Arch Pharm Res Vol 25, No 6, 795-800, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis and Antifungal Activity of 6,7-Bis-[*S*-(Aryl)thio]-5,8-Quinolinediones

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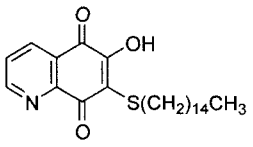
(Received August 1, 2002)

6,7-Bis-[*S*-(aryl)thio]-5,8-quinolinediones **4** and **5** were synthesized by the substitution of 6,7-dichloro-5,8-quinolinediones with appropriate arylthiols. Their antifungal activity were tested *in vitro* for their growth inhibitory activities against pathogenic fungi in comparison with flucytosine. The antifungal activities were significantly improved by S-(aryl)thio moieties of the compounds **4** and **5**. The all tested compounds **4** and **5** showed generally good activities against *C. albicans* and *A. niger* ranging from 0.8 to 25 μg/ml. Among them, compounds **4d-4h** and **5a-5c** exhibited also good activities against *C. krusei* and *C. tropicalis*. The activities of compounds **4j** and **4l** were comparable to those of flucytosine against all tested fungi.

Key words: 6,7-Bis-[*S*-(aryl)thio]-5,8-quinolinediones, Antifungal activity, MIC minimum inhibitory concentration, *Candida* species, *A. niger*

INTRODUCTION

Due to an increase of mycotic infections and frequent accounts of resistance there has been a renewed interest in developing of new antifungal agents (Groll *et al.*, 1998; Tkacz *et al.*, 2001). To identify new antifungal agents, we focused on developing 5,8-disubstituted-quinolinediones with novel mode of antifungal action (Roberts *et al.*, 1978). Among them, the 7-[(n-hexadecyl)thio]-6-hydroxy-5,8-quinolinedione **1** blockaded a mitochondrial electron transport in *Saccharomyces cerevisiae*, that may be correlated with the antifungal activity (Roberts *et al.*, 1978). 6,7-Disubstituted-5,3-quinolinediones (Fig. 1) showed antifungal activities (Jaschke *et al.*, 1993, Cheng *et al.*, 1995). Our previous works showed that some 6-arylamino-7-chloro-/ 6-arylamino-7-methylthio-5,8-quinolinediones (**2-3**) exhibited potent antifungal activities against pathogenic fungi (Ryu *et al.*, 1994; Ryu *et al.*, 1998). On the line of this study, we further extended to synthesize 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinedione derivatives **4-5**, and evaluated their antifungal activities (Fig. 1). A variety of quinones with different substituents could exhibit the activities with different action and sometimes improve the activities. The presence of thio, halo and alkyl substituents of quinones improved sometimes their antigungl activities (Ryu *et al.*, 1998; Ryu *et al.*, 2000). Based on these considerations, the 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones (**4a-4o**), 6,7-bis-[*S*-(aryl)thio]-2-chloro-4-methyl-5,8-quinolinediones (**5a-5b**) and 2,6,7-tri-



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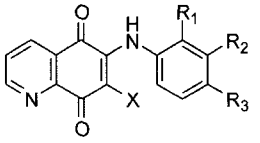
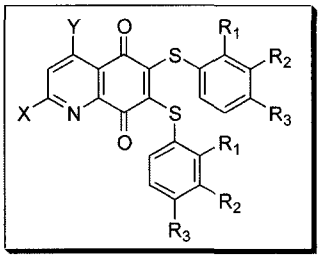
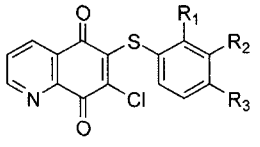


R_1, R_2, R_3 = H, F, Cl, ..
2: X= Cl or Br
3: X= S-CH_3



R_1, R_2, R_3 = H, F, Cl, ..
4: X= Y= H
5: X= Cl or S-Ph, Y= CH_3



R_1, R_2, R_3 = H, F, Cl, ..

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Fig. 1. 6,7-Disubstituted-5,8-quinolinediones

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[*S*-(aryl)thio]-4-methyl-5,8-quinolinedione (**5c**) with various substituents were synthesized and evaluated for their antifungal activities (Scheme 1, Scheme 2, Table I and Table II). The *in vitro* antifungal activities of the 5,8-quinolinediones **4-5** against pathogenic fungi were determined by the twofold broth dilution method.

MATERIALS AND METHODS

All melting points were measured BÜCHI melting point B-545 and were uncorrected. IR spectra were taken from Bio-Rad FTS-135 FT-IR spectrometer with KBr pellets. ^{1}H-NMR spectra were recorded on Varian Technology 400 MHz FT-NMR spectrometer using $CDCl_3$ or DMSO-d_6 as solvent, and chemical shifts were given in δ ppm with TMS as standard. Mass spectra were obtained on JMS AX 505 WA spectrometer (electronic impact at 70 eV). High resolution mass spectra (HRMS) were taken from JMS. AX505. WA (Jeol, Japan) to High resolution mode. UV spectrophotometer from Shimadzu UV-120-02 was used. Column chromatography was performed on silica gel G60 (70-230 mesh, ASTM, Merck). 8-Hydroxyquinoline, cerium(III) chloride heptahydrate, arylthiols, $CDCl_3$, $DMSO_{-d6}$ and other reagents were obtained from Aldrich Chemical Co. Modified Sabouraud dextrose broth and brain heart infusion (BHI) broth were purchased from Difco Lab. Other chemicals such as flucytosine were reagent grade commercially available. The microorganisms were incubated in an incubator bath from Sanyo Co.

General procedure for synthesis of 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones (4)

6,7-Dichloro-5,8-quinolinedione (**6**) was preparec according to a procedure described in the previous paper (Pratt *et al.*, 1960, Ryu *et al.*, 1994). A solution of 6,7-dichloro-5,8-quinolinedione **6** (0.227 g, 1 mmol) in 15 mL of 95% EtOH was added to the solution of the arylthiol (2.1 mmol) in 5 mL of 95% EtOH and stirred at room temperature for 2 h and then refluxed for 45 h. After the reaction mixture was kept overnight in the refrigerator or poured into 20 mL of ice water, the precipitate was collected by filtration. The crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH or MeOH. Crystallization from aq. EtOH afforded the 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones (**4a-4o**).

6,7-Bis-[*S*-(phenyl)thio]-5,8-quinolinedione (4a) Yield 66%; orange powder; mp: 164-166°C; IR (KBr): ν3354, 3058, 1671 (s, C=O), 1440-1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.69 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.99 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.81 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.45 (d, 2H, H14, H21), 7.21 (d, 2H, H17, H24), 6.93 (d, 2H, H16, H23); MS (m/z): 375 (M$^+$), 268, 266, 77, 76; HRMS: Anal. calcd for $C_{21}H_{13}NO_2S_2$ 375.4704, Found: 375.4740.

6,7-Bis-[*S*-(4-methylphenyl)thio]-5,8-quinolinedione (4b) Yield 81%; dark orange powder; mp: 175-178°C; IR (KBr): ν3452, 3010, 1668 (s, C=O), 1494-1582 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.42 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.61 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 8.15 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.62 (d, 2H, H14, H18), 7.12 (d, 2H, H15, H17), 2.45 (s, 3H, CH_3); MS (m/z): 403 (M$^+$), 388, 280, 77, 76; HRMS: Anal. calcd for $C_{23}H_{17}NO_2S_2$ 403.5207, Found: 403.5102.

6,7-Bis-[*S*-(3-methylphenyl)thio]-5,8-quinolinedione (4c) Yield 47%; orange powder; mp: 156-158°C; IR (KBr): ν3587, 3049, 1665 (s, C=O), 1454-1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.98 (dd, *J*=4.7, 7.8, 1.5, 1H, H2), 8.73 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.86 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.83(d, 2H, H14, H25), 7.31 (d, 2H, H15, H24), 7.09 (d, 2H, H18, H29), 2.50 (s, 3H, CH_3); MS (m/z): 403 (M$^+$), 388, 280, 77, 76; HRMS: Anal. calcd for $C_{23}H_{17}NO_2S_2$ 403.5207, Found: 403.5099.

6,7-Bis-[*S*-(3,4-dimethylphenyl)thio]-5,8-quinolinedione (4d) Yield 50%; brown powder; mp: 132-135°C; IR (KBr): ν3558, 3082, 1667 (s, C=O), 1453-1582 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.95 (dd, *J*=4.7, 7.8, 1.5, 1H, H2), 8.27 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.80 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.22 (d, 2H, H18, H21), 7.13 (d, 2H, H14, H25), 7.06 (d, 1H, H15, H24), 2.40 (s, 3H, CH_3), 2.22 (s, 3H, CH_3); MS (m/z): 431(M$^+$), 294, 77, 76; HRMS: Anal. calcd for $C_{25}H_{21}NO_2S_2$ 431.5710, Found: 431.5657.

6,7-Bis-[*S*-(2-chlorophenyl)thio]-5,8-quinolinedione (4e) Yield 44%; dark navy blue powder; mp: 178-180°C; IR (KBr): ν3329, 3063, 1676 (s, C=O), 1430-1581 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.01 (dd, 1H, H2), 8.35 (dd, 1H, H4), 7.85 (dd, 1H, H3), 7.80 (dd, 4H, H15, H22, H18, H25), 7.53 (d, 2H, H17, H24), 7.32 (d, 2H, H16, H23); MS (m/z): 443 (M$^+$), 408, 300, 77, 76; HRMS: Anal. calcd for $C_{21}H_{11}Cl_2NO_2S$ 444.3596, Found: 444.3539.

6,7-Bis-[*S*-(3-chlorophenyl)thio]-5,8-quinolinedione (4f) Yield 65%; dark brown powder; mp: 162-164°C; IR (KBr): ν3337, 3024, 1685 (s, C=O), 1461-1550 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.00 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.74 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.54 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.91 (dd, 4H, H14, H21, H18, H25), 7.35 (d, 2H, H17, H24), 6.90 (d, 2H, H16, H23); MS (m/z): 443 (M$^+$), 408, 300, 77, 76; HRMS: Anal. calcd for $C_{21}H_{11}Cl_2NO_2S$ 444.3596, Found: 444.3547.

6,7-Bis-[*S*-(4-chlorophenyl)thio]-5,8-quinolinedione (4g) Yield 48%; brown powder; mp: 184-187°C; IR (KBr):

ν3389, 3055, 1691 (s, C=O), 1474-1579 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.00 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.66 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.82 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.5 (d, 2H, H15, H22), 7.38 (d, 2H, H17, H24), 7.23 (d, 2H, H14, H21), 6.9 (d, 2H, H18, H25); MS (m/z): 443 (M^+), 408, 300, 77, 76; HRMS: Anal. calcd for $C_{21}H_{11}Cl_2NO_2S$ 444.3596, Found: 444.3542.

6,7-Bis-[*S*-(3,4-dichlorophenyl)thio]-5,8-quinolinedione (4h) Yield 58%; red powder; mp: 185-188°C; IR (KBr): ν3389 3066, 1662 (s, C=O), 1457-1584 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.00 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.32 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.61 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.40 (d, 2H, H13, H17), 6.85 (d, 2H, H14, H16), 3.80 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3); MS (m/z): 510 (M^+), 475, 384 77), 76.

6,7-Bis-[*S*-(2-fluorophenyl)thio]-5,8-quinolinedione (4i) Yield 56%; brown powder; mp: 122-123.0°C; IR (KBr): ν3438, 3037, 1662 (s, C=O), 1407-1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.00 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.35 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.85 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.60 (d, 2H, H14, H21), 7.50 (d, 2H, H18, H25), 7.45 (d, 2H, H17, H24); MS (m/z): 411 (M^+), 284, 77, 76.

6,7-Bis-[*S*-(3-fluorophenyl)thio]-5,8-quinolinedione (4j) Yield 52%; reddish brown needle; mp: 199-201°C; IR (KBr): ν3372, 3052, 1673 (s, C=O), 1473-1550 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.98 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.33 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.70 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.44 (d, 2H, H14, H25) 7.28 (d, 2H, H18, H21), 7.01 (d, 2H, H16, H23); MS (m/z): 411 (M^+), 284, 77, 76.

6,7-Bis-[*S*-(4-fluorophenyl)thio]-5,8-quinolinedione (4k) Yield 48%; dark brown powder; mp: 209-203°C; IR (KBr): ν3538, 3091, 1697 (s, C=O), 1461-1554 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.04 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.41 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.81 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.69 (d, 2H, H14, H21), 7.40 (d, 2H, H18, H25) 7.15 (d, 2H, H15, H22), 6.94 (d, 2H, H17, H24); MS (m/z): 411 (M^+), 284, 77.

6,7-Bis-[*S*-(2,4-difluorophenyl)thio]-5,8-quinolinedione (4l) Yield 31%; brown powder; mp: 142-143°C; IR (KBr): ν3387, 3085, 1678 (s, C=O), 1421-1550 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.96 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.29 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.81 (d, 2H, H18, H25), 7.69 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.45 (d, 2H, H15, H22) 7.14 (d, 2H, H17, H24); MS (m/z): 439 (M^+), 420, 294, 77, 76.

6,7-Bis-[*S*-(4-ethylphenyl)thio]-5,8-quinolinedione (4m) Yield 46%; reddish brown amorphous; mp: 118-120°C; IR (KBr): ν3458, 3069, 1688 (s, C=O), 1404-1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.95 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.27 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.80 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.36 (d, 2H, H14, H21), 7.18 (d, 2H, H17, H24), 2.63 (q, *J* = 7.5, 2H, $-CH_2-$), 1.50 (t, *J* = 7.5, 3H, $-CH_3$); MS (m/z): 431(M^+), 416, 402, 293, 77, 76.

6,7-Bis-[*S*-(4-methoxyphenyl)thio]-5,8-quinolinedione (4n) Yield 71%; gold dark purple powder; mp: 229-230°C; IR (KBr): ν3558, 3077, 1695 (s, C=O), 1460-1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.00 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.25 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 7.78 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.40 (d, 2H, H14, H21), 6.85 (d, 2H, H17, H24), 3.76 (s, 3H, OCH_3), 2.50 (s, 3H, CH_3); ;MS (m/z): 435 (M^+), 404, 296, 77.

6,7-Bis-[*S*-(naphthophenyl)thio]-5,8-quinolinedione (4o) Yield 42%; orange flake; mp: 189-190°C; IR (KBr): ν3307, 3051, 1670 (s, C=O), 1494-1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.97 (dd, *J* = 4.7, 7.8, 1.5, 1H, H2), 8.29 (dd, *J* = 4.7, 7.8, 1.5, 1H, H4), 8.03 (d, 2H, H18, H29), 7.62 (dd, *J* = 4.7, 7.8, 1.5, 1H, H3), 7.86 (d, 2H, H15, H26), 7.59 (d, 2H, H22, H33), 7.51 (d, 2H, H19, H30), 7.38 (d, 2H, H21, H32), 7.25 (d, 2H, H20, H31); MS (m/z): 475 (M^+), 316, 77, 76.

2,6,7-Trichloro-4-methyl-5,8-quinolinedione (8)

2-Chloro-4-methyl-5,8-quinolinedione (**7**) were prepared according to a procedure described in the previous paper (Potts *et al.*, 1986, Lee *et al.*, 2000). The compound **7** (0.5 g, 2.4 mmol) was dissolved in 20 ml of CH_3COOH and Cl_2 gas was passed through for 1 hours. The reaction mixture allowed to stand for 2 hours at rt and then poured into 50 ml of water. The precipitate was collected and crystallization from aq. EtOH afforded 2,6,7-trichloro-4-methyl-5,8-quinolinedione **(8):** yield 87%; orange powder; m.p.: 169-170°C.

General procedure for synthesis of 6,7-Bis-[S-(aryl) thio]-2-chloro-4-methyl-5,8-quinolinediones 5

A solution of 2,6,7-trichloro-4-methyl-5,8-quinolinedione **(8)** (0.274 g, 1 mmol) in 15 mL of 95% EtOH was added to the solution of the arylthiols (2.1 or 3.1 mmol) in 5 mL of 95% EtOH and stirred at RT for 2 h and then refluxed for 45 h. After the mixture was kept overnight in the refrigerator, the precipitate was collected by filtration. Crystallization from aq. EtOH afforded the 6,7-bis-[*S*-(aryl)thio]-2-chloro-4-methyl-5,8-quinolinedions **5a-5b** and 2,6,7-tri-[*S*-(aryl) thio]-4-methyl-5,8-quinolinedione (**5c**).

6,7-Bis-[*S*-(4-chlorophenyl)thio]-2-chloro-5,8-quinolinedione (5a) Yield 54%; dark violet powder; m.p.: 67-68°C; IR (KBr): ν3330, 1630 (s, C=O), 1472-1300, 1282

cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.4 (d, 2H, benzene), 7.3 (d, 2H, benzene), 2.37 (s, CH_3, pyridine); MS (m/z): 493 (M^+), 349, 314, 144.

6,7-Bis-[*S*-(4-methylphenyl)thio]-2-chloro-5,8-quinolinedione (5b) Yield 56%; dark violet powder; m.p.: 68-70°C; IR (KBr): ν3356 2940, 1680 (s, C=O), 1535-1490, 1280 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.8 (s, 1H, pyridine), 7.1 (d, 2H, benzene), 6.9 (d, 2H, benzene), 2.35 (s, 3H, CH_3); MS (m/z): 451 (M^+), 328, 124, 91, 65.

2,6,7-Tris-[*S*-(phenyl)thio]-2-chloro-5,8-quinolinedione (5c) Yield 43%; brown powder; m.p.: 124-125°C; IR (KBr): ν3056, 1630 (s, C=O), 1561-1370, 1283(m) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.4 (d, 3H, benzene), 7.3 (t, 3H, benzene), 7.2 (t, 3H, benzene), 2.4 (s, CH_3, pyridine); MS (m/z): 499 (M^+), 399, 223, 110.

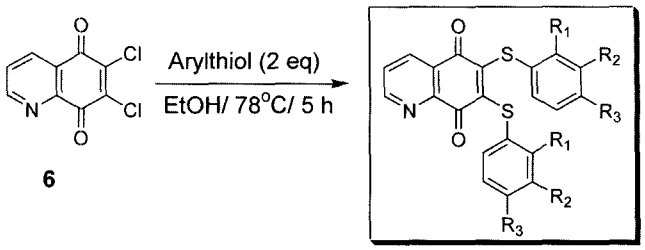
Antifungal *in vitro* Susceptibility Testing

The MIC (minimum inhibitory concentration) values of compounds **4a-4o** and **5a-5c** were determined by the standard broth dilution method (Mcginnis *et al.*, 1996). The antifungal activities were tested in modified Sabouraud dextrose broth against the following fungal strains: *Candida albicans* ATCC 10231, *C. glabrata* ATCC 2001, *C. krusei* ATCC 749, *C tropicalis* ATCC 28775 and and *Aspergillus niger* KCTC 1231. Flucytosine as an antifungal standard agent was used. The compounds were tested in the 0.1-100 μg/ml range. That was added to the modified Sabouraud dextrose broth for fungi over a final concentration range of 0.1 to 100 μg/ml. The inoculum sizes contained approximately 1×10^5 CFU/mL. They were incubated at 37°C for appropriate periods of time that sufficed to show clearly visible growth on drug-free control broths. The MIC value was defined, as the lowest concentration of the antifungal agent at which there showed optically clear. MIC values were read after 1 day for *Candida* species and 2 days for *A. niger* in 37°C.

RESULTS AND DISCUSSION

Chemistry

The method used to synthesize the 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones **4** is shown in Scheme 1. 6,7-Dichloro-5,8-quinolinedione **6** was prepared according to a procedure described in the previous paper (Pratt *et al.*, 1960, Ryu *et al.*, 1994). The 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones **4a-4o** (Table I) were synthesized by nucleophilic substitution on the compound **6** with two equivalent of appropriate arylthiols. Most of these substitutions went as expected and had an overall high yield of 32-81%. We also attempted to synthesize 6-[*S*-(aryl)thio]-7-chloro-5,8-quinolinediones **9** (Fig. 1) by the substitution on the compound **6** with the one equivalent arylthiols under various reaction conditions. Unfortunately, we failed to obtain the compounds **9**. By the reactions, only the 6,7-bis-arylthio-substituted compounds **4** were formed exclusively.



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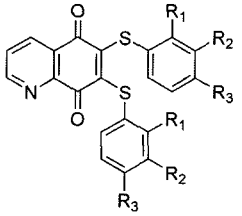
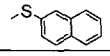
4a-4o: R_1, R_2 = H, F, Cl, ..

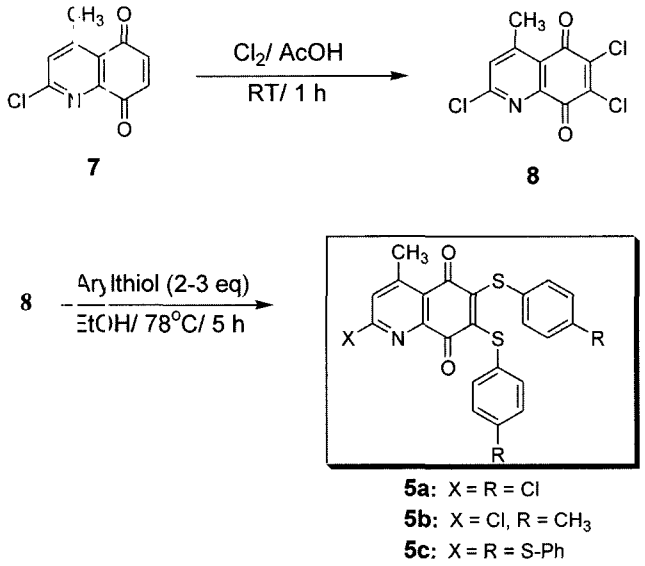
Scheme 1. Synthesis of 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones

Table I. The structures of 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinediones 4



Compounds	R_1	R_2	R_3
4a	H	H	H
4b	H	H	CH_3
4c	H	CH_3	H
4d	H	CH_3	CH_3
4e	Cl	H	H
4f	H	Cl	H
4g	H	H	Cl
4h	H	Cl	Cl
4i	F	H	H
4j	H	F	H
4k	H	H	F
4l	F	H	F
4m	H	H	C_2H_5
4n	H	H	OCH_3
4o			

In a similar manner, 6,7-bis-[*S*-(aryl)thio]-2-chloro-4-methyl-5,8-quinolinediones **5a-5b** or 2,6,7-tris-[*S*-(aryl)thio]-4-methyl-5,8-quinolinediones **5c** were prepared from 2-chloro-4-methyl-5,8-quinolinedione (**7**) (Scheme 2). 2-Chloro-4-methyl-5,8-quinolinedione (**7**) are reported in the literature (Potts *et al.*, 1986, Lee *et al.*, 2000). Chlorination of the compound **7** with the Cl_2 gas in HOAc yielded the 2,6,7-trichloro-4-methyl-5,8-quinolinedione (**8**) in a 87% yield. The 5,8-quinolinediones **5a-5c** were obtained by nucleophilic substitution on the compound **7** with two or three equivalent



Scheme 2. Synthesis of 6,7-bis-[*S*-(aryl)thio]-2-chloro- and 2,6,7-tris-[*S*-(aryl)thio]-4-methyl-5,8-quinolinediones.

of appropriate arylthiols. Most of these substitutions went as expected and had an overall yield of 43-56%.

Antifungal activities

The synthesized the 5,8-quinolinedione derivatives **4** and **5** were tested *in vitro* for their growth inhibitory activities against pathogenic fungi by comparison with flucytosine as a fungicidal standard agent. As indicated in the Table II, most of the 5,8-quinolinediones **4** and **5** showed generally good activities against *C. albicans* and *A. niger* at the MIC level ranging from 0.8 to 25 μg/ml. Among them, compounds **4d-4h** and **5a-5c** exhibited good activity against all tested fungi. Compounds **4l, 5a, 5b** and **5c** had more potent antifungal activities against *C. tropicalis* than flucytosine. Actualy, the activities of compounds **4j** and **4l** were comparable to those of flucytosine against all tested fungi. The 5,8-quinolinedione **4l** completely inhibited the growth of all fungal species tested at the MIC level of ≤ 6.3 μg/mL.

In terms of structure-activity relationship, observations presented in Table II and previously reported data (Ryu *et al.*, 1998 Ryu *et al.*, 1994), the 6,7-bis-[*S*-(aryl)thio]-5,8-quinolinedione series **4** and **5** showed, in general, more potent inhibitory activities than 6-arylamino-7-chloro- and 6-arylamino-7-methylthio-5,8-quinolinedione series **2** and **3**.

In addition, the 5,8-quinolinediones **6** and **7** without an *S-(aryl)thio group exhibited poor antifungal activities.* Thus, S-(aryl)thio moieties of the 6,7-bis-[S-(aryl)thio]-5,8-quinolinediones **4** and **5** improves the antifungal activity significantly. However, the 2-chloro or 5-methyl moiety of compounds **5a-5c** did not appear to contribute significantly toward antifungal potency. The structure-activity relationship may not exist between properties of substituents (R_1, R_2, R_3: F, Cl, ..) for the 6/7-S-arylthio moieties of 5,8-quinolinediones **4** and **5**.

Table II. *In vitro* antifungal activities of 5,8-quinolinediones 4 and 5

Compounds	MIC (μg/ml)[a]			
	C. albicans[b]	C. tropicalis	C. krusei	A. niger
4a	3.2	>100	6.3	25
4b	6.3	100	25	12.5
4c	12.5	25	6.3	12.5
4d	12.5	>100	50	12.5
4e	25	>100	6.3	25
4f	6.3	>100	6.3	6.3
4g	6.3	>100	50	6.3
4h	6.3	100	6.3	12.5
4i	12.5	<0.8	50	25
4j	12.5	6.3	12.5	3.2
4k	50	1.6	>100	25
4l	6.3	3.2	6.3	6.3
4m	6.3	50	6.3	12.5
4n	6.3	>100	3.2	6.3
4o	12.5	100	25	25
5a	3.2	1.6	>100	6.3
5b	6.35	1.6	>100	12.5
5c	3.2	1.6	>100	25
6	100	50	>100	50
7	50	50	100	50
flucytosine	3.2	12.5	6.3	6.3

[a]The MIC value was defined as the lowest concentration of the antifungal agent at which there showed optically clear. MIC values were read after 1 day for *Candida* species and 2 days for *A. niger* in 37°C. The inoculum sizes contained approximately 10^5 CFU/mL. Culture media tested were the modified Sabouraud dextrose broth (Difco Lab.). The final concentration of antifungal agents was between 0.4 and 100 μg/mL.
[b]Fungi tested: *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 28775, *C. krusei* ATCC 749 and *Aspergillus niger* KCTC 1231.

In conclusion, the results indicate that some 6,7-bis-[S-(aryl)thio]-5,8-quinolinediones would be potent antifungal agents. Moreover, the results should encourage the synthesis of 6,7-bis-[S-(aryl)thio]-5,8-quinolinedione analogs for improving antifungal properties.

ACKNOWLEDGMENTS

This study was supported by grants from Research Foundation of Ewha Womans University (2001) and Research Institute of Pharmaceutical Science, Ewha Womans University (2001).

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