 (C, C-1′), 149.7 (C, C-5), 140.0 (CH, C-2), 137.4 (CH, C-3), 134.9 (CH, C-7), 133.6 (C, C-8a), 129.9 (CH, C-6), 125.0 (CH, C-8), 123.5 (C, C-4a), 34.0 (CH_2, C-2′), 26.6 (CH_2, C-3′), 22.4 (CH_2, C-4′), 13.9 (CH_3, C-5′); HRESIMS *m/z* (pos) 259.0936 $C_{15}H_{15}O_4$ $[M + H]^+$ (calcd.: 259.0970).

5-O-Heptanoyloxy-1,4-naphthoquinone ***(9)*** This compound was obtained as a brown oil in 56 % yield; IR (KBr) ν_{max} 3079, 2929, 1768, 1667, 1596, 1135 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.03$ (1H, dd, $J = 1.2$, 7.8 Hz, H-8), 7.75 (1H, dd, $J = 8.0$, 7.8 Hz, H-7), 7.36 (1H, dd, $J = 1.2$, 8.1 Hz, H-6), 6.92 (1H, d, $J = 10.3$ Hz, H-3), 6.83 (1H, d, $J = 10.3$ Hz, H-2), 2.72 (2H, t, J = 7.5 Hz, H-2′), 1.80 (2H, tt, J = 7.5, 7.5 Hz, H-3′), 1.48–1.31 (6H, m, H-4′, H-5′, H-6′), 0.90 (3H, t, $J = 7.1$ Hz, H-7′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.2$ (C, C-4), 183.2 (C, C-1), 172.1 (C, C-1′), 149.6 (C, C-5), 139.9 (CH, C-2), 137.3 (CH, C-3), 134.8 (CH, C-7), 133.5 (C, C-8a), 129.8 (CH, C-6), 124.9 (CH, C-8), 123.3 (C, C-4a), 34.2 (CH_2, C-2′), 31.5 (CH_2, C-5′), 28.8 (CH_2, C-4′), 24.4 (CH_2, C-3′), 22.5 (CH_2, C-6′), 14.0 (CH_3, C-7′); HRESIMS *m/z* (pos) 287.1320 $C_{17}H_{19}O_4$ $[M + H]^+$ (calcd.: 287.1283).

5-O-Octanoyloxy-1,4-naphthoquinone ***(10)*** This compound was obtained as a brown oil in 49 % yield; IR (KBr) ν_{max} 3038, 2924, 1768, 1668, 1596 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.03$ (1H, dd, $J = 1.2$, 7.8 Hz, H-8), 7.75 (1H, dd, $J = 8.1$, 7.8 Hz, H-7), 7.36 (1H, dd, $J = 1.2$, 8.1 Hz, H-6), 6.92 (1H, d, $J = 10.3$ Hz, H-3), 6.83 (1H, d, $J = 10.3$ Hz, H-2), 2.72 (2H, t, J = 7.5 Hz, H-2′), 1.80 (2H, tt, J = 7.5, 7.5 Hz, H-3′), 1.48–1.24 (8H, m, H-4′, H-5′, H-6′, H-7′), 0.89 (3H, t, $J = 6.9$ Hz, H-8′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.2$ (C, C-4), 183.6 (C, C-1), 172.1 (C, C-1′), 149.6 (C, C-5), 139.9 (CH, C-2), 137.3 (CH, C-3), 134.7 (CH, C-7), 133.5 (C, C-8a), 129.8 (CH, C-6), 124.9 (CH, C-8), 123.4 (C, C-4a), 34.2 (CH_2, C-2′), 31.7 (CH_2, C-6′), 30.9 (CH_2, C-4′), 29.0 (CH_2, C-5′), 24.4 (CH_2, C-3′), 22.6 (CH_2, C-7′), 14.1 (CH_3, C-8′); HRESIMS *m/z* (pos) 301.1422 $C_{18}H_{21}O_4$ $[M + H]^+$ (calcd.: 301.1440).

5-O-Nonanoyloxy-1,4-naphthoquinone ***(11)*** This compound was obtained as a yellow solid in 64 % yield; mp 56–61 °C; IR (KBr) ν_{max} 3079, 2926, 1767, 1667, 1595, 1133 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 8.02$ (1H, dd, $J = 1.3$, 7.8 Hz, H-8), 7.73 (1H, dd, $J = 8.0$, 7.8 Hz, H-7), 7.36 (1H, dd, $J = 1.3$, 8.0 Hz, H-6), 6.91 (1H, d, $J = 10.3$ Hz, H-3), 6.82 (1H, d, $J = 10.3$ Hz, H-2), 2.72 (2H, t, J = 7.5 Hz, H-2′), 1.80 (2H, tt, J = 7.5, 7.5 Hz,

H-3′), 1.48–1.24 (10 H, m, H-4′, H-5′, H-6′, H-7′, H-8′), 0.87 (3H, t, $J = 6.9$ Hz, H-9′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.2$ (C, C-4), 183.6 (C, C-1), 172.1 (C, C-1′), 149.6 (C, C-5), 139.9 (CH, C-2), 137.2 (CH, C-3), 134.7 (CH, C-7), 133.5 (C, C-8a), 129.8 (CH, C-6), 124.8 (CH, C-8), 123.3 (C, C-4a), 34.2 (CH_2, C-2′), 31.8 (CH_2, C-7′), 29.23 (CH_2, C-4′), 29.13 (CH_2, C-6′), 29.11 (CH_2, C-5′), 24.4 (CH_2, C-3′), 22.6 (CH_2, C-8′), 14.1 (CH_3, C-9′); HRESIMS *m/z* (pos) 315.1586 $C_{19}H_{23}O_4$ $[M + H]^+$ (calcd.: 315.1596).

5-O-Decanoyloxy-1,4-naphthoquinone (***12***) This compound was obtained as a brown solid in 84 % yield; mp 44–49 °C; IR (KBr) ν_{max} 3076, 2924, 1766, 1667, 1594, 1137 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.02$ (1H, dd, $J = 1.3$, 7.9 Hz, H-8), 7.73 (1H, dd, $J = 7.9$, 7.9 Hz, H-7), 7.36 (1H, dd, $J = 1.3$, 7.9 Hz, H-6), 6.91 (1H, d, $J = 10.3$ Hz, H-3), 6.82 (1H, d, $J = 10.3$ Hz, H-2), 2.72 (2H, t, J = 7.7 Hz, H-2′), 1.80 (2H, tt, J = 7.7, 7.7 Hz, H-3′), 1.40–1.24 (12 H, m, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′), 0.87 (3H, t, $J = 6.7$ Hz, H-10′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.2$ (C, C-4), 183.6 (C, C-1), 172.1 (C, C-1′), 149.6 (C, C-5), 139.9 (CH, C-2), 137.2 (CH, C-3), 134.7 (CH, C-7), 133.5 (C, C-8a), 129.8 (CH, C-6), 124.8 (CH, C-8), 123.3 (C, C-4a), 34.2 (CH_2, C-2′), 31.8 (CH_2, C-8′), 29.41 (CH_2, C-6′), 29.28 (CH_2, C-7′), 29.24 (CH_2, C-4′), 29.13 (CH_2, C-5′), 24.4 (CH_2, C-3′), 22.6 (CH_2, C-9′), 14.1 (CH_3, C-10′); HRESIMS *m/z* (pos) 329.1787 $C_{20}H_{25}O_4$ $[M + H]^+$ (calcd.: 329.1753).

5-O-Palmitoyljuglone (***15***) This compound was obtained as a yellow solid in 49 % yield; mp 66–69 °C; IR (KBr) ν_{max} 3076, 2921, 1760, 1664, 1590, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.03$ (1H, dd, $J = 1.1$, 7.8 Hz, H-8), 7.74 (1H, dd, $J = 8.0$, 7.8 Hz, H-7), 7.36 (1H, dd, $J = 1.1$, 8.0 Hz, H-6), 6.92 (1H, d, $J = 10.3$ Hz, H-3), 6.83 (1H, d, $J = 10.3$ Hz, H-2), 2.72 (2H, t, J = 7.6 Hz, H-2′), 1.81 (2H, m, H-3′), 1.48–1.25 (24 H, m, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′, H-10′, H-11′), 0.87 (3H, t, $J = 6.4$ Hz, H-12′); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 184.2$ (C, C-4), 183.2 (C, C-1), 172.1 (C, C-1′), 149.6 (C, C-5), 139.9 (CH, C-2), 137.2 (CH, C-3), 134.7 (CH, C-7), 133.5 (C, C-8a), 129.8 (CH, C-6), 124.8 (CH, C-8), 123.3 (C, C-4a), 34.2 (CH_2, C-2′), 31.9 (CH_2, C-10′), 29.58 (CH_2, C-6′), 29.56 (CH_2, C-8′), 29.4 (CH_2, C-7′), 29.24 (CH_2, C-4′), 29.31 (CH_2, C-5′), 29.28 (CH_2, C-9′), 29.14 (CH_2, C-4′), 24.4 (CH_2, C-3′), 22.6 (CH_2, C-11′), 14.1 (CH_3, C-12′); HRESIMS *m/z* (pos) 357.2024 $C_{22}H_{29}O_4$ $[M + H]^+$ (calcd.: 357.2060).

Procedure for the synthesis of compound 25

To a stirred solution of commercially available 1-bromo-2,5-dimethoxybenzene (500 mg, 2.3 mmol) in dichloroethane (15 mL) at 0 °C was added maleic anhydride (237 mg, 2.42 mmol) followed by $AlCl_3$ (644 mg, 4.83 mmol) in small portions over 2 h 30 min. The reaction mixture was stirred at 0 °C and warmed slowly to room temperature and then stirred for 72 h. The reaction mixture was poured into ice water, and then, aqueous $NaHCO_3$ 5 % was added until the solid had dissolved. The resulting mixture was concentrated under reduced pressure to remove dichloroethane. The aqueous solution was acidified to pH 1 by addition of 5 % HCl. The mixture was stirred for 15 min and extracted with ethyl acetate (3 × 20 mL), and the organic extracts were combined and washed with water and brine and dried over anhydrous Na_2SO_4. The crude material was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (1:1) with 0.5 % acetic acid to afford **25** as a yellow solid (348.4 mg).

4′-(4-bromo-2,5-dimethoxyphenyl)-4′-oxobut-2′-enoic acid (***25***) This compound was obtained as a yellow solid in 48 % yield; mp 153–154 °C; IR (KBr) ν_{max} 3082, 3013, 2944, 1712, 1658, 1595, 1488, 1388, 1289, 1269, 1215 cm^{-1}; ^{1}H NMR (Pyridine-d_5, 400 MHz): $\delta = 8.23$ (1H, d, $J = 15.6$, H-3́), 7.47 (1H, s, H-6), 7.46 (1H, s, H-3), 7.31 (1H, d, $J = 15.6$, H-2́), 5.72 (1H, s, COOH), 3.75 (3H, s, C-2–O–CH_3), 3.74 (3H, s, C-5–O–CH_3); ^{13}C NMR (Pyridine-d_5, 100 MHz): $\delta = 191.2$ (C, C-4′), 169.0 (C, C-1′), 154.0 (C, C-5), 153.0 (C, C-2), 140.8 (CH, C-3′), 133.4 (CH, C-2′), 128.2 (C, C-4), 118.9 CH, C-6), 118.7 (C, C-1), 113.9 (CH–C-3), 57.2 (CH_3, C-2–O–CH_3), 57.1 (CH_3, C-5–O–CH_3); HRESIMS *m/z* (pos) 314.9861, 316.9842 $C_{12}H_{12}O_5Br$ $[M]^+$ (calcd.: 314.9864).

Biological evaluation

The compounds were subjected to an evaluation of their level activity using the broth microdilution method to estimate the minimal inhibitory concentration (MIC) according to the recommendations of (CLSI 2008; PA, 2009).

The microorganisms assessed were *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *S. aureus*, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. neoformans*.

A series of twofold dilutions from 1024 to 2 µg/mL (dissolved in DMSO up to 2 % final DMSO concentration) were prepared in a 96-well sterile microplate.

For bacteria In each well was introduced 50 µL of the dilution compound in Muller Hinton broth. Subsequently, 50 µL of an inoculum containing 5×10^6 CFU was added to each well. Gentamicin and doxycycline were used as the antibacterial references. The microplate was incubated at 37 °C for 24 h.

For yeasts 100 µL of an inoculum containing 1×10^3–5×10^3 CFU was added to each well. In each well was

introduced 100 μL of the dilution compound in RPMI 1640 with L-glutamine and without sodium bicarbonate (Sigma-Aldrich). Amphotericin B was used as the antifungal reference. The microplate was incubated at 37 °C for 48 h in *Candida* sp. and 72 h in *C. neoformans*.

MIC was considered as the weakest concentration at which turbidity could not be observed with the naked eye.

The effect of DMSO at a concentration of 2 % was checked and eliminated; at these concentrations, DMSO has no apparent effect on the microbial and fungal growth. The wells used as a negative control were prepared using the inoculum alone.

All experiments were repeated three times, and the results are expressed as average values.

Conclusions

Several known and new naphthoquinone derivatives have been tested for antibacterial and antifungal activity. In bacteria, compound **42** displayed the highest activity against *S. aureus* at MIC 16 μg/mL. Compound **37** was the most active against *Candida* spp. with MIC of 2–4 μg/mL, similar to amphotericin B. Structure–activity relationships of these compounds showed that halogen substituents at the C2 and/or C3 positions are the functional groups that have the most influence on the antibacterial and antifungal activities. The carbonyl groups at C-1 and C4 enhance the antimicrobial activity. The presence of side chains with more than ten carbon atoms increases the lipophilicity of these compounds. These chains led to a progressive decrease in the activity until they became inactive. The two rings of the naphthoquinone core are essential for the antimicrobial activity of these compounds. The presence of additional rings in the structure or the lack of either ring results in inactive compounds. The presence of hydroxyl groups at the C-5 and/or C-8 positions is critical for antibacterial activity. The replacement of these groups with other functional groups leads to the loss of activity.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest connected to this study.

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