**In vitro Investigation of the Antimicrobial Activity of a Series of Lipophilic Phenols and Naphthols**

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**ABSTRACT**

Five groups of phenols/naphthols (42 compounds in total) were synthesized and screened against Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative *Escherichia coli* and *Klebsiella pneumoniae*, and the fungus *Candida albicans*. Whereas compounds were found inactive against Gram-negative bacteria, potent activities against Gram-positive bacteria were observed. The activities correlate with the ability of molecules to form quinone methides, suggesting potential new modes of action.

**KEYWORDS**

Antimicrobial activity, phenols, naphthols, quinone methides.

**1. Introduction**

Bacterial infections are responsible for a vast number of human diseases. Moreover, development of bacterial resistance to common antibiotics is stimulating intensive research devoted to discovery of new targets in combating the bacteria. In search for new and improved antibiotics with new mechanism of action, it is important to screen many new libraries of compounds.

Antimicrobial activity of simple phenols, and their carboxylic acid derivatives has been investigated. It is generally accepted that phenols and benzyl alcohols exert antimicrobial action on the non-specific ability to alter membranes in Gram-negative bacteria. On the other hand, a more specific mode of action of the non-specific ability to alter membranes in Gram-negative bacteria. On the other hand, a more specific mode of action of phenolic compounds has been suggested, especially against Gram-positive bacteria, since the bactericidal concentrations are not dependent on the type of cell wall. It was suggested that phenolic compounds inhibit DNA synthesis, due to an effect associated with the inhibition of RNA and protein synthesis. Furthermore, antibacterial properties are ascribed to a large number of food products due to the presence of phenolic natural compounds. Examples include berries, vine, tropical vegetables, and mushrooms. Recently, an investigation of antimicrobial activities have been conducted on a series of naphthol derivatives of Mannich bases. The latter series of compounds can in principle be transformed to quinone methides (QM) intermediates during metabolic processes. QMs are interesting substrates in the development of antimicrobial agents since it was demonstrated that they act as alkyllating agents and inhibitors of serine proteases and \( \beta \)-lactamases. Depending on their structure, they are mechanism-based enzyme inhibitors, or suicide substrates. When QMs are formed from cyclic compounds such as coumarins, they usually induce cross-linking of the enzyme in the reaction with a histidine. On the contrary, free QM formed in the active site of the enzyme usually reacts with neighbouring tryptophan residues (Scheme 1).

We became interested in the photochemical generation of QMs with sterically congested lipophilic substituents and their antiproliferative activities. A series of simple phenols, biphenyls, naphthols, and anthols were synthesized and their antiproliferative activities were investigated. Since compounds that generate QMs are known \( \beta \)-lactamase inhibitors, we were also prompted to investigate their antimicrobial activities. Potentially, they can be used in combination with \( \beta \)-lactam derivatives as antibiotics. Herein we report an investigation of the antimicrobial activity of five series of lipophilic derivatives of phenols and naphthols and/or benzyl alcohols (Figs. 1–5) with the aim of developing a SAR relationship. Moreover, many of our derivatives bear an adamantyl substituent, and the antibacterial activity of a number of adamantane derivatives has been reported. The *in vitro* antimicrobial testing was performed on five strains of microorganisms, Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative *Escherichia coli* and *Klebsiella pneumoniae*, and the fungus *Candida albicans*.

**Scheme 1**
2. Materials and Methods

2.1. Compounds

The structures of the investigated compounds comprise phenols (Fig. 1), 2-hydroxybiphenyls (Fig. 2), 3-hydroxybiphenyls (Fig. 3), 4-hydroxybiphenyls (Fig. 4), or their corresponding methoxy or QM derivatives. All compounds were prepared according to previously published procedures. Chiral compounds 7–9 were synthesized as racemic mixtures.

2.1.1. Synthesis of Phenol Derivatives 1–9

2-Hydroxy-2-(2-methoxyphenyl)adamantane (1) was prepared from 2-adamantanone and 2-bromanisol in 50% yield according to the published procedure. Characterization of product 1 compares well with literature (mp = 170–171°C). 1H NMR (CDCl₃, 300 MHz) δ/ppm: 9.28 (s, 1H, OH), 7.32 (dd, 1H, J = 1.2 Hz, J = 7.7 Hz), 7.05 (dt, 1H, J = 1.5 Hz, J = 8.5 Hz), 6.73–6.82 (m, 2H), 4.93 (s, 1H, OH), 2.61 (br s, 2H), 2.44 (br s, 1H), 2.40 (br s, 1H), 1.60–1.50 (m, 8H), 1.51 (d, 2H, J = 12 Hz).

2-Hydroxy-2-(2-hydroxyphenyl)adamantane (2) was prepared from 1 by treatment with BBr₃ in 45% yield according to the published procedure. Characterization of product 2 compares well with literature (mp = 162–164°C). 1H NMR (DMSO-d₆, 300 MHz) δ/ppm: 9.14 (s, 1H), 7.07 (dd (t), 1H, J = 7.8 Hz), 7.00 (dd (t), 1H, J = 2.0 Hz), 6.96 (d, 1H, J = 7.8 Hz), 6.56 (dd, 1H, J = 2.0, 7.8 Hz), 4.81 (s, 1H), 2.61–2.50 (m, 2H), 2.13–1.95 (m, 2H), 1.91–1.65 (m, 7H), 1.59–1.33 (m, 5H).

exo-4-(3-Hydroxyphenyl)protoadamantan-4-ol (9) was prepared from exo-4-(3-methoxyphenyl)protoadamantan-4-ol by treatment with sodium thiolate in 46% yield according to the published procedure (mp = 176–178°C). Characterization of product 9 compares well with literature. 1H NMR (DMSO-d₆, 300 MHz) δ/ppm 9.13 (s, 1H), 7.07 (dd (t), 1H, J = 7.8 Hz), 6.91–6.86 (m, 2H), 6.57 (dd, 1H, J = 1.6, 7.6 Hz), 4.56 (s, 1H), 2.58 (dd (t), 1H, J = 8.2 Hz), 2.46–2.36 (m, 1H), 2.33–2.24 (m, 1H), 2.07 (bs, 4H), 1.74 (d, 1H, J = 13.0 Hz), 1.67–1.40 (m, 3H), 1.29 (dd (t), 2H, J = 13.0 Hz), 1.19 (d, 1H, J = 12.0 Hz).

Figure 2 2-Hydroxybiphenyl derivatives.
2-(2-Hydroxyadamantan-2-yl)-2'-methoxybiphenyl (10) was prepared in a Grignard reaction from adamantan-2-one and 2-bromo-2'-methoxybiphenyl in 76 % yield according to the published procedure.20 Characterization of product 10 compares well with literature (mp = 114–115 °C).20 1H NMR (CDCl3, 600 MHz) δ/ppm: 7.68 (d, 1H, J = 8.1 Hz), 7.38 (dt, 1H, J = 1.6 Hz, J = 8.1 Hz), 7.34 (dt, 1H, J = 1.7 Hz, J = 8.1 Hz), 7.27 (dt, 1H, J = 1.1 Hz, J = 7.4 Hz), 7.18 (dd, 1H, J = 1.6 Hz, J = 7.4 Hz), 7.00–7.04 (m, 2H), 6.93 (d, 1H, J = 8.3 Hz), 3.86 (br s, 1H, OH), 3.74 (s, 3H, OCH3) 2.59 (br s, 1H), 2.34 (dt, 1H, J = 2.2 Hz, J = 12.4 Hz), 2.11 (dt, 1H, J = 2.2 Hz, J = 12.4 Hz), 1.80–1.90 (m, 2H), 1.60–1.72 (m, 5H), 1.54 (dt, 1H, J = 2.2 Hz, J = 12.4 Hz), 1.40 (dt, 1H, J = 2.2 Hz, J = 12.4 Hz), 1.32 (dq, 1H, J = 3.2 Hz, J = 12.4 Hz), 1.23 (dq, 1H, J = 3.2 Hz, J = 12.4 Hz).

2-(2-Hydroxyadamantan-2-yl)-2' hydroxybiphenyl (11) was prepared from 10 by treatment with sodium thiolate in 60 % yield according to the published procedure.20 Characterization of product 11 compares well with literature (mp = 194–196 °C).20 1H NMR (DMSO-d6, 300 MHz) δ/ppm: 9.83 (s, 1H, OH), 7.70 (d, 1H, J = 7.4 Hz), 7.36 (dt, 1H, J = 1.5 Hz, J = 7.3 Hz), 7.26 (dt, 1H, J = 0.9 Hz, J = 7.3 Hz), 7.17 (dt, 1H, J = 1.5 Hz, J = 7.5 Hz), 7.09 (dd, 1H, J = 1.5 Hz, J = 7.5 Hz), 6.95 (dd, 1H, J = 1.5 Hz, J = 7.5 Hz), 6.83–6.90 (m, 2H), 5.30 (s, 1H, OH), 2.50 (br s, 1H), 2.24 (d, 1H, J = 12.1 Hz), 2.08 (br s, 2H), 1.75 (d, 1H, J = 11.6 Hz), 1.44–1.69 (m, 6H), 1.36 (d, 1H, J = 12.1 Hz), 1.29 (d, 1H, J = 12.1 Hz), 1.21 (d, 1H, J = 11.6 Hz).

Figure 3 3-Hydroxybiphenyl derivatives.
treatment with BBr₃ in 96 % yield according to the published procedure.²² Characterization of product 20 compares well with literature (mp = 141–142 °C).²² ¹H NMR (DMSO-d₆, 600 MHz) δ/ppm: 9.47 (s, 1H, OH), 7.50 (t, 1H, J = 1.4 Hz), 7.48 (dd, 1H, J = 1.7 Hz, J = 7.7 Hz), 7.36 (t, 1H, J = 7.7 Hz), 7.32 (dd, 4H, J = 7.2 Hz, J = 7.6 Hz), 7.23–7.28 (m, 6H), 7.21 (t, 1H, J = 7.8 Hz), 7.13 (dd, 1H, J = 1.5 Hz, J = 7.7 Hz), 6.95 (dd, 1H, J = 1.3 Hz, J = 7.7 Hz), 6.92 (t, 1H, J = 2.0 Hz), 6.73 (dd, 1H, J = 8.0 Hz, J = 2.3 Hz), 6.51 (s, 1H, OH).

(3’-Hydroxybiphenyl-4-yl)diphenylmethanol (21) was prepared from (3'-methoxybiphenyl-4-yl)diphenylmethanol by treatment with BBr₃ in 69 % yield according to the published procedure.²² Characterization of product 21 compares well with literature (mp = 141–142 °C).²² ¹H NMR (DMSO-d₆, 600 MHz) δ/ppm: 9.47 (s, 1H, OH), 7.50 (t, 1H, J = 1.4 Hz), 7.48 (dd, 1H, J = 1.7 Hz, J = 7.7 Hz), 7.36 (t, 1H, J = 7.7 Hz), 7.32 (dd, 4H, J = 7.2 Hz, J = 7.6 Hz), 7.23–7.28 (m, 6H), 7.21 (t, 1H, J = 7.8 Hz), 7.13 (dd, 1H, J = 1.5 Hz, J = 7.7 Hz), 6.95 (dd, 1H, J = 1.3 Hz, J = 7.7 Hz), 6.92 (t, 1H, J = 2.0 Hz), 6.73 (dd, 1H, J = 8.0 Hz, J = 2.3 Hz), 6.51 (s, 1H, OH).

2.1.4. Synthesis of 4-Hydroxybiphenyl Derivatives 22–31

Figure 4 4-Hydroxybiphenyl derivatives.

2-(2-Hydroxyadamantan-2-yl)-4'-methoxybiphenyl (22) was prepared in a Grignard reaction from 3-bromo-4'-methoxybiphenyl and 2-adamantanone in 78 % yield according to the published procedure.²² Characterization of product 22 compares well with literature (mp = 155–157 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 7.54 (d, 2H, J = 8.4 Hz), 7.32 (dd, 4H, J = 7.2 Hz, J = 7.7 Hz), 7.22–7.30 (m, 9H), 7.06 (d, 1H, J = 1.0 Hz, J = 7.7 Hz), 7.02 (t, 1H, J = 2.0 Hz), 6.75 (dd, 1H, J = 1.6 Hz, J = 8.0 Hz), 6.46 (s, 1H, OH).

2-(2-Hydroxyadamantan-2-yl)-4'-hydroxybiphenyl (23) was prepared from 22 by treatment with sodium thiolate in 69 % yield according to the published procedure.²² Characterization of product 23 compares well with literature (mp = 168–170 °C).²² ¹H NMR (DMSO-d₆, 600 MHz) δ/ppm: 9.49 (s, 1H, OH), 7.54 (d, 2H, J = 8.4 Hz), 7.32 (dd, 4H, J = 7.2 Hz, J = 7.7 Hz), 7.22–7.30 (m, 9H), 7.06 (d, 1H, J = 1.0 Hz, J = 7.7 Hz), 7.02 (t, 1H, J = 2.0 Hz), 6.75 (dd, 1H, J = 1.6 Hz, J = 8.0 Hz), 6.46 (s, 1H, OH).

3-(2-Hydroxyadamantan-2-yl)-4'-hydroxybiphenyl (24) was prepared in a Grignard reaction from 3-bromo-4’-methoxybiphenyl and 2-adamantanone in 78 % yield according to the published procedure.²² Characterization of product 24 compares well with literature (mp = 103–105 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 7.71 (br s, 1H), 7.51 (d, 2H, J = 8.8 Hz), 7.27–7.49 (m, 3H), 6.97 (d, 2H, J = 8.8 Hz), 3.84 (s, 3H), 2.62 (br s, 2H), 2.45 (br s, 1H), 2.41 (br s, 1H), 1.91 (br s, 1H), 1.68–1.80 (m, 10H).

3-(2-Hydroxyadamantan-2-yl)-4'-hydroxybiphenyl (25) was prepared from 24 by treatment with BBr₃ in 79 % yield according to the published procedure.²² Characterization of product 25 compares well with literature (mp = 135–137 °C).²² ¹H NMR (DMSO-d₆, 300 MHz) δ/ppm: 9.50 (br s, OH), 7.63 (br s, 1H), 7.46 (d, 2H, J = 8.6 Hz), 7.35–7.44 (m, 3H), 6.85 (d, 2H, J = 8.0 Hz), 2.52 (br s, 2H), 2.42 (br s, 1H), 2.38 (br s, 1H), 1.81 (br s, 1H), 1.55–1.70 (m, 10H).

3-(2-Hydroxyadamantan-2-yl)-4'-methoxybiphenyl (26) was prepared in a Grignard reaction from 4-bromo-4'-methoxybiphenyl and 2-adamantanone in 65 % yield according to the published procedure.²² Characterization of product 26 compares well with literature (mp = 186–187 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 9.48 (br s, OH), 7.42–7.61 (m, 6H), 6.84 (d, 2H, J = 8.5 Hz), 2.47 (br s, 2H), 2.41 (br s, 1H), 2.38 (br s, 1H), 1.81 (br s, 1H), 1.53–1.73 (m, 10H).

4-Hydroxymethyl-4'-hydroxybiphenyl (27) was obtained from 4-methoxy-4'-hydroxybiphenyl with sodium thiolate in 23 % yield according to the published procedure.²² Characterization of product 27 compares well with literature (mp = 195–196 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 9.50 (s, 1H, OH), 7.51 (d, 2H, J = 8.5 Hz), 7.46 (d, 2H, J = 8.5 Hz), 7.33 (d, 2H, J = 8.5 Hz), 6.83 (d, 2H, J = 8.6 Hz), 5.15 (br s, 1H, OH), 4.50 (s, 2H).

4-Thioethylmethyl-4'-hydroxybiphenyl (29) was obtained from 4-methoxy-4'-hydroxybiphenyl with sodium thiolate in 75 % yield according to the published procedure.²² Characterization of product 29 compares well with literature (mp = 195–196 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 9.48 (s, 1H, OH), 7.51 (d, 2H, J = 8.5 Hz), 7.46 (d, 2H, J = 8.5 Hz), 7.33 (d, 2H, J = 8.5 Hz), 6.83 (d, 2H, J = 8.6 Hz), 5.15 (br s, 1H, OH), 4.50 (s, 2H).

4-Hydroxy-4'-(2-hydroxypropan-2-yl)biphenyl (30) was obtained from 4-methoxy-4'-(2-hydroxypropan-2-yl)biphenyl with sodium thiolate in 80 % yield according to the published procedure.²² Characterization of product 30 compares well with literature (mp = 178–180 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 9.48 (s, 1H, OH), 7.48 (s, 4H), 7.45 (d, 2H, J = 8.6 Hz), 6.83 (d, 2H, J = 8.6 Hz), 4.98 (br s, 1H, OH), 1.44 (s, 6H).

4-((Hydroxydiphenylmethyl)benzyl)-4-0 (31) was obtained from (4'-methoxybiphenyl-4-yl)diphenylmethanol with BBr₃ in 83 % yield according to the published procedure.²² Characterization of product 31 compares well with literature (mp = 274–276 °C).²² ¹H NMR (CDCl₃, 300 MHz) δ/ppm: 9.50 (br s, 1H, OH), 7.49 (t, 4H, J = 8.7 Hz), 7.18–7.34 (m, 12H), 6.83 (d, 2H, J = 8.6 Hz), 6.61 (br s, 1H, OH).
2.1.5. Synthesis of Naphthols 32–40 and the Corresponding QM Derivatives 41 and 42

The synthesis of naphthols and their corresponding QM derivatives is described in detail. The structures and properties of the compounds are illustrated in Figure 5.

![Figure 5](image)

Figure 5: Naphthols and the corresponding QMs.

2-(2-Hydroxy-2-adamantyl)-3-methoxynaphthalene (32) was prepared in a Grignard reaction from 2-bromo-3-methoxynaphthalene and 2-adamantanone in 87% yield according to the published procedure. Characterization of product 32 compares well with literature (mp = 158–160 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 8.14 (d, 1H, J = 7.8 Hz), 7.52–7.57 (m, 3H), with a discernible doublet at 7.55, 6.75 (d, 1H, J = 8.2 Hz), 7.41–7.49 (m, 4H), 7.35–7.38 (m, 1H), 6.79 (d, 1H, J = 8.3 Hz), 5.26 (s, 2H), 2.67 (br s, 2H), 1.65 (br s, 1H), 1.70–1.82 (m, 6H).

1-Benzyl(2-hydroxy-2-adamantyl)naphthalene (39) was prepared by the reaction of 1-bromo-4-methoxynaphthalene with BBr₃ in 64% yield according to the published procedure. Characterization of product 39 compares well with literature (mp = 152–153 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 8.78–8.81 (m, 1H), 8.39–8.48 (m, 1H), 7.52–7.57 (m, 3H), with a discernible doublet at 7.55, 7.41–7.49 (m, 4H), 7.35–7.38 (m, 1H), 6.79 (d, 1H, J = 8.3 Hz), 5.26 (s, 2H), 2.67 (br s, 2H), 2.65 (br s, 1H), 1.95 (br s, 1H), 1.85 (s, 1H), 1.70–1.82 (m, 6H).

1-Hydroxy-5-(2-hydroxy-2-adamantyl)naphthalene (40) was obtained by catalytic hydrogenation of 1-benzyl(2-hydroxy-2-adamantyl)naphthalene in 82% yield according to the published procedure. Characterization of product 40 compares well with literature (mp = 152–153 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 8.28 (d, 1H, J = 8.8 Hz), 8.17 (d, 1H, J = 8.5 Hz), 7.65 (d, 1H, J = 7.4 Hz), 7.32 (dd, 1H, J = 8.2, 7.5 Hz), 7.16 (dd, 1H, J = 8.8, 7.5 Hz), 6.75 (d, 1H, J = 7.5 Hz), 2.70 (s, 1H), 2.69 (s, 1H), 1.87 (br s, 1H), 1.62–1.81 (m, 6H).

2-(2-Hydroxy-2-adamantyl)-4-methoxynaphthalene (38) was prepared from 2-amino-2-adamantyl-6-hydroxynaphthalene by reduction with Raney Ni in 19% yield according to the published procedure. Characterization of product 38 compares well with literature (mp = 256–258 °C). 1 H NMR (CDCl₃, 300 MHz) δ/ppm: 9.92 (s, 1H), 8.12 (br s, 3H), 7.98 (br s, 1H), 7.75–7.85 (m, 2H), 7.56–7.62 (m, 2H), 7.11–7.16 (m, 2H), 2.86 (br s, 2H), 2.34 (br s, 1H), 2.29 (br s, 1H), 1.63–1.97 (m, 10H).

2-(2-Amino-2-adamantyl)-6-hydroxynaphthalene hydrochloride (37) was obtained from 2-(2-azido-2-adamantyl)-6-hydroxynaphthalene by reduction with Raney Ni in 19% yield according to the published procedure. Characterization of product 37 compares well with literature (mp = 256–258 °C). 1 H NMR (CDCl₃, 300 MHz) δ/ppm: 9.92 (s, 1H), 8.12 (br s, 3H), 7.98 (br s, 1H), 7.75–7.85 (m, 2H), 7.56–7.62 (m, 2H), 7.11–7.16 (m, 2H), 2.86 (br s, 2H), 2.34 (br s, 1H), 2.29 (br s, 1H), 1.63–1.97 (m, 10H).

1-(2-Hydroxy-2-adamantyl)-4-methoxynaphthalene (39) was prepared in a Grignard reaction from 1-bromo-4-methoxynaphthalene and 2-adamantanone in 87% yield according to the published procedure. Characterization of product 39 compares well with literature (mp = 152–153 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 8.78–8.81 (m, 1H), 8.39–8.48 (m, 1H), 7.52–7.57 (m, 3H), with a discernible doublet at 7.55, 7.41–7.49 (m, 4H), 7.35–7.38 (m, 1H), 6.79 (d, 1H, J = 8.3 Hz), 5.26 (s, 2H), 2.67 (br s, 2H), 2.65 (br s, 1H), 1.95 (br s, 1H), 1.85 (s, 1H), 1.70–1.82 (m, 6H).

1-Hydroxy-5-(2-hydroxy-2-adamantyl)naphthalene (40) was obtained by catalytic hydrogenation of 1-benzyl(2-hydroxy-2-adamantyl)naphthalene in 82% yield according to the published procedure. Characterization of product 40 compares well with literature (mp = 152–153 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 8.28 (d, 1H, J = 8.8 Hz), 8.17 (d, 1H, J = 8.5 Hz), 7.65 (d, 1H, J = 7.4 Hz), 7.32 (dd, 1H, J = 8.2, 7.5 Hz), 7.16 (dd, 1H, J = 8.8, 7.5 Hz), 6.75 (d, 1H, J = 7.5 Hz), 2.70 (s, 1H), 2.69 (s, 1H), 1.87 (br s, 1H), 1.62–1.81 (m, 6H).

1-(2-Adamantylidene)naphthalene-2(1H)-one (41) was obtained in a reaction of 1-bromo-2-hydroxynaphthalene with BuLi and 2-adamantanone in 48% yield according to the published procedure. Characterization of product 41 compares well with literature (mp = 152–153 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 7.48–7.52 (m, 2H), 7.30–7.37 (m, 3H), 6.22 (d, 1H, J = 9.8 Hz), 4.10 (br s, 1H), 3.43 (br s, 1H), 1.94–2.02 (m, 6H), 1.84–1.90 (m, 4H), 1.77 (d, 2H, J = 12.2 Hz).

4-(2-Adamantylidene)naphthalene-1(4H)-one (42) was obtained by hydrogenation of 39 in 83% yield according to the published procedure. Characterization of product 42 compares well with literature (mp = 158–160 °C). 1 H NMR (CDCl₃, 600 MHz) δ/ppm: 8.14 (d, 1H, J = 10.3 Hz), 8.04 (dd, 1H, J = 7.8 Hz, J = 1.4 Hz), 7.71 (d, 1H, J = 7.8 Hz), 7.64 (dt, 1H, J = 1.3 Hz, J = 8.0 Hz), 7.51 (dt, 1H, J = 0.9 Hz, J = 7.4 Hz), 6.3 (d, 1H, J = 10.3 Hz), 3.73 (s, 1H), 3.61 (s, 1H), 1.85 2.17 (m, 12H).

2.2. Antimicrobial Testing

Preliminary antimicrobial screening of the compounds was
determined in duplicate using a modification of the Kirby-Bauer disc diffusion method. All compounds were dissolved in dimethyl sulfoxide (DMSO) and tested against *Staphylococcus aureus* ATCC 25923 (Gram-positive), *Bacillus subtilis* ATCC 6633 (Gram-positive), *Candida albicans* ATCC 10231 (fungus) *Escherichia coli* ATCC 25922 (Gram-negative) and *Klebsiella pneumoniae* ATCC 31488 (Gram-negative). The microbial cultures were grown overnight at 37 °C on Nutrient Agar plates (Biolab, South Africa), adjusted to 0.5 McFarlands standard using distilled water and lawn inoculated onto Mueller-Hinton agar (MHA) plates (Biolab, South Africa). 30 μL of each sample was inoculated onto antibiotic assay discs (6 mm diameter) and placed on the MHA plates which were incubated overnight at 37 °C and zones of inhibition were measured. DMSO was used as a control. Thereafter, minimum inhibitory concentrations (MIC) were determined in triplicate with compounds displaying antimicrobial activity using the broth dilution method. Serial dilutions (10000–19.56 μg/mL) of the compounds (with the exception of compounds 16 [4200–8.20 μg/mL] and 37 [4700–9.18 μg/mL]) were prepared from the stock solutions and tested against the cultures used in the preliminary antimicrobial activity studies.

3. Results and Discussion

MICs as determined by the broth dilution method are presented in Table 1. Generally, the compounds are not active against Gram-negative bacteria. The data suggest that the mode of action can be correlated with the ability of compounds to from QMs. The most active compound is QM 41. However, the structure of QM is also important since the other QM derivative 42 exerts no activity. To verify the proposed action mechanism of the investigated compounds it would be of significant importance to detect QMs formed in a metabolic process inside the living cells. However, such a detection is not warranted since all QMs except 41 and 42 are transient species, reactive intermediates with submillisecond lifetimes.

4. Conclusion

Five groups of compounds containing in total 42 phenol and naphthol derivatives were synthesized and screened for antimicrobial activity. Generally, compounds were found to be more active against Gram-positive bacteria suggesting some new mechanism of action. From the SAR studies it can be concluded that the mode of action can be correlated with the ability of compounds to from QMs. This hypothesis is to be further tested, and if shown to be true, will have an impact in the further design of antimicrobial agents.

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Supplementary Material

The online supplement contains a table with zones of inhibition (in mm) of the compounds (n = 3).

Table 1: MIC (μg/mL) of the compounds *(n = 3).*

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>Staphylococcus aureus</em> ATCC 25923</th>
<th>SD</th>
<th><em>Bacillus subtilis</em> ATCC 6633</th>
<th>SD</th>
<th><em>Candida albicans</em> ATCC 10231</th>
<th>SD</th>
<th><em>Escherichia coli</em> ATCC 25922</th>
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<td>–</td>
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<td>1</td>
<td>0.1</td>
<td>2</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>175</td>
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*The MIC is defined as the minimum drug concentration that inhibits growth of the organism and is represented as an average. The MICs were determined in triplicate, using the broth dilution method. The compounds were dissolved in DMSO and DMSO was used as a control.

(-) indicates no compound activity.

SD= standard deviation.
References


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<th><em>Bacillus subtilis</em> ATCC 6633</th>
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