Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 438–444
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Synthesis and antifungal activity of 2/3-arylthio- and 2,3-bis(arylthio)-5-hydroxy-/5-methoxy-1,4-naphthoquinones

Chung-Kyu Ryu *, Ju-Yeon Shim, Mi Jin Chae, Ik Hwa Choi, Ja-Young Han, Ok-Jai Jung, Jung Yoon Lee, Seong Hee Jeong

College of Pharmacy, Ewha Womans University, Seodaemun-ku, Seoul 120-750, South Korea

Received 10 July 2004; received in revised form 16 December 2004; accepted 21 December 2004

Available online 11 March 2005

Abstract

2/3-Arylthio- and 2,3-bis(arylthio)-5-hydroxy-/5-methoxy-1,4-naphthoquinones **5–9** were synthesized and tested for in vitro antifungal activity against *Candida* species and *Aspergillus niger*. The synthesized compounds **5–9** generally showed good activities against *Candida albicans* and *C. tropicalis*. The results suggest that the 1,4-naphthoquinones **5–9** would be potent antifungal agents.

Keywords: 1,4-Naphthoquinones; Juglone; Antifungal activity; Fungi

1. Introduction

Naphthoquinone compounds possess various biological activities [1–9]. An interesting sub-group of naphthoquinones is the 5-hydroxy-1,4-naphthoquinone. A number of 5-hydroxy-1,4-naphthoquinones such as juglone (**1**) and plumbagin (**2**) display potent biological properties including antimalarial activity [1] as well as antibacterial [2], cytotoxic properties [3], inhibitory effect of dihydroorotate dehydrogenase [4], trypanothione reductase [5] and topoisomerase [6]. The 2-(*n*-dodecyl)thio-3-hydroxy-1,4-naphthoquinone (**3**) blockades mitochondrial electron transport in *Saccaromyces cerevisiae*, as an inhibitor of mitochondrial cytochrome complex in yeast [7]. In previous reports [8,9], 2,3-disubstituted-1,4-naphthoquinones (**4**), which are derivatives of compounds **1–3**, had demonstrated antifungal activity against pathogenic fungi (Fig. 1). A variety of quinones with different substituents could exhibit the antifungal activity through different action and sometimes improves the activity. We assumed that incorporating an additional arylamino, arylthio or halo moiety to quinones would contribute to the improvement in biological efficacy [9,10]. Based on this speculation, 2/3-arylthio- and 2,3-bis(arylthio)-derivatives **5–9** of 5-hydroxy-/5-methoxy-1,4-naphthoquinone, as bioisosteres of the quinones **1–4**, were synthesized and their antifungal activity was evaluated (Fig. 1).

There have been a few reports on 5-hydroxy-1,4-naphthoquinone derivatives, exhibiting potent biological properties including antimalarial activity as well as antibacterial [1], and antiparasitic properties [5]. However, the antifungal activity of 5-hydroxy-/5-methoxy-1,4-naphthoquinone classes against *Candida* and *Aspergillus* species has not been reported to the best of our knowledge. Therefore, we synthesized a series of quinones **5–9** to elucidate their contribution to the antifungal activity. The in vitro antifungal activity of quinones **5–9** against pathogenic fungi was determined by the two-fold broth dilution method. Additional data for the antifungal activity of another 1,4-naphthoquinone derivative are provided.

2. Chemistry

A method for the synthesis of 1,4-naphthoquinones **5–9** (Table 1) is shown in Scheme 1. 5-Methoxy-1,4-naphthoquinone (**10**) and 2-bromo-5-hydroxy-1,4-naphthoquinone (**11**) were prepared from 5-hydroxy-1,4-naphthoquinone (**1**, juglone) according to the known method [11] with minor modification. Compound **10** was formed by methylation of compound **1** with CH_3I and Ag_2O in $CHCl_3$.

* Corresponding author. Tel.: +82 2 3277 2027; fax: +82 2 3277 3052.
E-mail address: ckryu@mm.ewha.ac.kr (C.-K. Ryu).

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2004.12.004

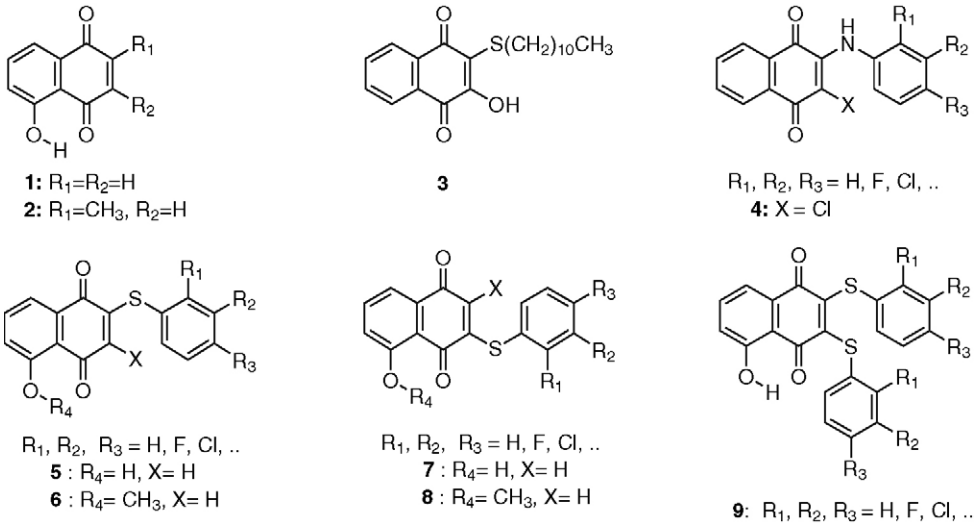


Fig. 1. 1,4-Naphthoquinone derivatives: juglone (**1**), plumbagin (**2**), 2-(*n*-dodecyl)thio-3-hydroxy-1,4-naphthoquinone (**3**) and 2,3-disubstituted-1,4-naphthoquinones (**4**).

Compounds **11** and **12** were prepared by bromination of compound **1** or **10**. The 2-arylthio-5-hydroxy-1,4-naphthoquinones (**5a**–**e**) were synthesized by regioselective nucleophilic substitutions of the compound **11** with appropriate arylthiols via palladium(0)-catalyzed reaction. The reaction of the compound **11** with the arylthiols smoothly proceeded in the presence of palladium catalyst, which might play a crucial role in the reactivity. Otherwise, the reaction in the absence of the palladium catalyst produced with very poor yields.

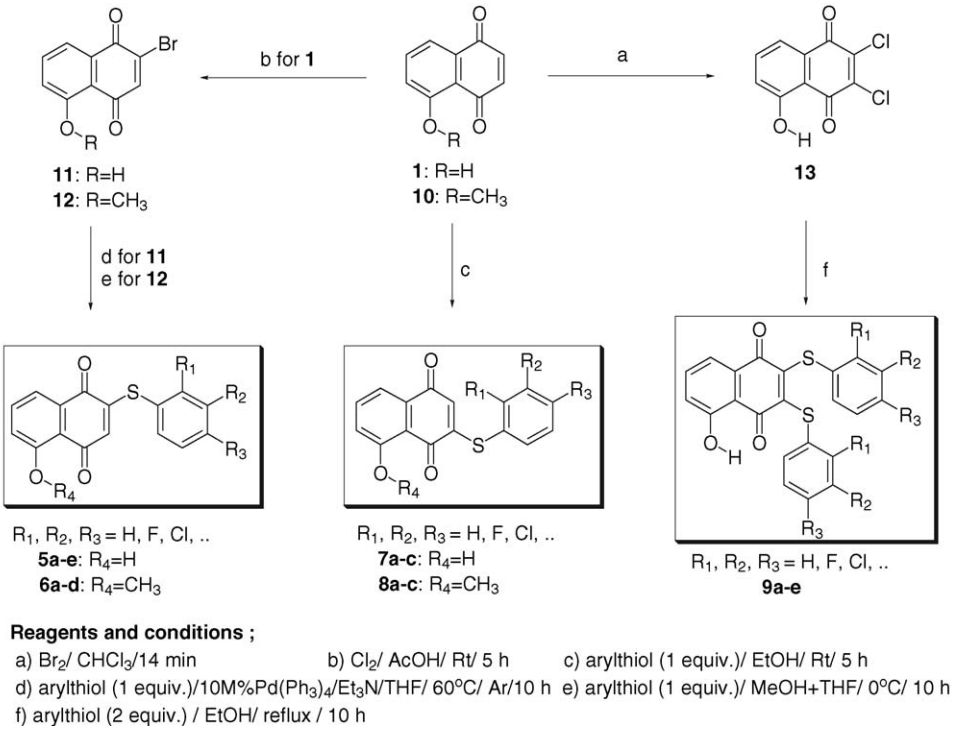
On the other hand, 2-arylthio-5-methoxy-1,4-naphthoquinones (**6a**–**d**) were prepared by regioselective nucleophilic substitutions of the compound **12** with arylthiols at 0 °C [12]. For the substitutions, the palladium catalyst is not essential. Without the catalyst, the substitution smoothly proceeded and had overall high yields of 65–80%.

Table 1
Structures and in vitro antifungal activities for 1,4-naphthoquinone series **5**–**9**

Compounds	R_1	R_2	R_3	MIC[a] (μg ml^{-1})			
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*A. niger*
5a	H	H	CH_3	4.0	4.0	>64.0	>64.0
5b	H	H	Cl	2.0	32.0	>64.0	8.0
5c	F	H	H	2.0	4.0	2.0	16.0
5d	H	H	F	2.0	2.0	8.0	4.0
5e	H	H	H	4.0	32.0	4.0	8.0
6a	H	H	CH_3	32.0	4.0	>64.0	>64.0
6b	H	H	Cl	16.0	64.0	16.0	>64.0
6c	F	H	H	8.0	4.0	4.0	>64.0
6d	H	F	H	32.0	32.0	4.0	>64.0
7a	H	H	CH_3	4.0	3.0	>64.0	8.0
7b	H	H	Cl	4.0	0.5	>64.0	4.0
7c	F	H	H	64.0	1.0	64.0	>64.0
8a	H	H	CH_3	2.0	4.0	2.0	8.0
8b	H	H	Cl	2.0	0.5	8.0	8.0
8c	H	F	H	16.0	32.0	16.0	>64.0
9a	H	H	CH_3	32.0	0.5	64.0	32.0
9b	H	H	Cl	4.0	0.5	4.0	>64.0
9c	H	H	F	2.0	0.5	4.0	2.0
9d	F	H	F	8.0	0.5	>64.0	4.0
9e	H	H	H	64.0	4.0	8.0	8.0
1				16.0	8.0	64.0	16.0
4a	H	H	F	16.0	32.0	32.0	16.0
10				64.0	2.0	32.0	8.0
Amphotericin **B**				0.25	0.5	1.0	2.0

[a] The MIC values of the compounds against *Candida* spp. and *A. niger* were determined by broth micro dilution testing in accordance with the guidelines in NCCLS document M27-A and M38-P [14].

[b] Fungi tested: *C. albicans* Berkout KCCM 50235, *C. tropicalis* Berkout KCCM 50662, *C. krusei* Berkout KCCM 11655 and *A. niger* KCTC 1231.



Scheme 1. Synthesis of juglone derivatives **5**–**9**.

It is well known that substitution of arylthiols to juglone (**1**) proceeds regioselectively, the substitutions giving mainly the 3-substituted juglone product along with the 2-substituted juglone as by-product [13]. 3-Arylthio-5-hydroxy-/5-methoxy-1,4-naphthoquinones (**7a**–**c**) and (**8a**–**c**) were prepared by the substitution on compound **1** or **10** with the arylthiols. The products **7a**–**c** and **8a**–**c** were separated by silica gel column chromatography with $CHCl_3$.

2,3-Bis(arylthio)-5-hydroxy-1,4-naphthoquinones (**9a**–**e**) were synthesized by nucleophilic substitution on 2,3-dichloro-5-hydroxy-1,4-naphthoquinone (**13**) with two equivalents of the arylthiols. Most of the substitutions went as expected and had an overall yield of 40–54%.

3. Results and discussion

The 5-hydroxy-1,4-naphthoquinone series **5**–**9** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard broth dilution method according to the NCCLS guidelines [13,14]. The minimum inhibitory concentration (MIC) values were determined by comparison with amphotericin **B** as a standard agent. As represented in Table 1, most of the 5-hydroxy-1,4-naphthoquinones series **5**–**9** generally showed potent antifungal activity against *Candida albicans* and *C. tropicalis*. Actually, the activity of compounds **5d**, **8a**, **8b** and **9c** was comparable to those of amphotericin **B** against *C. tropicalis*. The 5-hydroxy-1,4-naphthoquinones **5d**, **8a**, **8b** and **9c** completely inhibited the growth of all fungal species tested at the MIC level of 0.25–8.0 μg ml^{-1}. In addition, the compound **1** or **10** without the arylthio group exhibited weak antifungal activity. Thus, the arylthio moiety of compounds **5**–**9** appears to partially contribute to biological potency.

The structure–activity relationship may not exist between properties of substituents (R_1, R_2, R_3: H, CH_3, Cl, F, ..) for the arylthio moieties of compounds **5**–**9**. The compounds such as **5d** and **9c** containing 4-flouro-phenylthio substituent exhibited the potent antifungal activity.

The cytotoxic potential of compounds **5**–**9** has also been determined in human cancer cells according to the NCI protocols [15]. As represented in Table 2, the compounds **5d**, **8a**, **8b** and **9c** did not show significant cytotoxic activity but showed the selectivity, in that they possess potent antifungal activities.

4. Conclusions

2/3-Arylthio- and 2,3-bis(arylthio)-5-hydroxy-/5-methoxy-1,4-naphthoquinones **5**–**9** were synthesized from 5-hydroxy-1,4-naphthoquinones and tested for in vitro antifungal activity against *Candida* species and *Aspergillus niger*. The synthesized compounds **5**–**9** generally showed good activities against *C. albicans* and *C. tropicalis*. The results of this study suggest that 5-hydroxy-1,4-naphthoquinone series **5**–**9** would be potent antifungal agents. Moreover, the results should encourage the synthesis of 5-hydroxy-1,4-naphthoquinone analogs for improving antifungal properties.

Table 2
Cytotoxic activity of 1,4-naphthoquinone series **5–9**

Compounds	Cytotoxicity[a] IC_{50} (μg ml^{-1})		
	A 549[b]	SK-OV-3	SK-MEL-2
5a	27.90	1.28	0.61
5b	21.56	16.10	>100
5c	76.83	13.87	3.20
5d	25.00	36.01	32.50
5e	3.20	>100	3.20
6a	25.00	>100	>100
6b	16.10	5.89	12.50
6c	13.87	21.56	6.30
6d	36.01	76.83	16.21
7a	>100	16.10	27.90
7b	>100	13.87	21.56
7c	5.88	36.01	76.83
8a	27.90	>100	23.24
8b	21.56	>100	16.10
8c	76.83	5.88	13.87
9a	16.10	27.90	36.01
9b	13.87	21.56	>100
9c	36.01	76.83	>100
9d	>100	0.80	5.88
9e	>100	13.9	12.7
Adriamycin	0.07	0.28	0.02

[a] Cytotoxicity evaluation: SRB assay according to NCI protocols [14].
[b] Human tumor cell lines: A 549 (non-small cell lung), SK-OV-3 (ovarian), SK-MEL-2 (melanoma), HCT-15 (colon) and XF 498 (CNS) from National Cancer Institute (NCI) in USA.

5. Experimental protocols

5.1. Chemistry

All melting points (m.p.) were measured in open capillary tubes with a Büchi m.p. B-545 and were uncorrected. TLC was performed on precoated silica gel (60G 254, Merck) using chloroform as a solvent. The compounds were detected under UV light (254 nm) or by heating to 110 °C after spraying with a 30% H_2SO_4-vanillin solution. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). IR spectra were taken with Perkin Elmer 1420r IR spectrometer with KBr pellets. ^{1}H-NMR spectra were recorded on Unity Varian INOVA 400 MHz FT-NMR spectrometer using DMSO-d_6 as a solvent, and chemical shifts were given in ppm with TMS as a standard. High-resolution mass spectra (HRMS EI) were obtained on a Jeol JMS AX505 WA. 5-Hydroxy-1,4-naphthoquinone, arylamines, DMSO-d_6 and other reagents were obtained from Aldrich Chemical Co. Reagents for biological screening was obtained from Sigma Co.

*5.1.1. General procedure for the synthesis of 2-arylthio-5-hydroxy-1,4-naphthoquinones (**5**)*

A mixture of 2-bromo-5-hydroxy-1,4-naphthoquinone (**11**) (2 g, 0.8 mmol), $(Ph_3P)_4Pd$ (92 mg, 0.08 mmol), triethylamine (110 μl) and arylthiol (0.8 mmol) in 10 ml THF was heated in a capped heavy-walled pyrex tube under an argon atmosphere for 10 h at 60 °C. After cooling, the reaction mixture was filtered through celite to remove Pd catalyst, and then the mixture was extracted with three 100 ml portions of diethyl ether. The crude product was purified by silica gel column chromatography with *n*-hexane/EtOAc. Crystallization from aq. EtOH afforded the 2-arylthio-5-hydroxy-1,4-naphthoquinones (**5a–e**).

*5.1.1.1. 5-Hydroxy-2-(4-methylphenylthio)-1,4-naphthoquinone (**5a**).* Light brown needle (yield; 60%); m.p. 167–169 °C; ^{1}H-NMR (DMSO-d_6) δ 11.90 (s, 1H, OH), 7.74 (t, 1H, benzene), 7.61 (d, 1H, benzene), 7.36 (m, 4H, Ph–H), 5.86 (s, 1H, H3), 2.46 (s, 3H, CH_3); HRMS Anal. Calc. for $C_{17}H_{12}O_3S$, 296.0507, Found: 296.0508.

*5.1.1.2. 2-(4-Chlorophenylthio)-5-hydroxy-1,4-naphthoquinone (**5b**).* Brown needle (yield; 89%); m.p. 172–174 °C; ^{1}H-NMR (DMSO-d_6) δ 11.87 (s, 1H, OH), 7.75 (m, 1H, benzene), 7.68 (m, 4H, Ph–H), 7.61 (d, 1H, benzene), 5.91 (S, 1H, H3); HRMS Anal. Calc. for $C_{16}H_9ClO_3S$, 315.9961, Found: 315.9960.

*5.1.1.3. 2-(2-Fluorophenylthio)-5-hydroxy-1,4-naphthoquinone (**5c**).* Light brown needle (yield; 32%); m.p. 148–149 °C; ^{1}H-NMR (DMSO-d_6) δ 11.82 (s, 1H, OH), 7.72 (m, 3H, benzene), 7.63 (d, 1H, benzene), 7.54 (t, 1H, benzene), 7.45 (t, 1H, benzene), 7.38 (d, 1H, benzene), 5.75 (s, 1H, H2); HRMS Anal. Calc. for $C_{16}H_9FO_3S$, 300.0257, Found: 300.0256.

*5.1.1.4. 2-(4-Fluorophenylthio)-5-hydroxy-1,4-naphthoquinone (**5d**).* Light red needle (yield; 82%); m.p. 141–142 °C; ^{1}H-NMR (DMSO-d_6) δ 11.86 (s, 1H, OH), 7.75 (t, 1H, benzene), 7.70 (m, 2H, Ph–H), 7.62 (d, 1H, benzene), 7.48 (m, 2H, Ph–H), 7.38 (d, 2H, Ph–H), 5.87 (s, 1H, H3); HRMS Anal. Calc. for $C_{16}H_9FO_3S$, 300.0257, Found: 300.0257.

*5.1.1.5. 5-Hydroxy-2-(phenylthio)-1,4-naphthoquinone (**5e**).* Red-brown needle (yield; 60%); m.p. 142–143 °C, ^{1}H-NMR (DMSO-d_6) δ 11.88 (s, 1H, OH), 7.74 (t, 1H, benzene), 7.66–7.60 (m, 5H, Ph–H), 7.37 (d. 1H, benzene), 5.84 (s, 1H, H3); HRMS Anal. Calc. for $C_{16}H_{10}O_3S$, 282.0351, Found: 282.0350.

*5.1.2. General procedure for the synthesis of 5-arylthio-5-methoxy-1,4-naphthoquinones (**6**)*

A solution of arylthiol (5 mmol) and KOH (5 mmol) in 10 ml of MeOH was added dropwise, over 15 min, to a solution of 2-bromo-5-methoxy-1,4-naphthoquinone (**12**) (5 mmol) in 12 ml of THF and 2 ml of MeOH at 0 °C. The reaction mixture was stirred at this temperature for 2 h and then concentrated under reduced pressure. The residue was taken up in 100 ml of CH_2Cl_2 and washed with 30 ml of water and 30 ml of brine. The crude product was purified by silica

gel column chromatography with *n*-hexane/EtOAc. Crystallization from aq. EtOH afforded the 5-arylthio-5-methoxy-1,4-naphthoquinones (**6a**–**d**).

*5.1.2.1. 5-Methoxy-2-(4-methylphenylthio)-1,4-naphthoquinone (**6a**).* Brown plate (yield; 80%); m.p. 132–134 °C; ^{1}H-NMR (DMSO-d_6) δ 7.79 (t, 1H, benzene), 7.67 (d, 1H, benzene), 7.57 (d, 1H, benzene), 7.49 (d, 2H, Ph–H), 7.41 (d, 2H, Ph–H), 5.72 (s, 1H, H1), 3.88 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3); HRMS Anal. Calc. for $C_{18}H_{14}O_3S$, 310.0664, Found: 310.0663.

*5.1.2.2. 2-(4-Chlorophenylthio)-5-methoxy-1,4-naphthoquinone (**6b**).* Yellow powder (yield; 73%), m.p. 132–134 °C, ^{1}H-NMR (DMSO-d_6) δ 7.81 (t, 1H, benzene), 7.68 (d, 1H, benzene), 7.65 (s, 4H, Ph–H), 7.59 (d, 1H, benzene), 5.78 (s, 1H, H1), 3.89 (s, 3H, OCH_3). HRMS Anal. Calc. for $C_{17}H_{11}ClO_3S$, 330.0118, Found: 330.0117.

*5.1.2.3. 2-(2-Fluorophenylthio)-5-methoxy-1,4-naphthoquinone (**6c**).* Brown needle (yield; 68%); m.p. 181–182 °C; ^{1}H-NMR (DMSO-d_6) δ 7.80 (d, 1H, Ph–H), 7.70 (m, 3H, benzene), 7.60 (d, 1H, Ph–H), 7.52 (t, 1H, Ph–H), 7.43 (t, 1H, Ph–H), 5.72 (s, 1H, H2), 3.89 (s, 3H, OCH_3); HRMS Anal. Calc. for $C_{17}H_{11}FO_3S$, 314.0413, Found: 314.0414.

*5.1.2.4. 2-(3-Fluorophenylthio)-5-methoxy-1,4-naphthoquinone (**6d**).* Yellow powder (yield; 65%); m.p. 127–128 °C; ^{1}H-NMR (DMSO-d_6) δ 7.81 (t, 1H, benzene), 7.69 (m, 1H, benzene), 7.64 (m, 1H, benzene), 7.58 (m, 2H, Ph–H), 7.47 (m, 2H, Ph–H), 5.83 (s, 1H, H2), 3.89 (s, 3H, OCH_3); HRMS Anal. Calc. for $C_{17}H_{11}FO_3S$, 314.0413, Found: 314.0413.

*5.1.3. General procedure for the synthesis of 3-arylthio-5-hydroxy-1,4-naphthoquinones (**7**)*

A solution of 5-hydroxy-1,4-naphthoquinone (**1**, 0.1 g, 0.57 mmol) in 100 ml of 95% EtOH was added to the solution of the arylamine (0.57 mmol) in 5 ml of 95% EtOH and stirred at r.t. for 2 h and then refluxed for 5 h After the mixture was kept overnight in a refrigerator or poured into 20 ml of ice water, the precipitate was collected by filtration. The filtered crude product was purified by silica gel column chromatography with $CHCl_3$ and crystallized from 95% EtOH.

*5.1.3.1. 5-Hydroxy-3-(4-methylphenylthio)-1,4-naphthoquinone (**7a**).* Black powder (yield; 32%); m.p. 142–147 °C; IR (KBr) 3061 (w), 1650 (s, C=O), 1630 (s), 1452–1561, 1263 (s), 1094 (s) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.42 (s, 1H, OH), 7.75 (t, 2H, Ph–H), 7.52 (d, 2H, Ph–H), 7.45 (q, 3H, benzene), 7.34 (d, 1H, benzene), 5.8 (s, 1H, H2), 2.4 (s, 3H, CH_3); HRMS Anal. Calc. for $C_{17}H_{12}O_3S$, 296.0507, Found: 296.0509.

*5.1.3.2. 3-(4-Chlorophenylthio)-5-hydroxy-1,4-naphthoquinone (**7b**).* Brown powder (yield; 26%); m.p. 164–166 °C; IR (KBr) 3061 (w), 1650 (s, C=O), 1630 (s), 1452–1527, 1263 (s), 1094 (s) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.39 (s, 1H, OH), 7.76 (t, 1H, benzene), 7.67 (s, 4H, Ph–H), 7.48 (d, 1H, benzene), 7.35 (d, 1H, benzene), 5.9 (s, 1H, H2); HRMS Anal. Calc. for $C_{16}H_9ClO_3S$, 315.9961, Found: 315.9960.

*5.1.3.3. 3-(3-Fluorophenylthio)-5-hydroxy-1,4-naphthoquinone (**7c**).* Dark brown powder (yield; 50%); m.p. 169–171 °C; IR (KBr) 3072 (w), 1631 (s, C=O), 1459–1567, 1287 (s) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.4 (s, 1H, OH), 7.76 (t, 3H, benzene), 7.68 (q, 1H, benzene), 7.59 (dt, 1H, benzene), 7.49 (t, 3H, benzene), 7.59 (d, 1H, benzene), 5.89 (s, 1H, H2); HRMS Anal. Calc. for $C_{16}H_9FO_3S$, 315.9961, Found: 315.9962.

*5.1.4. General procedure for the synthesis of 3-arylthio-5-methoxy-1,4-naphthoquinones (**8**)*

A solution of 5-methoxy-1,4-naphthoquinone (**10**) (0.1 g, 0.57 mmol) in 100 ml of 95% EtOH was added to the solution of the arylamine (0.57 mmol) in 5 ml of 95% EtOH and stirred at r.t. for 2 h and then refluxed for 5 h. After the mixture was kept overnight in the refrigerator or poured into 20 ml of ice water, the precipitate was collected by filtration. The filtered crude product was purified by silica gel column chromatography with $CHCl_3$ and crystallized from 95% EtOH.

*5.1.4.1. 5-Methoxy-3-(4-methylphenylthio)-1,4-naphthoquinone (**8a**).* Orange powder (yield; 26%); m.p. 146–148 °C; IR (KBr) 3090 (w), 1663 (s, C=O), 1444–1584 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.81 (q, 1H, benzene), 7.54 (dd, 2H, benzene), 7.52 (d, 1H, benzene), 7.41 (d, 1H, benzene), 6.94 (dd, 1H, benzene), 6.82 (dd, 1H, benzene), 5.77 (s, 1H, H2), 3.95 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3); HRMS Anal. Calc. for $C_{18}H_{14}O_3S$, 310.0664, Found: 310.0663.

*5.1.4.2. 3-(4-Chlorophenylthio)-5-methoxy-1,4-naphthoquinone (**8b**).* Orange powder (yield; 27%); m.p. 135–138 °C; IR (KBr) 3041 (w), 1653 (s, C=O), 1461–1587 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.80 (q, 1H, benzene), 7.72 (dd, 2H, benzene), 7.66 (dd, 2H, benzene), 7.41 (d, 1H, benzene), 6.91 (dd, 1H, benzene), 6.80 (dd, 1H, benzene), 5.76 (s, 1H, H2), 3.98 (s, 3H, OCH_3); HRMS Anal. Calc. for $C_{16}H_9ClO_3S$, 330.0118, Found: 330.0117.

*5.1.4.3. 3-(3-Fluorophenylthio)-5-methoxy-1,4-naphthoquinone (**8c**).* Yellow powder (yield; 38%); m.p. 196–198 °C; IR (KBr) 3063 (w), 2838 (m), 1651 (s, C=O), 1470–1583, 1213 (m) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.82 (t, 1H, benzene), 7.66 (m, 2H, benzene), 7.55 (dd, 3H, benzene), 7.47 (d, 1H, benzene), 5.87 (s, 1H, H2), 3.98 (s, 3H, OCH_3); HRMS Anal. Calc. for $C_{17}H_{11}FO_3S$, 314.0413, Found: 314.0413.

*5.1.5. General procedure for the synthesis of 2.3-bisarylthio-5-hydroxy-1,4-naphthoquinones (**9**)*

A solution of 2,3-dichloro-5-hydroxy-1,4-naphthoquinone (**13**) (0.57 mmol), arylthiol (1.14 mmol) in 100 ml of 95%

EtOH was refluxed for 5 h. After the mixture was kept overnight in the refrigerator or poured into 20 ml of ice water, the precipitate was collected by filtration. The filtered crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH.

*5.1.5.1. 5-Hydroxy-2,3-bis(4-methylphenylthio)-1,4-naphthoquinone (**9a**).* Violet powder (yield; 54%); m.p. 154–157 °C; IR (KBr) 2927 (s), 1905 (m), 1670 (s, C=O), 1615 (s), 1454–1520, 1268 (s), 1015 (m) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.62 (s, 1H, OH), 7.54–7.48 (m, 3H, benzene), 7.36 (d, 1H, benzene), 7.28 (s, 2H, benzene), 7.20 (dd, 1H, benzene), 7.16 (d, 1H, benzene), 7.12 (d, 5H, benzene), 2.45 (s, 6H, $2CH_3$); HRMS Anal. Calc. for $C_{24}H_{18}O_3S_2$, 418.0697, Found: 418.0698.

*5.1.5.2. 2,3-Bis(4-chlorophenylthio)-5-hydroxy-1,4-naphthoquinone (**9b**).* Dark brown powder (yield; 43%); m.p. 186–189 °C; IR (KBr) 3077 (s), 1665 (s, C=O), 1626 (s), 1474–1510, 1267 (s), 1091 (s) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.42 (s, 1H, OH), 7.70 (t, 2H, benzene), 7.47 (m, 4H, benzene), 7.38 (m, 3H, benzene), 7.34 (d, 2H, benzene); HRMS Anal. Calc. for $C_{22}H_{12}Cl_2O_3S_2$, 457.9605, Found: 457.9606.

*5.1.5.3. 2,3-Bis(4-fluorophenylthio)-5-hydroxy-1,4-naphthoquinone (**9c**).* Brown powder (yield; 40%); m.p. 129–132 °C; IR (KBr) 3083 (s), 1652 (s, C=O), 1623 (s), 1453–1568 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.4 (s, 1H, OH), 7.70 (t, 1H, benzene), 7.51 (t, 2H, benzene), 7.45 (d, 1H, benzene), 7.40–7.26 (m, 5H, benzene), 7.19–7.15 (m, 2H, benzene); HRMS Anal. Calc. for $C_{22}H_{12}F_2O_3S_2$, 426.0196, Found: 426.0196.

*5.1.5.4. 2,3-Bis(2,4-difluorophenylthio)-5-hydroxy-1,4-naphthoquinone (**9d**).* Red brown powder (yield; 42%); m.p. 176–178 °C. IR (KBr) 3083 (s), 1672 (s, C=O), 1631 (s), 1421–1507, 1219 (m) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.4 (s, 1H, OH), 7.69 (t, 1H, benzene), 7.63 (qd, 2H, benzene), 7.44 (dd, 1H, benzene), 7.42–7.36 (m, 2H, benzene), 7.33 (dd, 2H, benzene), 7.14–7.08 (m, 2H, benzene); HRMS Anal. Calc. for $C_{22}H_{10}F_4O_3S_2$, 462.0007, Found: 462.0008.

*5.1.5.5. 5-Hydroxy-2,3-bis(phenylthio)-1,4-naphthoquinone (**9e**).* Dark brown powder (yield; 45%); m.p. 125–126 °C; IR (KBr) 3051 (s), 1669 (s, C=O), 1631 (s), 1453–1502, 1263 (s) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 11.51 (s, 1H, OH), 7.69 (q, 2H, benzene), 7.51 (t, 1H, benzene), 7.42–7.25 (m, 10H, Ph–H); HRMS Anal. Calc. for $C_{22}H_{14}O_3S_2$ 390.0384, Found: 390.0387.

5.2. Antifungal in vitro susceptibility testing

The MIC values of compounds **5**–**9** were determined by the standard broth dilution method [13,14]. MIC of the compounds against *Candida* spp. and *Aspergillus* spp. was determined by Broth micro dilution testing in accordance with the guidelines in NCCLS document M27-A and M38-P [13,14]. Briefly, stock solutions were prepared in water for fluconazole and DMSO for the compounds of the present study. Serial twofold dilution of all the compound and standard drug were made in RPMI1640 medium buffered to pH 7.0 with 0.165 M 4-morpholinepropanesulfonic acid (MOPS) buffer as outlined in NCCLS M27-A document. Aliquots of (0.1 ml) of each compound at a 2× final concentration were dispensed into the wells of micro titer plates. The final concentration of solvent did not exceed 1% in any well. An inoculum concentration of $(1.5 \pm 1.0) \times 10^3$ cells per ml was prepared by spectrometric method of inoculum preparation for each organism tested. Hundred microliters on individual fungal inoculum were added to each well of micro titer plate containing the compounds. The final concentration of all the compounds and drug were 64–0.12 μg ml^{-1}. The plates were incubated at 35 °C. MIC endpoints were read after 48 h incubation for *Candida* spp. and after 72 h for *Aspergillus* spp. After the completion of incubation, the growth in each well was compared with that of the growth control well. The MIC of each compound was defined as the lowest concentration that produced 90% inhibition in the growth of the organism compared with that of the drug free control.

Acknowledgements

This study was supported by a grant of the Korea Science and Engineering Foundation (KOSEF R06-2002-011-01004). We thanks to Dr. B.H. Lee of Wonkwang University for the SRB assay.

References

[1] J. Krungkrai, R. Kanchanarithisak, S.R. Krungkrai, S. Rochanakij, Exp. Parasitol. 100 (2002) 54–61.

[2] L. Cai, G.X. Wei, P. van der Bijl, C.D. Wu, J. Agric. Food Chem. 48 (2000) 909–914.

[3] J.J. Inbaraj, C.F. Chignell, Chem. Res. Toxicol. 17 (1) (2004) 55–62.

[4] W. Knecht, J. Henseling, M. Löffler, Chem. Biol. Interact. 124 (2002) 61–76.

[5] L. Salmon-Chemin, E. Buisine, V. Yardley, S. Kohler, M.-A. Debreu, V. Landry, et al., J. Med. Chem. 44 (2001) 548–565.

[6] H. Wang, Y. Mao, A.Y. Chen, N. Zhou, E.J. LaVoie, L.F. Liu, Biochemistry 40 (2001) 3316–3323.

[7] H. Roberts, W.M. Choo, S.C. Smith, S. Marzuki, A.W. Linnane, T.H. Porter, et al., Arch. Biochem. Biophys. 191 (1978) 306–315.

[8] C.-K. Ryu, D.Y. Kim, Arch. Pharm. Res. 16 (1993) 161–166.

[9] V.K. Tandon, R.B. Chhor, R.V. Singh, S.J. Rai, D.B. Yadav, Bioorg. Med. Chem. Lett. 14 (2004) 1079–1083.

[10] C.-K. Ryu, K.U. Choi, J.-Y. Shim, H.-J. You, I.H. Choi, M.J. Chae, Bioorg. Med. Chem. 11 (2003) 40039–44008.

[11] J. Grunwell, A. Karipides, C. Wigal, S. Heinzman, J. Parlow, J. Surso, et al., J. Org. Chem. 56 (1991) 91–95.

[12] S. Laugraud, A. Guingant, C. Chassagnard, J. D'Angelo, J. Org. Chem. 53 (1988) 1557–1560.

[13] National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing for Yeasts. Approval Standard. Document M27-A, National Committee for Clinical Laboratory Standards, Wayne, PA, 1997.

[14] National Committee for Clinical Laboratory Standards (NCCLS), Methods for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi. Proposed Standard (M38-P), National Committee for Clinical Laboratory Standards, Wayne, PA, 1998.

[15] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, et al., J. Natl. Cancer Inst. 82 (1990) 1107–1112.