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Synthesis and Antifungal Evaluation of 6-(N-Arylamino)-7-methylthio-5,8-quinolinediones

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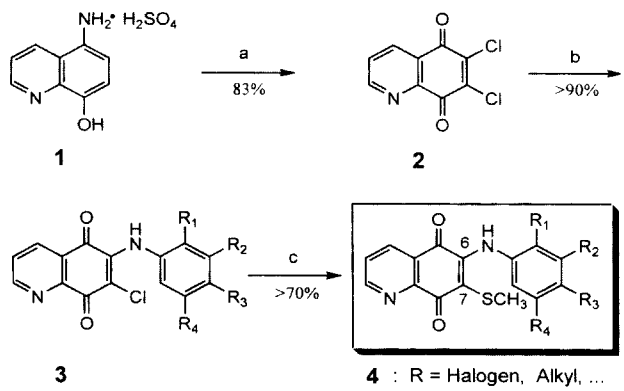
A series of 6-(N-arylamino)-7-methylthio-5,8-quinolinedione derivatives **4a-4l** was newly synthesized for the evaluation of antifungal activity. 6-(N-Arylamino)-7-methylthio-5,8-quinolinediones were prepared by regioselective nucleophilic substitution of 6,7-dichloro-5,8-quinolinediones with arylamines in the presence of Ce^{3+}, and Na_2S/dimethylsulfate. The MIC values of **4a-4l** were determined for antifungal susceptibility *in vitro* against *Candida* species by agar streak method. The derivatives **4a-4l** had generally potent antifungal activities against all human pathogenic fungi. Especially they had the most potent activity against *C. krusei* at 12.5~0.8 μg/ml. Compounds **4d, 4g, 4h, 4j** and **4k** had more potent antifungal activities than fluconazole. Compounds **4g** and **4h** completely inhibited the fungal growth at 0.8~6.3 μg/ml against all *Candida* species, while fluconazole inhibited the growth at 25 μg/ml. The compounds such as **4g** and **4h** containing an N-(4-bromo-2-methylphenyl)- or N-(4-bromo-3-methylphenyl)amino substituent exhibited the most potent antifungal activities.

Key words : 6-(N-Arylamino)-7-methylthio-5,8-quinolinedione, Antifungal activity, MIC minimum inhibitory concentration, *Candida* species

INTRODUCTION

The recent increase of fungal infections, especially among AIDS patients, has generated a renewed interest in antifungal drugs, including development of new antifungal agents (Georgopapadakou *et al.*, 1994, Sternberg *et al.*, 1994). In a program aimed for identifying novel antifungal agents, we focused on developing 6, 7-disubstituted-5,8-quinolinediones with new mode of antifungal action. The 5,8-quinolinedione derivatives were a selective and potent inhibitor of *de novo* pyrimidine biosynthesis (Hudson *et al.*, 1992) due to blockade of mitochondrial electron transport in yeast (Bowman *et al.*, 1973, Roberts *et al.*, 1978). Since the 6-(substituted)-7-chloro-5,8-quinolinediones, as antimetabolites of coenzyme Q, inhibit mitochondrial Co-Q dependent succinoxidase and electron transfer in yeast that may be correlated with antifungal activity (Bowman *et al.*, 1973), the 5,8-quinolinediones would be a potent fungicide. Among these compounds, 6-(substituted)-7-chloro- or 7-alkylthio-5,8-quinolinedione derivatives had especially potent antifungal activities (Bowman *et al.*, 1973, Jeschke *et al.*, 1993). However, the antifungal activity of these analogues against human pathogenic fungi was not tested or mentioned.

In the previous paper (Ryu *et al.*, 1997, 1994a, 1994b), we reported that 6-(N-arylamino)-7-chloro-5,8-quinolinediones exhibited the antifungal activity. For the continuous study on antifungal activities of 5,8-quinolinedione derivatives, a number of 6-(N-arylamino)-7-methylthio-5,8-quinolinediones **4a-4l** were synthesized (Scheme 1). Based on the observation that certain 6- or 7-alkylthio-5,8-quinolinediones have especially antifungal activity (Jeschke *et al.*, 1993), a series of 7-methylthio-5,8-quinolinediones was further extended by the preparation of other types of derivatives. The derivatives **4a-4l** were tested for their growth inhibitory activities against human pathogenic *Candida* species. The minimum inhibitory concentration (MIC) values of **4a-4l** were determined *in vitro* by the standard streak method (Mcginnis *et al.*, 1996, Ryu *et al.*, 1994a).



Scheme 1. a) HCl/$KClO_3$, 50~60°C, 1h, b) EtOH, $CeCl_3$, Arylamine, reflux, 5h, c) EtOH, Na_2S, $(CH_3O)_2SO_2$, RT, 1h

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MATERIALS AND METHODS

All melting points were measured in open capillary tubes with Thomas Hoover Capillary Apparatus Model and were uncorrected. Thin-layer chromatography were performed on precoated silica gel (60G 254, Merck) using $CHCl_3$ for solvent. The compounds were detected under UV light (254 nm) or by heating at 110°C after spraying 30% H_2SO_4-vanillin solution. The purity of compounds was verified by gas chromatography (Hewlett Packard 5890A, HP-5 capillary column at 260 °C, N_2, 17 ml/min as carrier gas, FID).

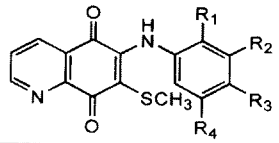
IR spectra were taken from Perkin Elmer 1420r IR spectrometer with KBr pellets. ^{1}H-NMR spectra were recorded on Brucker DPX 250 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvent, and chemical shifts are given in δ ppm with TMS as standard. Mass spectra were obtained on JMS AX 505 WA spectrometer (electronic impact at 70 eV). 8-Hydroxyquinoline, cerium (III) chloride heptahydrate ($CeCl_3 \cdot 7H_2O$), arylamines, dimethylsulfoxide (DMSO) and other reagents were obtained from Aldrich Chemical Co.

Modified Sabouraud dextrose agar and brain heart infusion (BHI) broth were purchased from Difco Lab. Other chemicals such as fluconazole and saline were reagent grade commercially available. UV spectrophotometer from Shimadzu UV-120-02 was used. The microorganisms were incubated in an incubator bath from Vision Scientific Co.

General procedure for synthesis of 6-(N-arylamino)-7-methylthio-5,8-quinolinedione (4)

5-Amino-8-hydroxyquinoline sulfate (**1**) and 6,7-dichloro-5,8-quinolinedione (**2**) were prepared according to the procedure described in the previous paper (Pratt *et al.*, 1960, Ryu *et al.*, 1994a). A solution of **2** (2.27 g, 0.01 mol) and $CeCl_3 \cdot 7H_2O$ (3.73 g, 0.01 mol) in 100 ml of 95% EtOH was added to a solution of the arylamine (0.011 mol) in 50 ml of 95% EtOH with stirring at room temperature for 2 hr and then refluxed for 4~5 hr. After the mixture was kept overnight in the refrigerator or poured into 150 ml of ice water, the precipitate was collected by filtration. The crude **3** obtained was purified by recrystallization from aq. EtOH. A solution of each **3** (0.01 mol) in 40 ml of EtOH was added to a solution of Na_2S (2.6 g, 0.011 mol) in 40 ml of water with stirring at RT for 1 hr. The dark blue solution was treated with dimethylsulfate (1 g, 0.011 mol) and further was refluxed for 1 hr. After the mixture was kept overnight in the refrigerator, the precipitate was collected by filtration. Crystallization form aq. EtOH afforded the 6-(N-arylamino)-7-methylthio-5,8-quinolinediones **4a-4l** (Scheme 1, Table I). Most of these reactions were proceeded in overall 60~95% yields. The used arylamines as reactants were

Table I. Structures of 6-(N-Arylamino)-7-methylthio-5,8-quinolinediones



Compound	R_1	R_2	R_3	R_4
4a	H	H	OH	H
4b	H	H	$(CH_2)_3COOH$	H
4c	H	OCH_3	H	OCH_3
4d	H	H	$COOC_2H_5$	H
4e	H	$COCH_3$	H	H
4f	H	CN	H	H
4g	CH_3	H	Br	H
4h	H	CH_3	Br	H
4i	H	H	OC_2H_5	H
4j	H	NO_2	Cl	H
4k	Cl	H	Br	H
4l	Br	CF_3	H	CF_3

4-aminophenol, 4-(4-aminophenyl)butyric acid, 3,5-dimethoxyaniline, ethyl-4-aminobenzoate, 3-aminoacetophenone, 3-cyanoaniline, 4-bromo-2-methylaniline, 4-bromo-3-methylaniline, phenetidine, 4-chloro-3-nitroaniline, 4-bromo-2-chloroaniline and 2-bromo-3,5-ditrifluoromethylaniline.

6-[N-(4-Hydroxyphenyl)amino]-7-methylthio-5,8-quinolinedione (4a): Yield: 94%, color: black brown plate, m.p.: 238~240°C, IR (KBr, cm^{-1}): 3500 (Ar-OH), 3250 (NH), 2800, 1700 (C=O), 1550, 1510, 1430, 1300, 1000, 830, ^{1}H-NMR (DMSO-d_6): δ 2.12 (3H, s, SCH_3), 6.99~7.10 (4H, m, benzene ring), 7.82~7.89 (3H, m, C_5H_3N), 9.31 (1H, m, NH), 9.54 (1H, s, OH), MS (m/e): 312 (M^+), 300, 265, 237, 209, 181.

6-[N-(4-Carboxypropylphenyl)amino]-7-methylthio-5,8-quinolinedione (4b): Yield: 64%, color: brown powder, m.p.: 246~247°C, IR (KBr, cm^{-1}): 3400 (NH), 3000 (COOH), 1700 (C=O), 1610, 1410, 1200, 900, 730, ^{1}H-NMR (DMSO-d_6): δ 1.12~1.18 (6H, m, $3CH_2$), 2.15 (3H, s, SCH_3), 7.11~7.22 (4H, m, benzene ring), 7.86~7.92 (3H, m, C_5H_3N), 9.41 (1H, m, NH), 9.51 (1H, s, COOH), MS (m/e): 382 (M^+), 370, 297, 275, 205, 116, 90.

6-[N-(3,5-Dimethoxyphenyl)amino]-7-methylthio-5,8-quinolinedione (4c): Yield: 75%, color: black plate, m.p.: 300~303°C, IR (KBr, cm^{-1}): 3000 (NH), 1650 (C=O), 1520, 1300, 1240, 830, 650, ^{1}H-NMR (DMSO-d_6): δ 2.60 (3H, s, SCH_3), 3.24, 3.57 (6H, s, 2-OCH_3), 7.11~7.17 (3H, m, benzene ring), 7.80~7.85 (3H, m, C_5H_3N), 9.26 (1H, m, NH), MS (m/e): 356 (M^+), 256, 227, 199, 164, 136, 100, 87, 77.

6-[N-(4-Ethylcarboxyphenyl)amino]-7-methylthio-5,8-quinolinedione (4d): Yield: 67%, color: bronze powder, m.p.: 359~360°C, IR (KBr, cm^{-1}): 3400 (NH), 1700 (C=O), 1530, 1430, 1300, 1150, 830, ^{1}H-NMR (DMSO-d_6): δ 1.47 (3H, t, CH_3), 2.25 (3H, s, SCH_3), 4.32 (2H,

g, $COCH_2$), 7.17~7.40 (4H, m, benzene ring), 7.82~7.94 (3H, m, C_5H_3N), 9.41 (1H, m, NH), MS (m/e): 368 (M^+), 352, 324, 279, 160, 128, 96, 64.

6-[N-(3-Acetophenyl)amino]-7-methylthio-5,8-quinolinedione (4e): Yield: 78%, color: black amorphous, m.p.: 115~118oC, IR (KBr, cm^{-1}): 3300 (NH), 1700 (C=O), 1540, 1250, 1020, 830, 650, ^{1}H-NMR (DMSO-d_6): δ 2.2 (3H, s, m-$COCH_3$), 2.62 (3H, s, SCH_3), 7.02 ~7.43 (4H, m, benzene ring), 7.78~8.50 (3H, m, C_5H_3N), 9.50 (1H, m, NH), MS (m/e): 338 (M^+), 311, 295, 263, 187, 94, 82, 65.

6-[N-(3-Cyanophenyl)amino]-7-methylthio-5,8-quinolinedione (4f): Yield: 81%, color: violet plate, m.p.: 220~223°C, IR (KBr, cm^{-1}): 3300 (NH), 2200 (C N), 1650 (C=O), 1500, 1400, 1200, 1000, 800, 700, ^{1}H-NMR (DMSO-d_6): δ 2.18 (3H, s, SCH_3), 6.98~7.67 (4H, m, benzene ring), 7.89~9.44 (3H, m, C_5H_3N), 9.61 (1H, m, NH), MS (m/e): 321 (M^+), 309, 274, 187, 102, 77.

6-[N-(4-Bromo-2-methylphenyl)amino]-7-methylthio-5,8-quinolinedione (4g): Yield: 84%, color: black powder, m.p.: 159~162°C, IR (KBr, cm^{-1}): 3250 (NH), 1650 (C=O), 1510, 1400, 1230, 830, ^{1}H-NMR (DMSO-d_6): δ 2.19 (3H, s, SCH_3), 2.60 (3H, s, CH_3), 7.05~7.52 (3H, m, benzene ring), 7.82~7.87 (3H, m, C_5H_3N), 9.06 (1H, m, NH), MS (m/e): 390 (M^+), 343, 309, 276, 187, 160, 90, 78.

6-[N-(4-Bromo-3-methylphenyl)amino]-7-methylthio-5,8-quinolinedione (4h): Yield: 61%, color: dark violet powder, m.p.: 182~184°C IR (KBr, cm^{-1}): 3300 (NH), 1650 (C=O), 1500, 1200, 1100, 1000, 700, ^{1}H-NMR (DMSO-d_6): δ 2.18 (3H, s, SCH_3), 2.50 (3H, s, m-CH_3), 6.93~7.13 (3H,m, benzene ring), 6.4~8.48 (3H, m, C $_5H_3N$), 9.23 (1H, s, NH), MS (m/e): 390 (M^+), 341, 309, 276, 219, 187, 90, 78.

6-[N-(4-Ethylphenyl)amino]-7-methylthio-5,8-quinolinedione (4i): Yield: 89%, color: black amorphous, m.p.: 123~125°C, IR (KBr, cm^{-1}): 3300 (NH), 1670 (C =O), 1550, 1300, 1200, 1000, 830, ^{1}H-NMR (DMSO-d_6): δ 1.31~1.44 (3H, t, CH_3) ,2.6 (3H, s, SCH_3), 4.06 (2H, g, OCH_2), 6.95~7.46 (4H, m, benzene ring), 8.36 ~8.96 (3H, m, C_5H_3N), 9.38 (1H, s, NH), MS (m/e): 340 (M^+), 324, 295, 267, 211, 82.

6-[N-(2-Chloro-3-nitrophenyl)amino]-7-methylthio-5,8-quinolinedione (4j): Yield: 62%, color: dark purple powder, m.p.: 151~154°C, IR (KBr, cm^{-1}): 3250 (NH), 2300, 1650 (C=O), 1500, 1300, 1100, 1000, 800, ^{1}H-NMR (DMSO-d_6): δ 2.19 (3H, s, SCH_3), 7.27~7.48 (3H, m, benzene ring), 7.74~9.09 (3H, m, C_5H_3N), 9.6 (1H, m, NH), MS (m/e): 375 (M^+), 358, 328, 282, 219, 187, 160, 77.

6-[N-(4-Bromo-2-chlorophenyl)amino]-7-methylthio-5,8-quinolinedione (4k): Yield: 94%, color: dark gray plate, m.p.: 238~241°C, IR (KBr, cm^{-1}): 3300 (NH), 1650 (C=O), 1600, 1500, 1350, 1120, 800, 670, ^{1}H-NMR (DMSO-d_6): δ 2.09 (3H, s, SCH_3), 7.42~7.91 (3H, m, benzene ring), 8.45~8.48 (3H, m, C_5H_3N), 9.31 (1H, m, NH), MS (m/e): 409 (M^+), 398, 363, 335, 254,191.

6-[N-(2-Bromo-3,5-difluoromethylphenyl)amino]-7-methylthio-5,8-quinolinedione (4l): Yield: 84%, color: purple plate, m.p.: 251~255°C, IR (KBr, cm^{-1}): 3350 (NH), 1700 (C=O), 1550, 1300, 1200, 1150, 780, 700, ^{1}H-NMR (DMSO-d_6): δ 2.18(3H, s, SCH_3), 7.96~8.01 (4H, m, benzene ring), 8.2~8.57 (3H, m, C_5H_3N), 9.15 (1H, m, NH), MS (m/e): 511 (M^+), 227, 199, 164, 136, 87.

Antimicrobial activity

The antifungal activity of compounds **4a-4l** was tested in modified Sabouraud dextrose agar against the following fungal strains: *Candida albicans* ATCC 10231, *C. glabrata* ATCC 2001, *C. krusei* ATCC 749, *C. tropicalis* ATCC 28775 and *C. parapsilosis* ATCC 22019. The antifungal effects of compounds **4a-4l** were determined by modified agar streak method (Mcginnis *et al.*, 1996, Ryu *et al.*, 1994a). Fluconazole as antifungal standard substance was used. The compounds **4a-4l** were tested in the 0.1~100 μg/ml range. That was added to the melted modified Sabouraud dextrose agar for fungi over a final concentration range of 0.1 to 100 μg/ml. A 3 μl of fungal inocula containing about 2×10^5 microorganisms was incubated by making a 2 cm long streak with loop on solidified agar plates. All the plates were incubated at 30°C for appropriate periods of time that sufficed to show clearly visible growth of colonies on drug-free control plates. The MIC value was defined as the lowest concentration of a drug at which there was no visible colonial growth (Table II).

RESULTS AND DISCUSSION

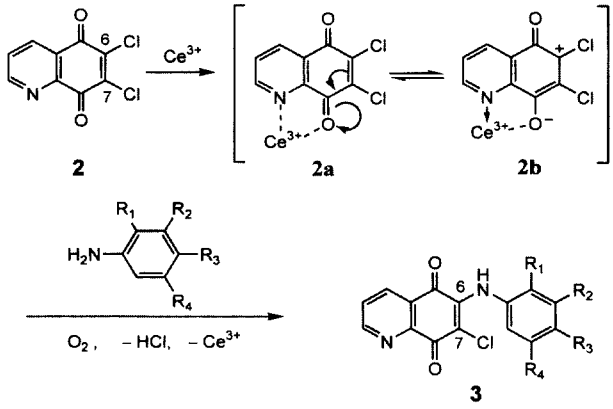
Chemistry

A convenient method for the synthesis of new compounds **4a-4l** form 6,7-chloro-5,8-quinolinedione (**2**) is showen in Scheme 1. The new 6-(N-arylamino)-7-methylthio-5,8-quinolinediones **4a-4l** (Table I) were synthesized by nucleophilic substitution of **2** with various arylamines and sodium sulfide/dimethylsulfate in high yields. The compound **2** was prepared according to the procedure described in the literature (Pratt *et al.*, 1962, Ryu *et al.*, 1994a). 6-(N-Arylamino)-7-chloro-5,8-quinolinedione **3** was formed exclusively by regiospecific nucleophilic substitution of **2** with the appropriate arylamines in the presence of $CeCl_3$ under atmospheric oxygen. This reaction in the presence of $CeCl_3$ gave no other isomeric mixture of products except **3**. From the results of the catalytic action of Ce^{3+} ions in the arylamine substitution reaction of **2**, the regioselectivity should originate from the selective increment of the electrophilicity of 6-position of **2** by the formation of Ce(III) chelate between

Table II. *In vitro* antifungal activities of 6-(N-arylamino)-7-methylthio-5,8-quinolinediones

Compound	MIC (μg/ml)				
	C. albicans	*C. glabrata*	*C. krusei*	*C. tropicalis*	*C. parapsilosis*
4a	25	6.3	3.2	100	100
4b	25	50	25	25	100
4c	50	50	25	100	25
4d	12.5	25	6.3	12.5	25
4e	25	25	25	25	50
4f	50	3.2	25	25	25
4g	1.6	3.2	>0.8	>0.8	3.2
4h	3.2	6.3	6.3	0.8	6.3
4i	6.3	25	25	50	25
4j	6.3	25	12.5	6.3	6.3
4k	25	>0.8	1.6	>0.8	25
4l	50	12.5	25	50	100
Fluc	25	25	25	50.0	12.5

a) Abbreviation: Fluc; fluconazole.
b) MIC values were read after 2-3 days for *Candida* species in 30°C. The inoculum sizes contained approximately 2×10^5 CFU/ml. MIC was defined as the lowest concentration of antifungal agent at which there was no visible colonial growth.
c) Culture media tested was modified Sabouraud dextrose agar.
d) Fungi tested: *Candida albicans ATCC 10231, C. glabrata ATCC 2001, C. krusei ATCC 749, C. tropicalis ATCC 28775 and C. parapsilosis ATCC 22019*



Scheme 2.

nitrogen at 1-position and carbonyl oxygen at 8-position (Scheme 2). The catalysis by the Ce^{3+} ion is understood as involving structure **2b**. The adduct formed by a Michael-type addition of arylamines to **2b** can be dehalogenated by both the uncharged **2a** and atmospheric oxygen to give arylaminated products **3**. Most of these reactions were proceeded in good overall yields.

Antifungal activity

As indicated in the Table II, the 6-(N-arylamino)-7-methylthio-5,8-quinolinediones had generally potent antifungal activities against all human pathogenic fungi. Especially they showed the most potent activity against *C. krusei* at 12.5~0.8 μg/ml. The control cultures exhibited no antifungal activities against all the strain of fungi. Compounds **4d**, **4g**, **4h**, **4j** and **4k** had more potent antifungal activities than fluconazole. Interestingly, compounds **4g** and **4h** completely inhibited the fungal growth at 0.8~6.3 μg/ml against all *Candida* species, while fluconazole inhibited the growth at 25 μg/ml. The compounds **4g** and **4h** containing N-(4-bromo-2-methylphenyl)- or N-(4-bromo-3-methylphenyl)amino substituent exhibited the most potent antifungal activities among the compounds tested. In conclusion, the results suggest that 6-[(N-arylamino)]-7-methylthio-5,8-quinolinedione derivatives may be developed as a potent antifungal agent. Moreover, the results should encourage the synthesis of new 7-methylthio-5,8-quinolinedione derivatives for improving antifungal properties. Further studies to explore the *in vivo* antifungal activities and selectivities of various new 6-(N-arylamino)-7-methylthio-5,8-quinolinediones are in progress in our laboratory.

ACKNOWLEDGMENT

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