Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1589–1591

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antifungal Activities of 5/6-arylamino-4,7-dioxobenzothiazoles

Chung-Kyu Ryu,[a,*] Hye-Young Kang,[a] Yu-Jin Yi,[a] Keun-Hwa Shin[a] and Byung-Hoon Lee[b]

[a]*College of Pharmacy, Ewha Womans University, Seodaemun-ku, Seoul 120–750, South Korea*
[b]*College of Pharmacy, Wonkwang University, Iksan, Chonbuk 570–749, South Korea*

Received 21 March 2000; accepted 11 May 2000

Abstract—5/6-Arylamino-4,7-dioxobenzothiazoles were synthesized and tested for in vitro antifungal activities against pathogenic fungi. Most of the tested 4,7-dioxobenzothiazoles exhibited potent antifungal activities against *Candida* species and *Aspergillus niger* © 2000 Elsevier Science Ltd. All rights reserved.

Due to an increase of invasive mycoses in immunocompromised patients and frequent accounts of resistance there has been a renewed interest in antifungal agents with novel mode of action.[1]

Quinones such as 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT, **1**) and 7-alkyl-6-hydroxy-5,8-quinolinediones blockade a mitochondrial electron transport in *Saccaromyces cerevisiae*.[2] The UHDBT inhibits a ubiquinol-cytochrome *c* reductase in *S. cerevisiae*.[3] In previous report,[4,5] 6-arylamino-7-chloro-5,8-quinolinediones **2** have demonstrated potent antifungal activities against pathogenic fungi. The arylamino-substituents of quinones **2** sometimes improve the activities.[5] From this information, 6-arylamino-4,7-dioxobenzothiazoles **3** and 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **4**, which would be bioisosteres of quinones **2**, were synthesized for evaluation of their antifungal activities (Fig. 1).

The UHDBT (**1**), one of 4,7-dioxobenzothiazole compounds, has been reported as inhibitors of mitochondrial cytochrome complex in malaria,[6] yeast,[7] bacteria[8] and mammalians.[9] Also, the other 4,7-dioxobenzothiazole derivatives exhibited inhibitory activities of mitochondrial cytochrome complex *bc* in mammalians[10] and cytotoxic activities against cancer cell lines.[11] However, the antifungal activities of the 4,7-dioxobenzothiazole classes against pathogenic fungi have not been reported. Therefore, we describe herein the antifungal activities of 5/6-arylamino-4,7-dioxobenzothiazole derivatives **3a**–**3f** and **4a**–**4k** in comparison with their cytoxic potential (Scheme 1 and Table 1).

Chemistry

A method for the synthesis of the 4,7-dioxobenzothiazoles **3a**–**3f** and **4a**–**4k** (Table 1) is shown in Scheme 1. 6-Methoxy-7-aminobenzothiazole (**5**) was prepared according to the known method.[6] Oxidation of the compound **5** with Fremy's salt (potassium nitrosodisulfonate) gave 6-methoxy-4,7-dioxobenzothiazole (**6**) in about 70% yield. The 6-arylamino-4,7-dioxobenzothiazoles **3a**–**3f** were prepared from the compound **6**. The 6-arylamino-4,7-dioxobenzothiazoles **3a**–**3f** were formed by regioselective nucleophilic substitution of the compound **6** with the appropriate arylamines in the presence of $CeCl_3$. The

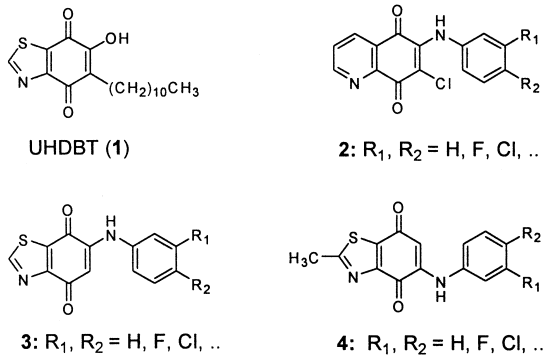
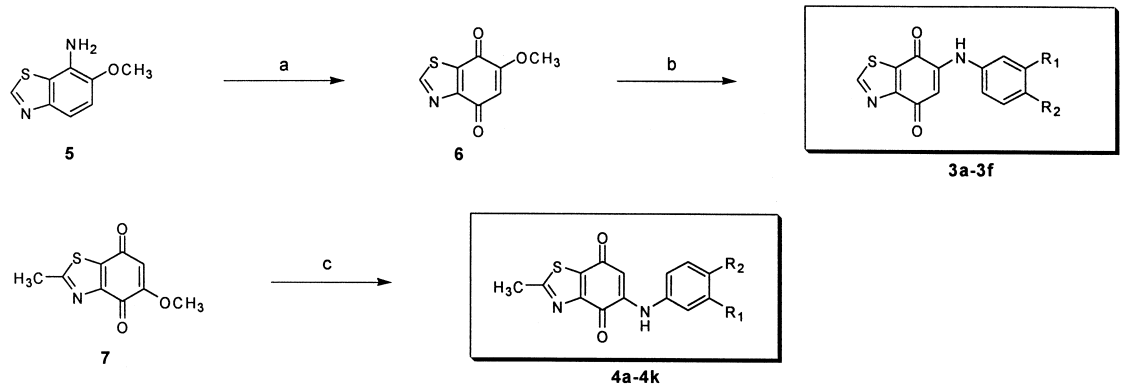


Figure 1. 5,8-Quinolinedione and 4,7-dioxobenzothiazole derivatives.

*Corresponding author. Tel.: +82-2-3277-2027; fax: +82-2-3277-3051; e-mail: ckryu@mm.ewha.ac.kr

PII: S0960-894X(00)00301-2



Scheme 1. Synthesis of 5/6-arylamino-4,7-dioxobenzothiazoles. Reagents and conditions: (a) Fremy's salt (2 equiv) in 0.3 M NaH_2PO_4/acetone/rt/1 h/67%; (b) arylamine (1 equiv)/$CeCl_3$ (0.5 equiv)/EtOH/reflux/5 h/80–95%; (c) arylamine (1 equiv)/EtOH/reflux/5 h/75–95%.

Table 1. Structures and in vitro antifungal and cytotoxic activities for 5/6-arylamino-4,7-dioxobenzothiazoles

Compounds	R_1	R_2	MIC[a] (μg/mL)				IC_{50} (μg/mL)
			C. albicans[b]	*C. tropicalis*	*C. krusei*	*A. niger*	Cytotoxicity for HepG2[c]
3a	H	H	12.5	6.3	6.3	3.2	14.7
3b	H	F	12.5	6.3	3.2	3.2	17.9
3c	F	H	12.5	12.5	3.2	1.6	100 >
3d	H	Cl	12.5	12.5	25	12.5	16.4
3e	H	OCH_2CH_3	12.5	12.5	6.3	3.2	23.6
3f	H	OCF_3	12.5	12.5	3.2	3.2	100 >
4a	H	H	6.3	1.6	0.4	12.5	1.5
4b	H	F	3.2	6.3	3.2	3.2	1.6
4c	H	Cl	12.5	25	25	6.3	3.4
4d	H	CH_3	6.3	1.6	1.6	12.5	1.2
4e	H	OCH_2CH_3	3.2	6.3	3.2	1.6	4.9
4f	H	CF_3	12.5	12.5	3.2	6.3	100 >
4g	H	OCF_3	12.5	0.8	25	6.3	100 >
4h	H	n-C_6H_{13}	12.5	6.3	12.5	12.5	22.0
4i	Cl	H	12.5	6.3	6.3	12.5	5.7
4j	Br	H	12.5	0.8	6.3	12.5	15.0
4k	CH_3	CH_3	12.5	0.4	3.2	12.5	4.8
6			50	25	12.5	25	NT[d]
7			25	25	50	12.5	NT
Flucytosine			3.2	50	3.2	1.6	NT
Cisplatin			NT	NT	NT	NT	0.33

[a]MIC was defined as the lowest concentration of the agent at which there was no visible colonial growth. MIC values were read after 1 day for *Candida* species and 2 days for *A. niger* in 30 °C. The inoculum sizes contained approximately 1×10^5 CFU/mL. Culture media tested were the modified Sabouraud dextrose agar (Difco Lab.). The final concentration of antifungal agents was between 0.4 and 100 μg/mL.
[b]Fungi tested: *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 28775, *C. krusei* ATCC 749 and *Aspergillus niger* KCTC 1231.
[c]Cytotoxicity evaluation for HepG2 (hepatocarcinoma from ATCC): according to MTT assay.[13]
[d]NT = not tested.

nucleophilic displacement of the 6-methoxy group with the amines produced the 4,7-dioxobenzothiazoles **3a**–**3f**. Most of these substitutions went as expected and had overall high yields of 80–95%.

In a similar manner, the 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **4a**–**4k** were prepared by regioselective nucleophilic substitutions of the 5-methoxy-2-methyl-4,7-dioxobenzothiazole (**7**) with the appropriate arylamines according to previously reported method.[11]

Biological Activities

The quinones **3** and **4** were tested in vitro for their growth inhibitory activities against pathogenic fungi by the standard method.[12] The MIC (minimum inhibitory concentration) values were determined by comparison with flucytosine[12] as a fungicidal standard agent. As indicated in the Table 1, the 6-arylamino-4,7-dioxobenzothiazoles **3a**–**3f** showed generally potent antifungal activities against all tested fungal species. However, the activities against many fungi were less potent than those of flucytosine. Most of the 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **4a**–**4k** showed potent antifungal activities against all tested fungal species, and the activity against *C. tropicalis* was prominent. All compounds tested had more potent antifungal activities against *C. tropicalis* than flucytosine. Actually, the activities of compounds **4b**, **4d** and **4e** were superior or comparable to those of flucytosine against many tested fungi. The 4,7-dioxobenzothiazoles **4b** and **4e** completely inhibited the growth of all fungal species tested at 6.3 μg/mL. The cytotoxic potential of compounds **3** and **4** was determined in human cancer

cell HepG2 by the MTT assay according to the protocol as described previously.[13] Cisplatin was used as a cytotoxic reference agent. As indicated in Table 1, some 4,7-dioxobenzothiazoles **3** and **4** showed very weak or even no cytotoxicities.[14] Among the quinones tested, the compounds **3c**, **3e**, **3f**, **4f**, **4g** and **4h** showed the selectivity, in that they possess potent antifungal activities without cytotoxicities in mammalian cells.

In contrast, the compounds **4a**, **4b**, **4d** and **4e** showed both potent cytotoxic and antifungal activities.[11]

In terms of structure–activity relationship, the 5-arylamino-4,7-dioxobenzothiazoles **4a**–**4k** showed, in general, more potent antifungal activities than the 6-arylamino-4,7-dioxobenzothiazoles skeletons **3a**–**3f**. However, the compounds **3a**–**3f** exhibited relatively more selective activities than the compounds **4a**–**4k**. In addition, the 4,7-dioxobenzothiazoles **6** and **7** without an arylamino group exhibited the poor antifungal activities. Thus, 5/6-arylamino groups of quinones **3** and **4** partially improve the antifungal activities. The structure–activity relationship may not exist between properties of substituents (R_1 and R_2) of 5/6-arylamino moiety of compounds **3a**–**3f** and **4a**–**4k**.

In conclusion, the results of this study suggest that 5/6-arylamino-4,7-dioxobenzothiazoles would be potent antifungal agents. Moreover, the results should encourage the synthesis of 4,7-dioxobenzothiazoles analogues for improving antifungal properties.

Acknowledgements

This research was supported by the Brain Korea 21 Project and the MOST through National R&D Program (97-N6-01-01-A-18) for Women's Universities.

References and Notes

1. Georgopapadakou, N. H.; Walsh. T. J. *Science* **1994**, *264*, 371. Groll, A. H.; De Lucca, A. J.; Walsh. T. J. *Trend Microbiol.* **1998**, *6*, 117.
2. Roberts, H.; Choo, W. M.; Smith, S. C.; Marzuki, S.; Linnane, A. W.; Porter, T. H.; Folkers, K. *Arch. Biochem. Biophys.* **1978**, *191*, 306.
3. Di Rigo, J.-P.; Bruel, C.; Graham, L. A.; Slonimski, P.; Trumpower, B. L. *J. Biol. Chem.* **1996**, *271*, 15341.
4. Jeschke, P.; Linder, W.; Mueller, N.; Harder, A.; Mencke, N. *Eur. Patent* 519,290, 1993; *Chem. Abstr.* **1993**, 118, 233893.
5. Ryu, C. K.; Kim, H. J. *Arch. Pharm. Res.* **1994**, *17*, 139. *Chem. Abstr.* **1995**, *122*, 9883.
6. Friedman, M. D.; Stotter, P. L.; Porter, T. H.; Folkers, K. *J. Med. Chem.* **1973**, *16*, 1314.
7. Tsai, A. L.; Kauten, R.; Palmer, G. *Biochim. Biophys. Acta.* **1985**, *806*, 418.
8. Musser, S. M.; Stowell, M. H. B.; Lee, H. K.; Rumbley, J. N.; Chan, S. I. *Biochemistry* **1997**, *36*, 894.
9. Kim, H.; Xia, D.; Yu, C.-A.; Xia, J.-X.; Kachurin, A. M.; Zhang, L.; Yu, L.; Deisenhofer, H. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 8026 and references therein.
10. Yang, F. D.; Yu, L.; Yu, C.-A. *J. Biol. Chem.* **1989**, *264*, 891 and references therein.
11. Ryu, C.-K.; Kang, H.-Y.; Lee, S.-K.; Nam, K. A.; Hong, C. Y.; Ko, W.-G.; Lee, B.-H. *Bioorg. Med. Chem. Lett.* **2000**, *10* (5), 461; in this paper, we have reported the physical data and cytotoxicities of five compounds **4**–**4e**.
12. Mcginnis, M. R.; Rindali, M. G. *Antibiotics in Laboratory Medicine* (4th ed.); Lorian, V., Ed.; Williams and Wilkins: Baltimore, 1996; pp 176–211.
13. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P. A.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
14. Unpublished data; we also tested cytotoxicities of the 4,7-dioxobenzothiazoles **3** and **4** against human tumor cell lines such as A 549 (human lung carcinoma) and Col 1 (human colon carcinoma). Among the quinones tested, the compounds **3c**, **3e**, **3f**, **4f**, **4g** and **4h** showed no cytotoxic activities.