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The Antifungal Activities of some 6-[N-(Halophenyl)amino]-7-Chloro-5,8-Quinolinediones against *Candida* Species

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A series of 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinedione derivatives 1-10 were tested for antifungal susceptibilities, *in vitro*, against pathogenic *Candida* species such as *C. albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis*. The MICs were determined by the standard macrodilution techniques, according to the NCCLS 1992 guidelines. The 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinedione derivatives showed generally potent antifungal activities against pathogenic *Candida* species. Among them, derivative **1**, **2**, **5** and **7** showed more potent antifungal activities than ketoconazole. All derivatives **1-10** had specially potent activities against *C. tropicalis*. Derivative **1** and **2** containing (N-3,4-dihalo-phenyl)amino moiety exhibited the potent antifungal activities. Derivative **2** with (3,4-dichlorophenyl)amino substituent was the most effective in preventing the growth of *Candida* species at MICs 4 μg/ml respectively.

Key words: 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinedione, Antifungal activities, Susceptibility test, *Candida* species, MIC

INTRODUCTION

Infections caused by fungi have become increasingly frequent owing to the increasing number of patients who receive treatment with antibiotics and chemotherapeutic agent or who are immunocompromised (Rex *et al.*, 1993; Sheehan *et al.*, 1993). The emerging magnetitude of fungal infections has generated a renewed interest in aspects of antimycotic drugs, including development of new antifungal agent (Dupouy-Camet *et al.*, 1991).

The 5,8-quinolinedione ring is the biophore for development of antifungal agents. 5,8-Quinolinedione derivatives have potent antifungal (Roberts *et al.*, 1978; Wagner *et al.*, 1962; Ryu *et al.*, 1994), antibacterial (Roberts *et al.*, 1978; Wagner *et al.*, 1962), antimalarial (Bowman *et al.*, 1973; Porter *et al.*, 1971) and antiviral (Inouye *et al.*, 1987; Yasuda *et al.*, 1987; Hafuri *et al.*, 1988) acitivities. The mechanism of cytotoxicities of 5,8-quinolinediones is due to inhibition of electron transfer in respiratory chain of mitochondria and production of oxygen free semiquinone radical (Oyanagui *et al.*, 1989). As antimetabolites of coenzyme Q, the 6-(substituted)-7-chloro-5,8-quinolinediones inhibited malarial mitochondrial Co-Q dependent succinoxidase (Bowman *et al.*, 1973; Porter *et al.*, 1971). The 5,8-quinolinediones produce superoxide (Cadenas *et al.*, 19 90) and inhibit reverse transcriptase of virus (Inouye *et al.*, 1987; Hafuri *et al.*, 1988). Certain 7-chloro-5,8-quinolinediones have specially antifungal and antibacterial activities (Roberts *et al.*, 1978; Wagner *et al.*, 1962).

In previous paper (Ryu *et al.*, 1994), newly prepared 6-(N-arylamino)-7-chloro-5,8-quinolinediones were tested for antifungal activities by unstandardized antifungal susceptibility test (Mcginnis *et al.*, 1991), *in vitro*, against *Candida albicans, Aspergillus niger* and *Tricophyton mentagrophytes*. And N-(halophenyl)amino compounds among these N-arylamino derivatives showed more potent antifungal activities than fluconazole and griseofulvin.

For the continuous study on antifungal susceptibilities of 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinediones, their antifungal activities were determined, *in vitro*, against five pathogenic *Candida* species such as *C. albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis*. The MICs (*Minimal Inhibitory Concentration*) of 5,8-quinolinedione derivatives **1-10** (Table I) were determined by the standard macrodilution tech-

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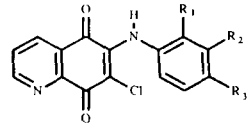
Table I. The Structure of 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinediones



No.	N-(halophenyl)amino	R_1	R_2	R_3
1	(3,4-difluoro-phenyl)amino	H	F	F
2	(3,4-dichloro-phenyl)amino	H	Cl	Cl
3	(2,4-dichloro-phenyl)amino	Cl	H	Cl
4	(2,4-dibromo-phenyl)amino	Br	H	Br
5	(4-fluoro-phenyl)amino	H	H	F
6	(4-chloro-phenyl)amino	H	H	Cl
7	(4-bromo-phenyl)amino	H	H	Br
8	(4-iodo-phenyl)amino	H	H	I
9	(4-chloro-3-nitro-phenyl)amino	H	NO_2	Cl
10	(3-chloro-4-methyl-phenyl)amino	H	Cl	CH_3

nique, according to the NCCLS 1992 guidelines that had better reproducibility than another unstandardized antifungal susceptibility testings (Espinel-Ingroff *et al.*, 1991, 1992; Galgiani, 1993, 1993; Rex *et al.*, 1993, Sheehan *et al.*, 1993).

MATERIALS AND METHODS

Material and Apparatus

The 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinedione derivatives **1-10** (Table I) prepared previously (Ryu *et al.*, 1994) were used for antifungal susceptibility testings.

RPMI 1640 medium, morpholinepropanesulfonic acid (MOPS), L-glutamine and sodium bicarbonate were purchased from Sigma, and Sabouraud Agar and Brain Heart Infusion (BHI) broth from Difco Lab. Dimethyl sulfoxide (DMSO) was obtained from Aldrich Chemical Co., ethanol and bromine from Shinyo Pure Chemicals Co. Other chemicals such as ketoconazole and saline were reagent grade commercially available.

UV spectrophotometer from Shimadzu UV-120-02 was used. The microorganisms were incubated in shaking water bath from Vision Scientific Co.

Antifungal Agents

The MICs of the derivatives **1-10** were determined simultaneously by broth macrodilution technique. Ketoconazole was used for antifungal reference substance

The derivatives **1-10** and ketoconazole were dissolved in DMSO. Stock solutions were prepared in 6400 μg/ml concentrations.

Cultures

MICs were determined against pathogenic *Candida* species. The following fungal strains were used as target microorganisms: *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 2001, *Candida krusei* ATCC 749, *Candida parapsilosis* ATCC 22019 and *Candida tropicalis* ATCC 28775. The *Candida* species were maintained on Sabouraud dextrose agar until tested.

Procedures

The following recommendations provided by the NCCLS 1992 (Espinel-Ingroff *et al.*, 1991, 1992; Galgiani *et al.*, 1993; Sheehan *et al.*, 1993) for broth macrodilution susceptibility tests were used for both technique.

Mediums: The medium used were RPMI 1640 containing L-glutamine; it was buffered with 0.165 M MOPS and adjusted to pH 7.0 by using 10 M NaOH. This medium and sterility control were performed for each batch of prepared medium before use.

Inoculum preparation and quantitation (Pfaller *et al.*, 1988): All *Candida* species were sunbultured at least twice onto Sabouraud dextrose agar for 24 hr at 35℃. The inocula were prepared by picking five colonies ≥1 mm in diameter 24 hr culture of individual *Candida* species and suspending the cells in 5 ml sterile 0.85% saline. This each suspension was vortexed for 15 seconds and the turbidity of each suspension was measured and adjusted spectrophotometrically at 530 nm to a final transmission that ranged from 75 to 77%. This yielded a working suspension of approximately $1\text{-}5\times10^6$ CFU/ml. For the tests, the adjusted suspensions were diluted 1 : 100 with RPMI 1640 medium to obtain the first inoculum size of approximately $1\text{-}5\times10^4$ CFU/ml. For the second final inoculum size, the diluted suspensions were further diluted 1 : 20. This resulted in inoculum sizes of approximately $0.5\text{-}2.5\times10^3$ CFU/ml.

Drug dilutions: Broth macrodilution tests were performed by using the same additive twofold drug dilutions. The broth macrodilution tests were performed with sterile tubes. Drug dilutions were made 10 times by using medium as the diluent. The drug dilutions were dispensed in 0.1 ml volumes into each tube. When each tube was inoculated with 0.9 ml of adjusting final working suspension, final drug concentrations ranged from 64 to 0.12 μg/ml for both tests.

Incubation and scoring of MIC endpoints: All tubes were incubated at 35℃, and MIC endpoints were read at 48 hr. Tubes were observed for the absence or presence of turbidity or growth by a visual method. MIC endpoints were scored as recommended by the NCCLS (Espinel-Ingroff *et al.*, 1991, 1992; Sheehan *et al.*, 1993). Growth in each drug concentration tube was compared with growth in the control (drug-free) tube and given a score as follow; 4+, no reduction

Table II. Antifungal activities of 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinediones MIC (μg/ml)

No.	*C. albicans*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*
1	16.0	8.0	8.0	8.0	2.0
2	1.0	4.0	2.0	4.0	0.25
3	32.0	4.0	16.0	32.0	0.5
4	16.0	8.0	4.0	32.0	8.0
5	8.0	16.0	8.0	8.0	2.0
6	32.0	32.0	64.0	32.0	8.0
7	16.0	8.0	8.0	8.0	0.5
8	32.0	64.0	32.0	32.0	1.0
9	32.0	64.0	32.0	32.0	1.0
10	64.0	64.0	64.0	64.0	2.0
Ketoconazole	16.0	32.0	8.0	8.0	16.0

[a]MICs were determined by using RPMI 1640 medium containing L-glutamine buffered with 0.165 M MOPS (pH 7.0) and were read after incubation at 35℃ for 48 hr. The inoculum sizes contained approximately 0.5-2.5×10^3 CFU/ml. [b]Fungi tested; *Candida albicans* ATCC 10231, *C. glabrata* ATCC 2001, *C. krusei* ATCC 749, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 28775. [c]MICs were defined as the lowest drug concentration in which showed slightly hazy turbidity or optically clear.

of turbidity or growth; 3+, slight reduction in turbidity; 2+, about 50% reduction in turbidity; 1+, slightly hazy turbidity; and optically clear. MICs were defined as the lowest drug concentration in which showed 1+ or less growth or turbidity (Table II).

RESULTS AND DISCUSSION

The 6-[N-(halophenyl)amino]-7-chloro-5,8-quinolinediones **1-10** were tested for determination of antifungal activities against pathogenic *Candida* species. The MICs were determined by the standard macrodilution techniques according to the NCCLS guidelines. The results are given in Table II by comparison with MICs of ketoconazole. The control cultures showed no antifungal activities against all the strain of *Candida* species.

As indicated in the Table II, the 6-N-(halophenyl) amino-7-chloro-5,8-quinolinedione derivatives showed generally potent antifungal activities with widely expanded spectra. **1-10** had not only antifungal activities against *Aspergillus niger* and *Tricophyton mentagrophytes* (Ryu *et al.*, 1994) but also the five pathogenic *Candida* species. All derivatives showed specially very potent activity against *C. tropicalis* at 8-0.2 μg/ml. Among these derivatives, **1**, **2**, **5** and **7** had more potent antifungal activities than ketoconazole.

1 completely inhibited the fungal growth at 4 μg/ml against all *Candida* species. On the other hand, ketoconazole inhibited the growth at 16 μg/ml respectively. In fact, activities of **3** and **4** were superior to that of ketoconazole against many fungi. The compounds such as **1** and **2** containing (N-3,4-dihalo-phenyl)amino moiety exhibited the potent antifungal activities. 1 with (3,4-dichlorophenyl)amino substituent exhibited most potent antifungal activities.

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