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Synthesis and Antifungal Activity of Naphthalene-1,4-diones Modified at Positions 2, 3, and 5

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A series of 2-arylamino-5-hydroxy-naphthalene-1,4-diones, 3-arylamino-5-methoxy-naphthalene-1,4-diones, and 2-arylamino-3chloro-5-hydroxy-naphthalene-1,4-diones were synthesized and tested for *in vitro* antifungal activity against the species *Candida* and *Aspergillus niger*. Among those tested, 3-arylamino-5-methoxy-naphthalene-1,4-diones exhibited potent antifungal activity. In general, the 3-arylamino-5-methoxy-naphthalene-1,4-diones showed more potent antifungal activity than the 2-arylamino-5-hydroxy-naphthalene-1,4-diones and the 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones.

Key words: 5-Methoxy-naphthalene-1,4-diones, Antifungal, MIC, *Candida* species, *A. niger*

INTRODUCTION

Naphthalene-1,4-dione compounds possess various biological activities (Thomson *et al.*, 1987). An interesting subgroup of naphthoquinones is the 5-hydroxy-naphthalene-1,4-dione (Middleton and Parrick, 1988). A number of 5-hydroxy-naphthalene-1,4-diones, such as juglone (**1**) and plumbagin (**2**), display potent biological characteristics : (i) antimalarial activity (Krungkrai *et al.*, 2002); (ii) antibacterial activity (Cai *et al.*, 2000); (iii) cytotoxic properties (Inbaraj *et al.*, 2004); (iv) inhibitory effects of topoisomerase (Wang *et al.*, 2001); and (v) trypanothione reductase (Salmon-Chemin *et al.*, 2001). However, the antifungal activity of the 5-hydroxy-naphthalene-1,4-dione classes has not been studied.

The naphthalene-1,4-dione derivatives blockade the mitochondrial electron transport in *Saccharomyces cerevisiae,* as inhibitors of the mitochondrial cytochrome complex in yeast (Roberts *et al.*, 1978). In our previous report (Ryu *et al.*, 1992), 2-halo-3-substituted-naphthalene-1,4-diones **3**, which could be an analog of compounds **1-2**, demonstrated antifungal activity against pathogenic fungi (Fig. 1).

A variety of quinones with different substituents could exhibit the potential to biological activity with various actions and sometimes improve the antifungal activity (Ryu *et al.*, 2003). Our research assumed that incorporating an additional arylamino, hydroxyl, methoxy or chloro moiety into the quinone would improve efficacy. Based on this speculation, a series of 2/3-arylamino-5-hydroxy-/5-methoxy-naphthalene-1,4-dione **4-6** were synthesized for the evaluation of their antifungal activities (Fig. 1). The *in vitro* antifungal activity of the quinones **4-6** against pathogenic fungi was determined using the twofold broth dilution method. In addition, the antifungal activity of another naphthalene-1,4-dione derivative was measured.

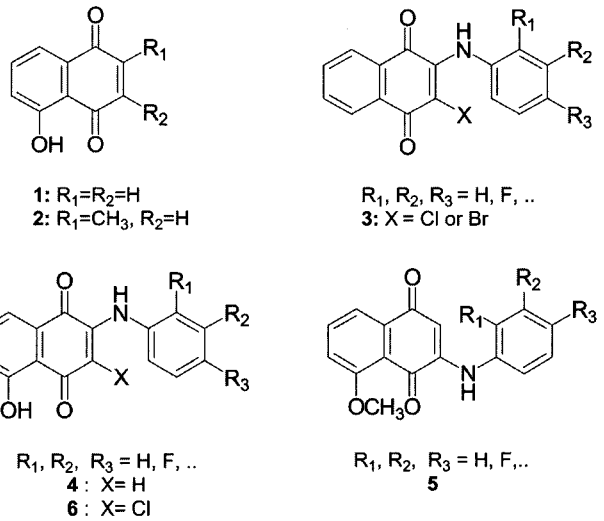

Fig. 1 Structures of naphthalene-1,4-dione derivatives (**4**, **5**, and **6**)

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MATERIALS AND METHODS

All melting points were measured in open capillary tubes using a Büchi melting point B-545 and were uncorrected. TLC was performed on pre-coated silica gel (60G 254, Merck) using $CHCl_3$ as a solvent. The compounds were detected under UV light (254 nm) or by heating to 110°C after being sprayed 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 a Perkin Elmer 1420r IR spectrometer using KBr pellets. ^{1}H-NMR spectra were recorded on a Unity Varian INOVA 400 MHz FT-NMR spectrometer using DMSO-d_6 as a solvent. 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 were obtained from Sigma Co.

General procedure for synthesis of the 2-arylamino-5-hydroxy-naphthalene-1,4-diones (4a-e)

The 2-bromo-5-hydroxy-naphthalene-1,4-dione (**7**) was prepared by brominating the 5-hydroxy-naphthalene-1,4-dione (**1**) with Br_2 in CH_2Cl_2, by methods as defined in previous report (Hannan *et al.*, 1979). A mixture of compound **7** (0.2 g, 0.8 mmol), $(Ph_3P)_4Pd$ (92 mg, 0.08 mmol), triethylamine (110 μL) and an appropriate arylamine (0.8 mmol) in 10 mL of THF was heated in a capped heavy-walled Pyrex tube under an argon atmosphere for 12 h at 60°C. After cooling, the reaction mixture was filtered through celite to remove the Pd catalyst. Then, the mixture was extracted using 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 compounds **4a-e**.

5-Hydroxy-2-(phenylamino)naphthalene-1,4-dione (4a)
A dark brown needle (yield 52%); m.p. 245-246°C; ^{1}H-NMR (DMSO-d_6) δ 13.06 (s,1H, OH), 9.60 (s, 1H, NH), 7.66 (t, *J*=8.4, 1H), 7.60 (d, 1H, *J*=7.8), 7.47 (t, *J*=8.4, 2H, Ph), 7.40 (d, *J*=8.4, 2H, Ph) 7.30 (d, *J*=7.8, 1H), 7.27 (t, *J*=8.4, 1H, Ph), 6.00 (s, 1H, H3); MS (m/z) 265 (M^+).

5-Hydroxy-2-(4-methylphenylamino)naphthalene-1,4-dione (4b)
A dark needle (yield 43%); m.p. 211-212°C; ^{1}H-NMR (DMSO-d_6) δ 13.11 (s, 1H, OH), 9.55 (s, 1H, NH), 7.64 (t, *J*=8.2, 1H), 7.59 (d, *J*=8.2, 1H), 7.31 (d, *J*=8.2, 1H), 7.28 (s, 4H, Ph), 2.33 (s, 3H, CH_3); MS (m/z) 279 (M^+).

5-Hydroxy-2-(4-methoxyphenylamino)naphthalene-1,4-dione (4c)
A black needle (yield 60%); m.p. 218-219°C; ^{1}H-NMR (DMSO-d_6) δ 13.16 (s, 1H, OH), 9.54 (s, 1H, NH), 7.66-7.45 (m, 3H), 7.31 (d, *J*=7.4, 2H, Ph), 7.03 (d, *J*=7.4, 2H, Ph), 5.84 (s, 1H, H3), 3.79 (s, 3H, OCH_3); MS (m/z) 295 (M^+).

5-Hydroxy-2-(4-hydroxyphenylamino)naphthalene-1,4-dione (4d)
Dark red powder (yield 52%), m.p. 218-219°C; ^{1}H-NMR (DMSO-d_6) δ 13.22 (s, 1H, OH), 9.65 (s, 1H, OH), 9.47 (s, 1H, NH), 7.63 (t, *J*=8.4, 1H), 7.57 (d, J=8.8, 1H), 7.30 (d, *J*=8.4, 2H), 7.18 (d, J=8.8, 2H, Ph), 7.34 (d, *J*=8.4, 2H, Ph), 5.79 (s, 1H, H3)); MS (m/z) 281 (M^+).

5-Hydroxy-2-[4-(trifluoromethoxy)phenylamino]naphthalene-1,4-dione (4e)
Red needle crystal (yield 30%); m.p. 192-194°C; ^{1}H-NMR (DMSO-d_6) δ 12.97 (s, 1H, OH), 9.66 (s, 1H, NH), 7.66 (t, *J*=8.4, 1H), 7.61 (d, *J*=8.1, 1H), 7.53 (d, *J*=8.1, 2H, Ph), 7.46 (d, *J*=8.4, 2H, Ph), 7.33 (d, *J*=8.4, 1H), 6.04 (s, 1H, H3) ; MS (m/z) 347 (M^+).

General procedure for synthesis of the 3-arylamino-5-methoxy-naphthalene-1,4-diones (5a-h)

The 5-methoxy-naphthalene-1,4-dione (**8**) was prepared by methylation of the 5-hydroxy-naphthalene-1,4-dione (**1**) with CH_3I and Ag_2O in $CHCl_3$, according to previously-reported method (Hannan *et al.*, 1979). A solution consisting of compound **8** (0.1 g, 0.53 mmol) in 20 mL of 95% EtOH was added to a solution of arylamine (0.53 mmol) in 10 mL of 95% EtOH, and then refluxed for 4-5 h. After the reaction mixture was maintained overnight, the precipitate was collected by filtration. The crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH. Crystallization from aq. EtOH-afforded compounds **5a-h**.

5-Methoxy-3-(4-methylphenyl)amino-naphthalene-1,4-dione (5a)
Dark red powder (14%); m.p. 161-164°C; IR (KBr) 3309 (s, NH), 3094 (w, aromatic ring), 2360 (w), 1672 (s, C=O), 1447-1519 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.0 (s, 1H, NH), 7.79 (t, *J*=7.6, 1H, Ph), 7.58 (dd, *J*=7.6, 1H, Ph), 7.47 (d, *J*=7.8, 1H, Ph), 7.24 (s, 2H, Ph), 7.2 (d, *J*=7.8, 2H, Ph), 5.9 (s, 1H, H3), 3.9 (s, 3H, OCH_3), 2.3 (s, 3H, CH_3); MS (m/z) 293 (M^+).

3-(4-Bromophenyl)amino-5-methoxy-naphthalene-1,4-dione (5b)
Dark red powder (27%); m.p. 181-183°C; IR (KBr) 3247 (s, NH), 3067 (w, aromatic ring), 2357 (w), 1671 (s, C=O),

1450-1510 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 8.95 (s, 1H, NH), 7.80 (t, *J*=8.4, 1H, Ph), 7.72 (d, *J*=8.8, 1H, Ph), 7.58 (d, *J*=8.8, 1H, Ph), 7.34 (m, 2H, Ph), 6.8 (d, *J*=8.4, 2H, Ph), 6.0 (s, 1H, H3), 3.9 (s, 3H, OCH_3); MS (m/z) 357 (M^+); HRMS Calcd for $C_{17}H_{12}BrNO_3$ 357.0001 Found: 356.9998.

3-(4-Fluorophenyl)amino-5-methoxy-naphthalene-1,4-dione (5c)

Red powder (22%); m.p. 149-152°C; IR (KBr) 3329 (s, NH), 3083 (w, aromatic ring), 2360 (w), 1666 (s, C=O), 1440-1529 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.1 (s, 1H, NH), 7.8-7.4 (m, 3H, Ph), 7.2-6.8 (m, 4H, Ph), 5.9 (s, 1H, H3), 3.9 (s, 3H, OCH_3); MS (m/z) 297 (M^+).

3-(4-Chlorophenyl)amino-5-methoxy-naphthalene-1,4-dione (5d)

Dark red powder (34%); m.p. 145-147°C; IR (KBr) 3260 (s, NH), 3010 (w, aromatic ring), 2360 (w), 1671 (s, C=O), 1460-1540 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.1 (s, 1H, NH), 7.8-7.5 (m, 3H, Ph), 7.4 (dd, 2H, Ph), 7.3 (dd, 2H, Ph), 6.0 (s, 1H, H3), 3.9 (s, 3H, OCH_3); MS (m/z) 313 (M^+); HRMS Calcd for $C_{17}H_{12}INO_3$ 404.9682, Found: 404.9685.

3-(2,3,4-Trifluorophenyl)amino-5-methoxy-naphthalene-1,4-dione (5e)

Dark yellow powder (33%); m.p. 131-133°C; IR (KBr) 3326 (s, NH), 3083 (w, aromatic ring), 2360 (w), 1658 (s, C=O), 1468-1518, 1282 (w), cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.1 (s, 1H, NH), 7.8-7.5 (m, 3H, Ph), 6.9 (dd, 2H, Ph), 3.9 (s, 3H, OCH_3); MS (m/z) 333 (M^+); HRMS Calcd for $C_{17}H_{10}F_3NO_3$ 333.0613, Found: 333.0609.

3-(3,4-Difluorophenyl)amino-5-methoxy-naphthalene-1,4-dione (5f)

Dark green powder (34%); m.p. 149-151°C; IR (KBr) 3287 (s, NH), 3061 (w, aromatic ring), 2360 (w), 1671 (s, C=O), 1471-1518 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.9 (s, 1H, NH), 8.4 (dd, 1H, Ph), 7.9 (dt, 2H, Ph), 7.7 (m, 1H, Ph), 7.6 (d, *J*= 7.6 Hz, 1H, Ph), 7.5 (d, *J*= 7.6 Hz, 1H, Ph), 6.0 (s, 1H, H3), 3.9 (s, 3H, OCH_3); MS (m/z) 315 (M^+).

3-(2-Fluorophenyl)amino-5-methoxy-naphthalene-1,4-dione (5g)

Bright brown powder (29%); m.p. 181-182°C; IR (KBr) 3332 (s, NH), 3024 (w, aromatic ring), 2362 (w), 1658 (s, C=O), 1470-1584 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.0 (s, 1H, NH), 7.8 (t, *J*=7.6, 1H, Ph), 7.6 (td, *J*=7.6, 2H, Ph), 6.9 (q, *J*=7.6, 2H, Ph), 5.6 (s, 1H, H3), 3.9 (s, 3H, OCH_3); MS (m/z) 297 (M^+).

3-(2,4-Difluorophenyl)amino-5-methoxy-naphthalene-1,4-dione (5h)

Dark red powder (7%); m.p. 103-105°C; IR (KBr) 3328 (s, NH), 3094 (w, aromatic ring), 2361 (w), 1670 (s, C=O), 1470-1528 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.7 (s, 1H, NH), 7.7 (m, 2H, Ph), 7.5 (dd, 1H, Ph), 7.4 (m, 2H, Ph), 7.1 (td, 1H, Ph), 5.3 (s, 1H, H3), 3.8 (s, 3H, OCH_3); MS (m/z) 315 (M^+).

General procedure for synthesis of the 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones (6a-g)

The 2,3-dichloro-5-hydroxy-naphthalene-1,4-dione (**9**) was prepared by chlorinating compound **1** according to common method (Thomson *et al.*, 1948). A solution of compound **9** (0.1 g, 0.41 mmol) in 20 mL of 95% EtOH was added to a solution of arylamine (0.41 mmol) in 10 mL of 95% EtOH, and then refluxed for 5 h. After the reaction mixture was maintained overnight, the precipitate was collected by the filtration. The crude product was purified using silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH. Crystallization from aq. EtOH afforded compounds **6a-g**.

3-Chloro-2-(4-chlorophenyl)amino-naphthalene-1,4-dione (6a)

Dark brown powder (69%); m.p. 250-251°C; IR (KBr) 3280 (s, NH), 1640 (s, C=O), 1600, 1480 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.33-7.39 (4H, m, Ph), 7.57-7.69 (3H, m), 9.6 (1H, s, NH), 12.4 (1H, s, OH); MS (m/z) 333 (M^+).

3-Chloro-2-(4-fluorophenyl)amino-naphthalene-1,4-dione (6b)

Red plate (54%); m.p. 247-249°C; IR (KBr) 3300 (s, NH), 1640 (s, C=O), 1480, 1520 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.15-7.22 (4H, m, Ph), 7.32-7.34 (3H, m), 9.6 (1H, s, NH), 12.5 (1H, s, OH); MS (m/z) 317 (M^+).

3-Chloro-2-(4-hydroxyphenyl)amino-naphthalene-1,4-dione (6c)

Dark purple plate (72%); m.p. 255-257°C; IR (KBr) 3400 (s, OH), 3290 (s, NH), 1650 (s, C=O), 1498, 1520 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 6.70-7.00 (4H, m, Ph), 7.20-7.60 (3H, m), 9.4 (1H, s, NH), 12.6 (1H, s, OH); MS (m/z) 315 (M^+).

2-(4-Bromophenyl)amino-3-chloro-naphthalene-1,4-dione (6d)

Dark brown powder (49%); m.p. 258-259°C; IR (KBr) 3290 (s, NH), 1690 (s, C=O), 1630, 1640, 1500, 1490 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.10-7.14 (4H, m, Ph), 7.50-7.70 (3H, m), 9.5 (1H, s, NH), 12.3 (1H, s, OH); MS (m/z) 377 (M^+).

3-Chloro-2-(3,4-dichlorophenyl)amino-naphthalene-1,4-dione (6e)

Red powder (58%); m.p. 246-247°C; IR (KBr) 3320 (s, NH), 1690 (s, C=O), 1660, 1630, 1120 cm^{-1}; ^{1}H-NMR

(DMSO-d_6) δ 7.10-7.40 (3H, m, Ph), 7.60-7.70 (3H, m), 9.5 (1H, s, NH), 12.3 (1H, s, OH); MS (m/z) 367 (M^+).

3-Chloro-2-(3,4-difluorophenyl)amino-naphthalene-1,4-dione (6f)

Dark red plate (81%); m.p. 240-250°C; IR (KBr) 3300 (s, NH), 1690 (s, C=O), 1630, 1520 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.10-7.30 (3H, m, Ph), 7.60-7.70 (3H, m), 9.6 (1H, s, NH), 12.4 (1H, s, OH); MS (m/z) 335 (M^+).

3-Chloro-2-(4-iodophenyl)amino-naphthalene-1,4-dione (6g)

Dark brown powder (43%); m.p. 271-272°C; IR (KBr) 3300 (s, NH), 1690 (s, C=O), 1650, 1610, 1500 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.11-7.16 (4H, m, Ph), 7.40-7.60 (3H, m), 9.4 (1H, s, NH), 12.5 (1H, s, OH); MS (m/z) 425 (M^+).

Antifungal *in vitro* susceptibility testing

The MIC (minimum inhibitory concentration) values of compounds **4**, **5**, and **6** were determined using the standard broth dilution method (Mcginnis and Rinaldi, 1996). The antifungal activity was tested in a modified Sabouraud dextrose broth against the following fungal strains: *Candida albicans* Berkout KCCM 50235; *C. tropicalis* Berkout KCCM 50662; *C. krusei* Berkout KCCM 11655; and *Aspergillus niger* KCTC 1231. Flucytosine and ketoconazole antifungal standard agents were used. The compounds were tested in the 0.1-100 μg/mL range and 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 cells/mL. They were incubated at 37°C for appropriate periods and showed visible growth on drug-free control broths. The MIC value was defined as the lowest concentration of the antifungal agent, which was shown as optically clear. MIC values were read after 1 day for *Candida* species and 2 days for *A. niger* at 37°C.

RESULTS AND DISCUSSION

Chemistry

A method for the synthesis of naphthalene-1,4-diones **4-6** (Table I) is shown in Scheme 1. The 2-bromo-5-hydroxy-naphthalene-1,4-diones (**7**, Hannan *et al.*, 1979), 5-methoxy-naphthalene-1,4-diones (**8**), and 2,3-dichloro-5-hydroxy-naphthalene-1,4-dione (**9**) (Thomson *et al.*, 1948) were prepared from juglone (**1**) according to known methods with minor modifications. Compounds **7** and **9** were prepared by bromination and chlorination of compound **1**, respectively. Compound **8** was formed by methylation of compound **1** with CH_3I and Ag_2O in $CHCl_3$.

The 2-arylamino-5-hydroxy-1,4-naphthoquinones (**4a-e**) were synthesized by regioselective substitutions of compound **7** with appropriate arylamines *via* a palladium(0)-catalyzed reaction. The reaction of compound **7** with arylamines proceeded smoothly in the presence of palladium catalyst, which may play a crucial role in the reactivity of substitutions. Otherwise, the reaction in the absence of the palladium catalyst gave very poor yields.

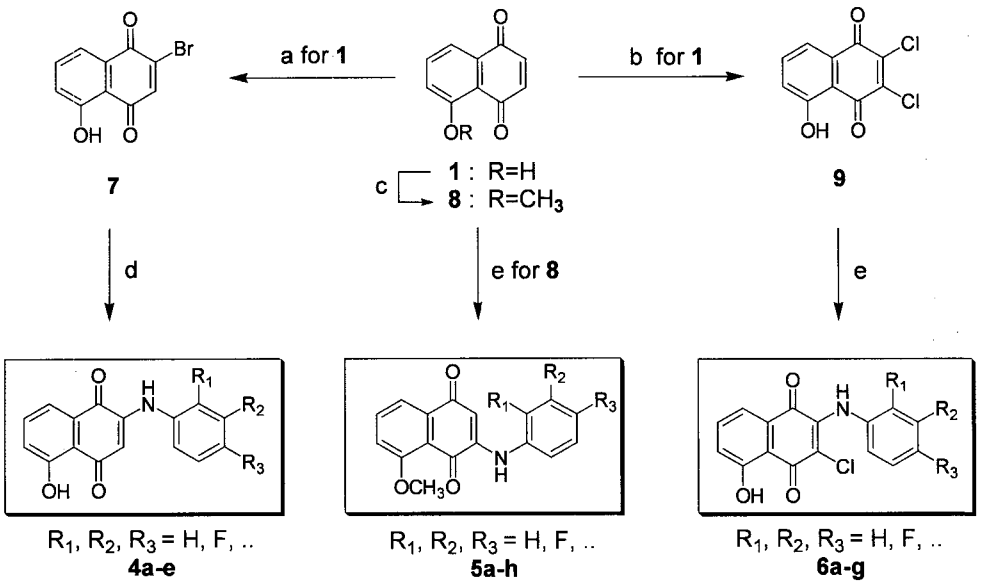
The 3-arylamino-5-methoxy-1,4-naphthoquinones (**5a-h**) were synthesized by the substitution on the 5-methoxy-naphthalene-1,4-dione (**8**) with arylamines. It is well known that the substitution of arylamines in relation to compound **8** proceeds with high selectivity at the C-3 position

Table I. Structures and *in vitro* antifungal activities for naphthalene-1,4-diones **4-6**

Compounds	R_1	R_2	R_3	MIC[a] (μg/mL)			
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*A. niger*
4a	H	H	H	>100	>100	>100	>100
4b	H	H	CH_3	>100	50	>100	>100
4c	H	H	OCH_3	>100	>100	>100	>100
4d	H	H	OH	>100	>100	>100	>100
4e	H	H	OCF_3	50	3.2	>100	>100
5a	H	H	CH_3	25	0.8	>100	50
5b	H	H	Br	50	0.8	>100	6.3
5c	H	H	F	12.5	0.8	6.3	3.2
5d	H	H	Cl	6.3	0.8	6.3	>100
5e	F	F	F	6.3	0.8	12.5	50
5f	H	F	F	25	0.8	50	25
5g	F	H	H	12.5	3.2	12.5	12.5
5h	F	H	F	12.5	0.8	12.5	6.3
6a	H	H	Cl	>100	>100	100	25
6b	H	H	F	>100	>100	50	25
6c	H	H	OH	>100	>100	>100	50
6d	H	H	Br	>100	>100	>100	100
6e	H	Cl	Cl	>100	>100	>100	50
6f	H	F	F	>100	>100	100	50
6g	H	H	I	>100	>100	100	100
1				12.5	0.8	50	25
8				50	1.6	50	12.5
flucytosine				3.2	6.3	6.3	6.3
ketoconazole				6.3	6.3	12.5	12.5

[a] The MIC value was defined as the lowest concentration of the antifungal agent at which 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 1×10^5 cells/mL. Culture media tested were the modified Sabouraud dextrose broth (Difco Lab.). The final concentration of antifungal agents was between 0.1 and 100 μg/mL.

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



Reagents and conditions

a) Br_2/ $CHCl_3$/14 min
b) Cl_2/ AcOH/ rt/ 5 h
c) CH_3I/ Ag_2O/ $CHCl_3$/ rt/ 24 h
d) arylamine/ $Pd(Ph_3)_4$/ Et_3N/ THF/ 60°C/ Ar/12 h
e) arylamine/ EtOH/ rt/ 5 h

Scheme 1. Synthesis of naphthalene-1,4-diones **4-6**

(Kelly *et al.*, 1982). The electronic effects of the 5-methoxyl group present in compound **8** have been extensively utilized in total syntheses to control regiochemistry in reactions involving the quinone double bond (Cameron *et al.*, 1999). Actually, the substitution in compound **8** primarily gave the 3-arylamino products **5a-h** along with the 2-arylamino compounds, as a by-product. Products **5a-h** were separated by silica gel column chromatography with $CHCl_3$ and showed poor yields.

We also synthesized the 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones (**6a-g**) by substituting the 2,3-dichloro-5-hydroxy-naphthalene-1,4-dione (**9**) with arylamines. In compound **9**, the nucleophilic substituting of chlorine by arylamines proceeded with high selectivity at the C-2 position (Naruta *et al.*, 1988).

Antifungal activity

The naphthalene-1,4-diones **4-6** were tested *in vitro* for their growth inhibitory activity against pathogenic fungi using the standard method (Mcginnis, *et al.*, 1996). The MIC values were determined by making comparisons with flucytosine as a fungicidal standard agent. As indicated in Table I, the most potential of the 5-hydroxy-naphthalene-1,4-dione series **4-6**, was found to be for the 3-arylamino-5-methoxy-naphthalene-1,4-diones (**5a-h**). They showed generally strong activity against all tested *Candida* and *A. niger* species. In contrast, the 2-arylamino-5-hydroxy-naphthalene-1,4-diones (**4a-e**) and the 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones (**6a-g**) showed poor antifungal activity, although many compounds exhibited good activity against *C. tropicalis*. Most of the 3-arylamino-5-methoxy-naphthalene-1,4-diones (**5a-h**) showed potent antifungal activity against all tested fungal species, and the activity against *C. tropicalis* was prominent. Much of compounds **5** had more potent antifungal activities against *C. tropicalis* than flucytosine. Actually, against all tested fungi, the activities of compounds **5c** and **5h** were comparable to those of flucytosine. The 5-hydroxy-naphthalene-1,4-diones **5c** and **5h** completely inhibited the growth of all fungal species tested at the MIC level of 0.8-12.5 μg/mL.

In terms of the structure-activity relationship, the 3-arylamino-5-methoxy-naphthalene-1,4-diones (**5a-h**) showed more potent antifungal activities compared to the 2-arylamino-5-hydroxy-naphthalene-1,4-diones (**4a-e**) and the 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones (**6a-g**). The 5-methoxy-substituted compounds **5** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action for these compounds. In contrast, the 5-hydroxy- or 3-chloro-moieties of compounds **5** and **6** did not significantly improve their antifungal activity in comparison with the 5-methoxy-substituted compound **5**.

In addition, the juglone (**1**) and 5-methoxy-naphthalene-1,4-diones (**8**) without an arylamino group also exhibited antifungal activities. Thus, the 3-arylamino group of

compounds **5a-h** partially improve the antifungal activity. The 2-arylamino- and 3-chloro-moieties of compounds **4** and **6** did not contribute toward biological potency. The structure-activity relationship may not exist between properties of substituents (R: H, F, Cl,) for the arylamino moiety of compounds **4-6**.

CONCLUSION

The 2-arylamino-5-hydroxy-naphthalene-1,4-diones (**4a-e**) were synthesized by regioselective substitution of the 2-bromo-5-hydroxy-naphthalene-1,4-dione (**7**). This was achieved with appropriate arylamines *via* a palladium-(0)-catalyzed reaction. The 3-arylamino-5-methoxy-1,4-naphthoquinones (**5a-h**) were synthesized by substituting the 5-methoxy-naphthalene-1,4-dione (**8**) with arylamines. Similarly, we also synthesized the 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones (**6a-g**) by substituting the 5-hydroxy-2,3-dichloro-naphthalene-1,4-dione (**9**) with arylamines. Most of the substitutions went as expected and had high overall yields.

The most active potential among the naphthalene-1,4-diones series **4-6** was found for the 3-arylamino-5-methoxy-naphthalene-1,4-diones (**5a-h**), which generally showed strong activity against all tested *Candida* and *A. niger* species. The 2-arylamino-5-hydroxy-naphthalene-1,4-diones (**4a-e**) and 2-arylamino-3-chloro-5-hydroxy-naphthalene-1,4-diones (**6a-g**) demonstrated antifungal activity, although many compounds exhibited good activity against *C. tropicalis*.

These results suggest that the 3-arylamino-5-methoxy-naphthalene-1,4-diones would be potent antifungal agents.

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