Arch. Pharm. Res. Vol. 16, No. 2, pp. 161-163, 1993

Note

The antimicrobial Activities of Some 1,4-Naphthalenediones (III)

Chung-Kyu Ryu[1] and Dong-Hyun Kim[2]

[1]*College of Pharmacy, Ewha Womans University, Seoul 120-750 and* [2]*College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*

(Received February 19, 1993)

In order to evaluate the antimicrobial effect of 2,3-disubstituted-1,4-naphthalenedione derivatives, we synthesized several 2-chloro, 2-bromo and 2-hydroxy-3-(substituted)-1,4-naphthalenediones (**1-25**). These derivatives were tested for antifungal and antibacterial activities, *in vitro*, against *Candida albicans* 10231 and local, *Aspergillus niger* KCTC 1231, *Tricophyton mentagrophytes* KCTC 6085, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* NCTC 10490, *Staphylococcus aureus* ATCC 6538p, *Escherichia coli* NIHJ. The MIC values were determined by the two-fold agar dilution/streak method. Among these derivatives, 4, 5 and 6 showed the potent antifungal activities. Also 5 and 6 had the antibacterial activities. 5 with (1,2,4-triazolyl)-amino moiety was the most effective in preventing the growth of fungi, such as *Candida albicans, Aspergillus niger* and *Tricophyton mentagrophytes*.

Key words: 2-Chloro- and 2-bromo-3-substituted-1,4-naphthalenedione, (1,2,4-Triazolyl)-amino moiety, MIC, Antifungal, Antibacterial activities

INTRODUCTION

Some 1,4-naphthalenedione derivatives possess various biological activities. 2,3-Disubstituted-1,4-naphthalenediones have potent antifungal (Ryu *et al.*, 1992; Takeda *et al.*, 1986, 1988), antibacterial (Silver *et al.*, 1968; Ryu *et al.*, 1992), antimalarial (Lin *et al.*, 1991), cytotoxic and antineoplastic (Hodnet *et al.*, 1983; Takeda *et al.*, 1988) activities. Many naphthoquinoid antibiotics such as rifamycin, tolypomycin, damavaricin and manumycin have 1,4-naphthalendione ring as minimum pharmacophore (Kirk-Othmer, 1978). Also the derivatives of 2-halo-1,4-naphthalenedione were capable of inhibiting growth of bacteria and fungi. 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).

The potentiating effect of a side chain attached to the 2 or 3 position of 1,4-naphthalenedione on antifungal activities may be increased (Kerkar *et al.*, 1987; Ryu *et al.*, 1992). Therefore, the alkyl or arylamino chains were also introduced to a 1,4-naphthalenedione ring, together with other halogen functional group and 1,2,4-triazolyl moiety, that was active entity of antifungal triazoles like flucoconazole and itraconazole (Lorian *et al.*, 1991).

For the continuous study on biological activities of 2-halo-3-substituted-1,4-naphthalenediones, a number of 1,4-naphthalenediones were determined their growth inhibitory activities against bacteria and fungi.

MATERIALS AND METHODS

Materials and Apparatus

Twentyfive compoounds of 2,3-disubstituted-1,4-naphthalenedione derivatives (**1-25**) were prepared by the method described previouslly (Ryu *et al.*, 1992). Mueller-Hinton broth and Sabouraud agar were purchased from Difco (Detroit, USA).

UV spectrophotometer from Shimadzu UV-120-02 (Japan) was used. The microorganisms were incubated in shaking water bath from Thomostat T-22S (Thomas Kagaku, Japan).

Antimicrobial activities of 2,3-disubstituted-1,4-naphthalenediones

The antimicrobial effect of the compounds was determined by the standard two-fold agar dilution/streak method (Ryu *et al.*, 1992). Minimal inhibitory concentration (MIC) 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

Correspondence to: Chung-Kyu Ryu, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

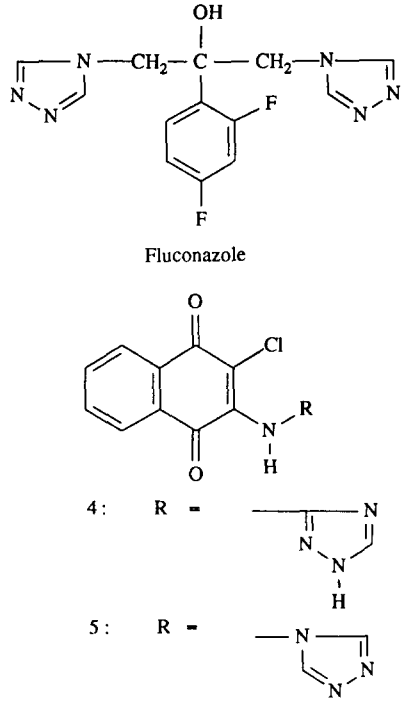


Fig. 1. Fluconazole and 1,4-Naphthalendiones with Triazolyl moiety

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

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.

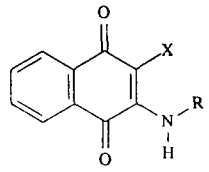
Test compounds (1 mg) were dissolved in 0.5 ml of DMSO and subjected to two-fold step dilution of the solution (0.1 ml). That was then added to the incubated Sabouraud agar containing had about 2×10^5 microorganisms. The MIC values were determined by judging visually the microbial growth in the series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

RESULTS AND DISCUSSION

Antifungal and antibacterial activities

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

Table I. 2,3-Disubstituted-1,4-naphthalendiones



NO	X	R
1	X=Cl	[2-(trifluoro-methyl)-phenyl]-amino
2	X=Cl	[1-(3-hydroxy-propyl)]-amino
3	X=Cl	N-pyrrolidino
4	X=Cl	(1,2,4-triazol-3-yl)-amino
5	X=Cl	(1,2,4-triazol-4-yl)-amino
6	X=Cl	(4-methyl-1,2,4-thiazol-2-yl)-amino
7	X=Cl	(isopropyl)-amino
8	X=Cl	(3-propyl)-amino
9	X=Cl	[N-1-(N-pyrrolidino-carboxy-methyl)-piperazino]-amino
10	X=Cl	(4-aceto-phenyl)-amino
11	X=Cl	(4-cyano-phenyl)-amino
12	X=Cl	(4-hexyl-phenyl)-amino
13	X=Cl	(4-hydroxy-phenyl)-amiino
14	X=Cl	(4-ethyl-phenyl)-amino
15	X=Cl	(5-ethyl-1,2,4-triazol-2-yl)-amino
16	X=Br	[3-(1-carboxy-propyl)]-amino
17	X=Br	(4-ethoxy-phenyl)-amino
18	X=Br	(3-amino-pheyl)-amino
19	X=Br	(4-methyl-phenyl)-amino
20	X=Br	(3-methyl-1,2-oxazol-5-yl)-amino
21	X=OH	[N-(2-hydroxy-ethyl)-pipezidino]-methyl
22	X=OH	(4-chloro-phenyl)-methyl
23	X=OH	(4-ethoxy-phenyl)-methyl
24	X=OH	(4-hydroxy-phenyl)-methyl
25	X=OH	(3-carboxy-phenyl)-methyl

As indicated in the Table II, compounds **4**, **5** and **6** has potent antimicrobial activities with widely expanded spectra against Gram positive, negative bacteria and fungi. Compund **5** completely inhibited the fungal growth at 12.5 μg/ml against *Candida albicans, Aspergillus niger* and *Tricophyton mentagrophytes*. On the other hand, Fluconazole inhibited the growth at 25 μg/ml against fungi, respectively.

Against Gram positive bacteria, copounds **6**, **9** and **10** displayed potent antimicrobial activities comparable or slightly inferior to that of ampicillin. In fact, activities of **1** and **11** were superior to that of fluconazole against many fungi.

The compounds such as **4** and **5** containing 1,2,4-triazolyl moiety, that was active entity of fluconazole,

Table II. Antibacterial and antifungal activity of 1,4-naphthalendione derivatives

Compound	MIC (μg/ml)						
	C. albicans	*A. niger*	*T. mentagrophytes*	*B. subtillus*	*S. aureus*	*E. coli*	*P. aeroginosa*
1	25	12.5	12.5	50	50	100	100
2	50	50	25	50	25	25	100
3	50	50	50	25	25	25	100
4	12.5	12.5	12.5	12.5	50	100	100
5	6.6	12.5	6.3	6.3	12.5	100	100
6	12.5	25	12.5	3.2	3.2	100	100
7	50	25	50	50	25	50	100
8	100	100	100	25	50	25	50
9	25	50	25	3.2	6.3	100	100
10	50	25	25	25	25	12.5	50
11	25	12.5	12.5	50	12.5	12.5	50
12	25	25	25	100	100	50	100
13	100	100	50	50	50	50	50
14	25	25	25	25	25	50	50
15	50	50	50	3.2	3.2	>100	100
16	25	25	25	50	100	50	25
17	100	100	100	100	100	>100	100
18	100	100	100	100	100	>100	100
19	100	100	100	100	100	>100	100
20	100	100	100	>100	>100	>100	>100
21	100	6.3	50	25	50	100	100
22	100	25	50	50	>100	100	100
23	100	25	100	100	100	100	100
24	100	100	100	25	>100	50	50
25	100	25	100	25	100	100	100
Ampicillin	*	*	*	12.5	3.2	25	6.3
Griseofulvin	50	25	50	*	*	*	*
Fluconazole	25	25	12.5	*	*	*	

*, not determined.

saperconazole and itraconazole, exhibited increase of the potent antifungal activities (Fig. 1).

The derivatives with 2-chloro substituent (**1-15**) exhibited generally more potent antimicrobial activities than those with 2-bromo and 2-hydroxyl substituent (**16-25**).

ACKNOWLEGEMENT

This report was supported, in part of C. K. Ryu's experiment, by the grant from Professor Research Foundation of Ewha Womans University (1992).

REFERENCES CITED

Hodnet, E. M., Wongweichintana, C., Dunn, W. and Marrs, M., Substituted 1,4-naphthoquinones vs. the ascitic sarcoma 180 of mice. *J. Med. Chem.*, 26, 570-574 (1983).

Kerkar, V. D., Mhaske, R. B., Kulkarni, P. L. and Kulkarni, A. R., Antimicrobial studies of some juglones. *Indian J. Pharm. Soc.*, 49, 184-186 (1987).

Kirk-Othmer, *Encyclopedia of Chemical Technology*, Vol. 2, 3rd Edn., John Wiley & Sons, New York, 1978, p. 852-868.

Lorian, V., *Antibiotics in Laboratory Medicine*, 3rd Edn., Williams and Wilkins, Baltimore, 1991, p. 198-239.

Lin, T. S., Zhu, L. Y., Xu, S. P., Divo, A. and Sartorelli, A., Synthesis and antimalarial activity of 2-aziridinyl- and 2,3-bis-(aziridinyl)-1,4-naphthoquinonyl sulfonate and acylate derivatives. *J. Med. Chem.*, 34, 1634-1639 (1991).

Ryu, C. K. and Kim, D. H., The synthesis and antimicrobial activities of 1,4-naphthoquinones (II). *Arch. Pharm. Res.*, 15(3), 263-268 (1992).

Ryu, C. K., Ryu, J. C., Chung, C. Y. and Kim, D. H., Antimicrobial activities of 1,4-naphthoquinone derivatives. *Yakhak Hoeji*, 36(2), 110-114 (1992).

Silver, R. F. and Holmes,H.L., Synthesis of some 1,4-naphthoquinones and reactions relating to their use in the study of bacterial growth inhibition. *Canadian J. Chem.*, 46, 1859-1854 (1968).

Takeda, Y., Sawada, M., Kunai, H., Inouye, Y. and Nakamura, S., Mechanism of inhibition of reverse transcriptase by quinone antibiotics. *J. Antibiotics*, 39, 557 (1986); 41, 1471-1478 (1988).

Wurm, G., Geres, U. and Schmidt, H., Untersuchungen an 1,4-Naphthoquinonen. *D. Apotheker Zeitung*, 43, 2045-2051 (1980).