The Antimicrobial Activities of some 1,4-Naphthalenediones (IV)

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A series of 2-chloro and 2-bromo-3-(substituted)-1,4-naphthalenedione derivatives (1-25) were tested for antifungal and antibacterial activities *in vitro* against *Candida albicans* 10231 and Local, *Aspergillus niger* KCTC 1231, *Tricophyton mentagrophytes* KCTC 6085, *Fusarium oxysporium* KTCC 6051, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* NCTC 10490, *Staphylococcus aureus* ATCC 6538p, *Escherichia coli* NIHJ and *Acinetobacter baumanii* Local. The MIC values were determined by the twofold agar dilution/streak method. Among these derivatives, 1, 9, 20, 21, 23 and 25 showed more potent antifungal activities than fluconazole. 20 and 23 completely inhibited the growth of fungi, such as *Candida albicans, Aspergillus niger, Tricophyton mentagrophytes* and *Fusarium oxysporium*, at 3.2 μg/ml. Also some derivatives had the antibacterial activities against Gram-positive bacteria.

Key words: 2-Chloro- and 2-bromo-3-substituted-1,4-naphthalenedione, MIC value, Antifungal, Antibacterial activities

INTRODUCTION

Many kinds of 1,4-naphthalenedione analogs possess various biological activities. 2-Halo-3-substituted-1,4-naphthalenediones have potent antifungal (Ryu *et al.*, 1992, 1993; Take *et al.*, 1988), antibacterial (Silver *et al.*, 1968; Ryu *et al.*, 1992, 1993), antimalarial (Lin *et al.*, 1991), cytotoxic and antineoplastic (Hodnet *et al.*, 1983; Take *et al.*, 1988) activities. The naphthoquinoid antibiotics such as nananomycin (Omura, 1986), sakyomicin (Hafuri *et al.*, 1986), rifamycin, tolypomycin, damavaricin and manumycin have 1,4-naphthalenedione ring as minimum pharmacophore (Kirk-Othmer, 1978). Also the derivatives of 2-halo-1,4-naphthalenedione were capable of inhibiting the growth of fungi and bacteria. 1,4-Naphthalendiones have function as bacterial growth inhibitors by interfering electron transport competitively with the endogenous vitamin K or ubiquinone (Wurm *et al.*, 1980).

A representive group of 1,4-naphthalendione derivatives, bearing alkoxy, phenoxy and acyloxy substituents in the 3-position , were assayed against fungi. The alkoxy compounds exhibited moderate activity , which was ehanced by the presence of a 2-chloro or bromo substituent. 3-Chloro-3-phenoxy derivatives were highly active, but the compound without 3-chloro or bromo were inactive (Clark, 1984, Niels *et al.*, 1986). The potentiating effect of a halo and aryl amino chain attached to the 2 and 3 position of 1,4-naphthalenedione on antifungal activities may be increased (Kerkar *et al.*, 1987; Ryu *et al.*, 1992). Therefore, the chloro and bromo were also introduced to a 1,4-naphthalenedione ring, together with other alkyl or aryl amino chains and 1,2,4-triazolyl moiety (Ryu *et al.*, 1991, 1992; Lorian *et al.*, 1991).

In previous paper (Ryu *et al.*, 1992, 1993), 2-halo-3-substituted-1,4- naphthalenediones were tested for antimicrobial activities. For the continuous study on biological activities of 2-halo-3-aryl or alkyl-1,4-naphthalenediones, a number of synthetic 1,4-naphthalenedione derivatives (Ryu *et al.*, 1991, 1992) were determined their growth inhibitory activities against fungi and bacteria.

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MATERIALS AND METHODS

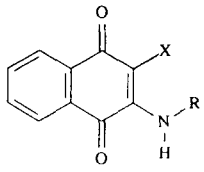
Materials and Apparatus

Twenty five compounds of 2-halo-3-alkyl- or aryl-amino-1,4-naphthalenedione derivatives (**1-25**) which were prepared previously (Ryu *et al.*, 1991, 1992) were used for determination of antifungal and antibacterial activities. Mueller-Hinton broth and Sabouraud agar were purchased from Difco Co.

Other chemicals such as DMSO, fluconazole and ampicillin were reagent grade commercially available.

UV spectrophotometer from Shimadzu UV-120-02

Table I. 2-Halo-3-(N-alkyl and N-arylamino)-1,4-naphthoquinones



NO	X	R
1	X=Cl	(benzothiazol-2-yl)-amino
2	X=Cl	(1,2,4-triazol-2-yl)-amino
3	X=Cl	N-(5-bromo)-pyridino
4	X=Cl	[4-(2-hydroxy-ethyl)-phenyl]-amino
5	X=Cl	[11-(1-carboxy)-undecanyl]-amino
6	X=Cl	N-(4-dihydroxy)-piperadino
7	X=Cl	[5-(1-hydroxy-pentyl)]-amino
8	X=Cl	[2-(1-hydroxy-ethyl)]-amino
9	X=Cl	(4-fluoro-phenyl)-amino
10	X=Cl	(furfur-2-yl)-amino
11	X=Cl	N-morpholino
12	X=Cl	[3-(1-hydroxy)-propanyl]-amino
13	X=Cl	(diethyl)-amino
14	X=Cl	isopropyl-amino
15	X=Cl	(pyridine-4-yl)-amino
16	X=Cl	N-imidazolino
17	X=Cl	(6-hexyl)-amino
18	X=Br	[2-(imidazol-3-yl)-ethyl]-amino
19	X=Br	N-[N-(2-hydoxy-ethyl)]-piperazino
20	X=Br	(7-heptyl)-amino
21	X=Br	(4-butyl)-amino
22	X=Br	(6-hexyl)-amino
23	X=Br	(ethyl-acetyl)-hydrazino
24	X=Br	(t-butyl)-amino
25	X=Br	(diethyl)-amino

was used. The microoraganisms were incubated in incubator from Thomostat T-22S, Thomas Kagaku Co.

Antimicrobial activities of 2,3-disubstituted-1,4-naphthalenediones *in vitro*

The antimicrobial effect of the compounds was determined by the standard twofold agar dilution/streak method (Ryu *et al.*, 1992). The MIC (Minimal Inhibitory Concentration) of the compounds was determined by judging visually the microbial growth in the series of test agar plates.

In the determination of antifungal activities, the following fungal strains were used as target organisms: *Candida albicans* ATCC 10231, *Aspergillus niger* KCTC 1231, *Tricophyton mentagrophytes* KCTC 6085 and *Fusarium oxysporium* KTCC 6051. In the determination of antibacterial activities, the following bacterial strains were used as target organisms: *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* NCTC 10490, *Staphylococcus aureus* ATCC 6538p, *Escherichia coli* NIHJ, and *Acinetobacter baumanii* Local.

Prior to determination of antifungal activity, the strains of fungi were cultured in Sabouraud agar at 30℃ for 3-7 days, whereas the strains of bacteria were cultured in liquid Mueller-Hinton broth at 37℃ for 24 hr, and subcultured again for 6 hr. The number of cells was adjusted with the same sterile broth to 2×10^5 microorganisms and then used for the tests.

Test compounds (1 mg) were dissolved in 0.5 ml of DMSO and subjected to twofold step dilution of the solution (0.1 ml). That was then added to the incubated Sabouraud agar containing had about 2×10^5 microorganisms. The MIC values were determined by judging visually the microbial growth in the series of test agar plates. Fluconazole as antifungal standard substance and ampicillin as antibacterial standard substance were used.

RESULTS AND DISCUSSION

These 1,4-naphthalenedione derivatives (Table I) subjected to the analysis of antifungal and antibacterial activities. The MIC values of the compounds were determined, *in vitro*, by the twofold agar dilution/streak method. The result is given in Table II, III by comparison with those of fluconazole and ampicillin. The control cultures showed no antimicrobial activities against all the strain of microorganisms.

As indicated in the Table II, **1**, **9**, **20**, **21**, **23** and **25** have potent antifungal activities with widely expanded spectra against fungi. **20** and **23** completely inhibited the fungal growth at 3.2 μg/ml against *Candida albicans, Aspergillus niger, Tricophyton mentagrophytes* and *Fusarium oxysporium*. On the other hand, fluconazole inhibited the growth at 25 μg/ml against fungi, respectively. In fact, activities of **1**, **9**, **10**, **17**, **21** and **25** were superior to that of fluconazole against many fungi.

Against Gram-positive bacteria, **7**, **10** and **2** displayed antibacterial activities comparable or slightly inferior to that of ampicillin. Otherwise, all the 1,4-naphthalediones (**1-25**) were found to be less active than ampicillin against Gram-negative bacteria. The compounds such as **20** and **21** containing alkyl and bromo moiety exhibited increase of the potent antifungal activities (Fig. 1). The derivatives with 2-bromo substituent (**18-25**) exhibited generally more potent antimicrobial activities than those with 2-chloro substituent (**1-17**).

In general, 1,4-naphthalenedione derivatives were quite active in vitro against fungi but less active against bacteria (Ryu *et al.*, 1992, 1993).

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Table II. Antifungal activities of 1,4-naphthalenediones

NO	*C. albicans*	*C. albicans* L	*A. niger*	*T. mentagrophyte*	*F. oxysporium*
1	12.5	12.5	6.3	6.3	12.5
2	25	25	25	25	25
3	25	25	12.5	12.5	25
4	50	50	50	50	50
5	25	25	25	25	25
6	50	50	50	50	50
7	25	25	50	25	50
8	25	25	25	25	25
9	6.3	6.3	25	12.5	12.5
10	12.5	12.5	25	12.5	25
11	25	25	25	25	12.5
12	50	12.5	50	25	6.4
13	25	25	25	25	25
14	50	6.4	25	50	6.4
15	25	25	25	12.5	25
16	25	25	25	25	25
17	12.5	25	12.5	12.5	25
18	50	50	100	50	100
19	50	25	100	100	50
20	1.6	1.6	3.2	1.6	3.2
21	3.2	3.2	3.2	3.2	3.2
22	50	50	100	50	100
23	3.2	6.3	6.3	3.2	6.3
24	25	25	25	25	25
25	6.3	6.3	6.3	6.3	6.3
Fluc	25	25	25	12.5	25
Gris	50	*	25	50	*

*: not determined, Fluc: fluconazole, Gris: griseofulvin.
Fungi: *Candida albicans* ATCC 10231, *Candida albicans* Local, *Aspergillus niger* KTCC 1231, *Trichophyton mentagrophytes* KTCC 6085, *Fusarium oxysporium* KTCC 6051.

Table III. Antibacterial activities of 1,4-naphthalenediones

NO	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. baumanii*
1	25	50	>100	100	100
2	6.3	50	>100	100	50
3	25	25	>100	>100	100
4	3.2	12.5	>100	>100	100
5	6.3	3.2	100	>100	>100
6	25	50	100	100	100
7	6.4	6.4	100	>100	100
8	25	12.5	>100	100	100
9	50	100	100	>100	100
10	6.3	3.2	100	>100	100
11	50	6.3	25	50	>100
12	50	25	25	100	100
13	25	6.3	25	100	>100
14	50	25	50	100	>100
15	25	100	12.5	100	100
16	25	100	50	50	100
17	50	100	100	25	>100
18	50	100	6.3	>100	100
19	12.5	25	25	50	>100
20	25	50	>100	>100	100
21	3.2	12.5	100	100	100
22	50	50	>100	>100	100
23	12.5	12.5	>100	100	100
24	50	100	100	100	100
25	50	50	>100	100	100
Amp	3.2	6.3	25	100	25

Amp: ampicillin, Bacteria: *Bacillus subtilis* ATCC 6633, *Staphylcoccus aureus* ATCC 6538p, *Escherchia coli* NIHJ, *Pseudomonas aeruginosa* NTCC 10490, *Acinetobacter baumanii* 48 Local

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