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Synthesis and Antifungal Activity of 6-Arylthio-/6-Arylamino-4,7-dioxobenzothiazoles

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Abstract—6-Arylthio-/6-arylamino-4,7-dioxobenzothiazoles were synthesized and tested for in vitro antifungal activity against *Candida* species and *Aspergillus niger*. 6-Arylamino-4,7-dioxobenzothiazoles **5** and **6** showed, in general, more potent antifungal activity than 6-arylthio-4,7-dioxobenzothiazoles **3** and **4**. The 6-arylamino-substituted compounds **5** and **6** exhibited the greatest activity. In contrast, 6-arylthio-, 2-/5-methyl- or 5-methoxy-moieties of compounds **3**–**4** did not improve their antifungal activity significantly. The results of this study suggest that 6-arylamino-4,7-dioxobenzothiazoles would be potent antifungal agents.

Introduction

Heterocyclic quinone compounds represent an important class of biologically active molecules.[1] The quinones such as 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT, **1**) blockade a mitochondrial electron transport in *Saccaromyces cerevisiae*.[2] The UHDBT (**1**) has been reported as inhibitors of mitochondrial cytochrome complex in yeast,[3,4] malaria,[5] bacteria[6] and mammalians.[7] In our previous reports,[8] 5-arylamino-4,7-dioxobenzothiazoles **2**, which could be analogues of UHDBT, have demonstrated potent antifungal activity against pathogenic fungi (Fig. 1). The arylthio/amino-substituents of quinones improve sometimes the activities.[8,9] On the line of this study, we further extended to synthesize, 6-arylthio-4,7-dioxobenzothiazoles **3**–**4** and 6-arylamino-4,7-dioxobenzothiazoles **5**–**6**, which would be bioisosteres of quinones **2**, and evaluated their antifungal activity.

A variety of quinones with different substituents could exhibit the activities with different action and sometimes improve the activities. The presence of thio, amino, halo and alkyl substituents of quinones improved sometimes their antifungal activity.[8,9] Based on these considerations, the 4,7-dioxobenzothiazoles **3**–**6** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity (Schemes 1 and 2).

The in vitro antifungal activity of the 4,7-dioxobenzothiazoles **3**–**6** against pathogenic fungi was determined by the 2-fold broth dilution method. Additional data for properties and antifungal activity of 4,7-dioxobenzothiazoles are provided.

Results and Discussion

Chemistry

A method for the synthesis of 6-arylthio-4,7-dioxobenzothiazoles **3a**–**3e**, and **4a**–**4e** (Table 1) is shown in Scheme 1. Nitration of 2,5-dimethylbenzothiazole (**7**) by HNO_3/H_2SO_4 afforded 4-nitro-2,5-dimethylbenzothiazole (**8**) in about 92% yield. 4-Amino-2,5-dimethylbenzothiazole (**9**) was prepared by reduction of the compound **8** with $SnCl_2$/HCl variation in about 70% yield. 2,5-Dimethyl-4,7-dioxobenzothiazole (**10**) was synthesized by oxidizing the compound **9** with Fremy's salt (potassium nitrosodisulfonate) in 59% yield. The 6-arylthio-2,5-dimethyl-4,7-dioxobenzothiazoles **3a**–**3e** were prepared from the compound **10**. The 4,7-dioxobenzothiazoles **3a**–**3e** were synthesized by regioselective nucleophilic substitution of the compound **10** with appropriate arylthiols.

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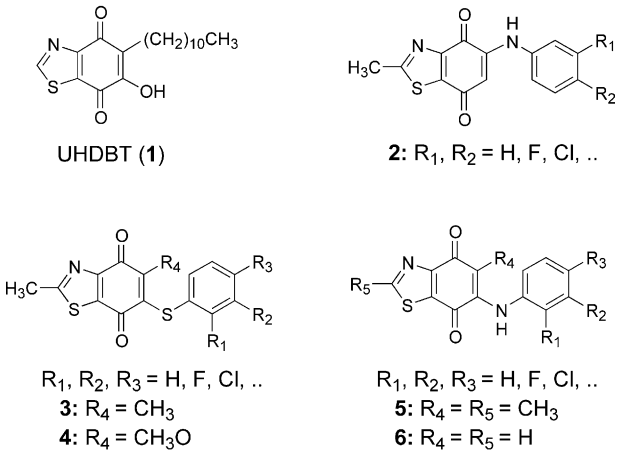
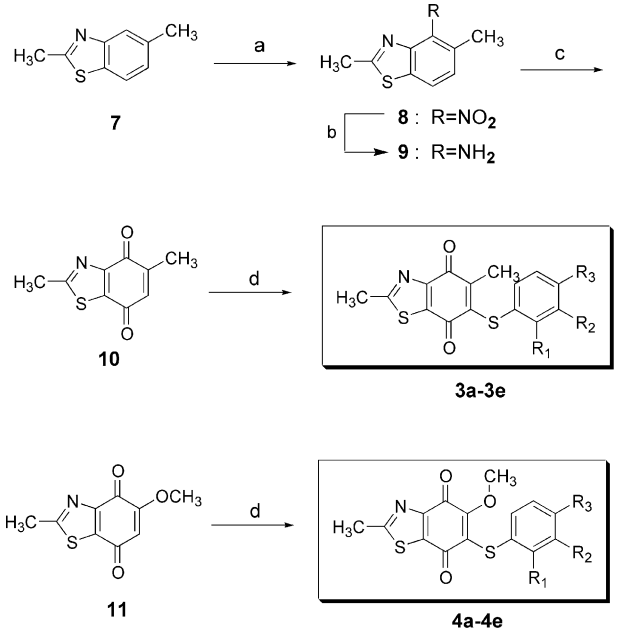
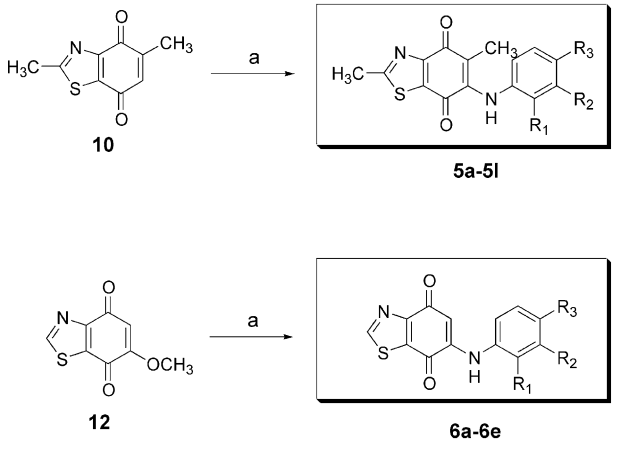


Figure 1. Antifungal 4,7-dioxobenzothiazole derivatives.



Scheme 1. Synthesis of 6-arylthio-4,7-dioxobenzothiazoles **3** and **4**. Reagents and conditions: (a) HNO_3/H_2SO_4/rt; (b) $SnCl_2$/HCl/60 °C; (c) Fremy's salt (2 equiv) in 0.3 M KH_2PO_4/acetone/rt; (d) arylthiol (1 equiv)/EtOH/reflux/4–10 h.



Scheme 2. Synthesis of 6-arylamino-4,7-dioxobenzothiazoles **5** and **6**. Reagents and conditions: (a) arylamine (1 equiv)/EtOH/reflux/4–6 h.

Table 1. Structures and in vitro antifungal activity for 6-arylthio-4,7-dioxobenzothiazoles **3** and **4**

Compd	R_1	R_2	R_3	MIC[a] (μg/mL)			
				C. albicans [b]	*C. tropicalis*	*C. krusei*	*A. niger*
3a	H	H	CH_3	50	12.5	>100	25
3b	H	H	Cl	100	0.8	>100	100
3c	H	H	F	12.5	3.2	50	12.5
3d	F	H	F	50	0.8	100	50
3e	Cl	H	H	25	0.8	>100	100
4a	H	H	OCH_3	>100	12.5	>100	100
4b	H	H	CH_3	>100	12.5	50	>100
4c	H	H	H	>100	25	>100	>100
4d	H	F	*H*	>100	12.5	>100	>100
4e	H			>100	12.5	>100	>100
10				>100	25	>100	>100
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 there 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 CFU/mL. Culture media tested were the modified Sabouraud dextrose broth (Difco Lab.). The final concentration of antifungal agents was between 0.4 and 100 μg/mL.
[b]Fungi tested: *C. albicans* ATCC 10231, *C. tropicalis* ATCC 28775, *C. krusei* ATCC 749 and *Aspergillus niger* KCTC 1231.

5-Methoxy-2-methyl-4,7-dioxobenzothiazole (**11**) was prepared according to previously reported method.[8] 6-Arylthio-5-methoy-2-methyl-4,7-dioxobenzothiazoles **4a–4e** were formed by regioselective nucleophilic substitution of the compound **11** with the appropriate arylthiols. Most of these substitutions went as expected and had overall high yields.

A convenient method for the synthesis of 6-arylamino-4,7-dioxobenzothiazoles **5a–5l** and **6a–6e** (Table 2) is shown in Scheme 2.

6-Arylamino-2,5-dimethyl-4,7-dioxobenzothiazoles **5a–5l** were synthesized by nucleophilic substitutions of the 2,5-dimethyl-4,7-dioxobenzothiazole (**10**) with the appropriate arylamines.

6-Methoxy-4,7-dioxobenzothiazole (**12**) was prepared according to previously reported method.[5] 6-Arylamino-4,7-dioxobenzothiazoles **6a–6e** were synthesized by regioselective nucleophilic substitution of the compound **12** with the appropriate arylamines.

Biological activities

The synthesized 4,7-dioxobenzothiazoles **3–6** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard method.[10] The MIC (minimum inhibitory concentration) values were determined by comparison with ketoconazole as a standard agent. As indicated in Tables 1 and 2, the most active potential among the 4,7-dioxobenzothiazole series **3–6** was found for 6-arylamino-4,7-dioxobenzothiazoles **5** and **6**, which showed generally good activity against all tested *Candida* species and *A. niger*. The 6-arylthio-4,7-dioxobenzothiazoles **3a–3e** showed also antifungal

Table 2. Structures and in vitro antifungal activity for 6-arylamino-4,7-dioxobenzothiazoles **5** and **6**

Compd	R_1	R_2	R_3	MIC[a] (μg/mL)			
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*A. niger*
5a	H	H	OCH_3	25	3.2	25	3.2
5b	H	H	OC_2H_5	12.5	3.2	25	3.2
5c	H	H	CH_3	6.3	1.6	6.3	6.3
5d	H	H	F	12.5	3.2	12.5	3.2
5e	F	H	H	6.3	1.6	12.5	12.5
5f	F	H	F	12.5	1.6	25	12.5
5g	H	H	Cl	3.2	1.6	6.3	6.3
5h	H	Cl	Cl	50	1.6	>100	>100
5i	H	H	I	12.5	3.2	>100	3.2
5j	H	H	Br	6.3	3.2	12.5	6.3
5k	H	Br	H	50	3.1	>100	>100
5l	H	H	C_4H_9	>100	1.6	>100	>100
6a	H	H	F	12.5	6.3	3.2	3.2
6b	H	F	H	12.5	12.5	3.2	1.6
6c	H	H	H	12.5	12.5	25	3.2
6d	H	H	Cl	12.5	12.5	3.2	12.5
6e	H	H	CF_3	12.5	12.5	6.3	3.2
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 there showed optically clear. MIC values were read after 1 day for *Candida* species and 2 days for *A. niger* in 37 °. The inoculum sizes contained approximately 1×10^5 CFU/mL. Culture media tested were the modified Sabouraud dextrose broth (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.

activity. In contrast, compounds **4a–4e** did not show significant antifungal activity, although many compounds of them exhibited good activity against *Candida tropicalis*. Most of the 6-arylamino-4,7-dioxobenzothiazoles **5a–5l** and **6a–6e** showed potent antifungal activity against all tested fungal species, and the activity against *C. tropicalis* was prominent. Most of compounds **5** and **6** had more potent antifungal activity against *C. tropicalis* than ketoconazole. Actually, the activities of compounds **5c**, **5e**, **5g** and **5j** were superior or comparable to those of ketoconazole against all tested fungi. The 4,7-dioxobenzothiazoles **5c** and **5g** completely inhibited the growth of all fungal species tested at the MIC level of 6.3 μg/mL.

The cytotoxic potential of compounds **3–6** was also determined in human cancer cells according to the NCI protocols.[11] All of the tested compounds did not show significant cytotoxic activity and showed the selectivity, in that they possess potent antifungal activities without the cytotoxicity in mammalian cells.[12]

In terms of structure–activity relationship, the 6-arylamino-4,7-dioxobenzothiazole series **5a–5l** and **6a–6e** showed, in general, more potent antifungal activity than 6-arylthio-4,7-dioxobenzothiazole series **3a–3e** and **4a–4e**. The 6-arylamino-substituted compounds **5** and **6** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds. In contrast, 6-arylthio-, 2-/5-methyl- or 5-methoxy-moieties of compounds **3–4** did not improve their antifungal activity in comparison to 6-arylamino-4,7-dioxobenzothiazoles **5** significantly.

In addition, the 4,7-dioxobenzothiazole **10** without a 6-arylamino group exhibited poor antifungal activity. Thus, 6-arylamino moiety of 4,7-dioxobenzothiazoles **5** and **6** improves the antifungal activity significantly. The 2,5-dimethyl moieties of compounds **5** did not appear to contribute partially toward biological potency. The structure–activity relationship may not exist between properties of substituents (R: F, Cl, Br, ...) for the 6-arylamino moieties of the 4,7-dioxobenzothiazoles **3–6**.

Conclusion

The 6-arylthio-4,7-dioxobenzothiazoles **3** and **4** were synthesized by regioselective nucleophilic substitution of the 2,5-dimethyl-4,7-dioxobenzothiazole (**10**) and 5-methoxy-2-methyl-4,7-dioxobenzothiazole (**11**) with the appropriate arylthiols. In similar manner, the 6-arylamino-4,7-dioxobenzothiazoles **5** and **6** were prepared by the regioselective substitution of compound **10** and 6-methoxy-4,7-dioxobenzothiazole (**12**) with the appropriate arylamines. Most of these substitutions went as expected and had overall high yields.

6-Arylamino-4,7-dioxobenzothiazoles **5** and **6** showed generally more potent antifungal activity than 6-arylthio-4,7-dioxobenzothiazoles **3** and **4**. The 6-arylamino-moieties of compounds **5** and **6** improved their antifungal activity significantly. The results of this study suggest that 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.

Experimental

All melting points were measured in open capillary tubes with Buchi melting point B-545 and were uncorrected. The TLC was performed on precoated silica gel (60G 254, Merck) using $CHCl_3$ for solvent. The compounds were detected under UV light (254 nm) or by heating at 110 °C after spraying 30% H_2SO_4–vanillin solution. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). The purity of 4,7-dioxobenzothiazoles **3–6** was also verified by GC (Hewlett Packard 5890A, HP-5 capillary column at 260 °C, N_2 gas, 17 mL/min as carrier gas, FID). The IR spectra were taken from Perkin Elmer 1420r IR spectrometer with KBr pellets. ^{1}H NMR spectra were recorded on Brucker DPX 250 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvent, and chemical shifts are given in ppm with TMS as a standard. High-resolution mass spectra (HRMS EI) were taken from Jeol JMS AX505 WA. 2,5-Dimethylbenzothiazole (**7**) was obtained from TCI Co. $CDCl_3$, DMSO-d_6 and other reagents were purchased from Aldrich Chemical Co.

2,5-Dimethyl-4-nitrobenzothiazole (8). 2,5-Dimethylbenzothiazole (**7**, 5 g, 30.7 mmol) in 18 mL of C–H_2SO_4 and 18 mL of C–HNO_3 was stirred at rt for 2 h. The precipitate was filtered and crystallized from $CHCl_3$.

2,5-Dimethyl-4-nitrobenzothiazole (**8**) was obtained (5.9 g, 92%): pale yellow powder; mp: 111–112 °C; IR (KBr): ν 3094 (s), 2990, 1550 (s, NO_2), 1310 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.4 (s, 1H), 7.6 (s, 1H), 2.8 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); HRMS Anal. calcd for $C_9H_8N_2O_2S$ 208.0306, Found: 208.0306.

4-Amino-2,5-dimethylbenzothiazole (9). To 12 g (64 mmol) of $SnCl_2$ in 25 mL of C–HCl was added 2 g (9.6 mmol) of compound **8**. The mixture was stirred at 60 °C for 2 h and was extracted twice with CH_2Cl_2. The extract was evaporated and crystallized from CH_2Cl_2. 4-Amino-2,5-dimethylbenzothiazole (**9**) was obtained (1.2 g, 70%): pale yellow plate; mp: 91–92 °C; IR (KBr): ν 3451, 3329 (s, NH_2), 3100 (s), 2915, 1473, 1326 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.4 (s, 1H), 7.2 (s, 1H), 5.2 (s, 2H, NH_2), 2.8 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); HRMS Anal. calcd for $C_9H_{10}N_2S$ 178.0565, Found: 178.0565.

2,5-Dimethyl-4,7-dioxobenzothiazole (10). To a solution of compound **9** (0.5 g, 2.8 mmol) in 100 mL of KH_2PO_4 buffer (0.3 M, 200 mL) was added a solution of potassium nitrosodisulfonate (1.5 g, 5.60 mmol) in the KH_2PO_4 buffer. The mixture was stirred at rt for 5 h and was extracted twice with $CHCl_3$. The extract was evaporated and purified by column chromatography with $CHCl_3$. 2,5-Dimethyl-4,7-dioxobenzothiazole (**10**) was obtained (0.32 g, 59%): yellow powder; mp: 138–139 °C. IR (KBr): ν 3045 (w), 2922, 1670 (s, C=O), 1475–1503 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.8 (s, 1H, H6), 2.8 (s, 3H, CH_3), 1.5 (s, 3H, CH_3); HRMS Anal. calcd for $C_9H_7O_2NS$ 193.0198, Found: 193.0196.

General procedure for synthesis of 6-arylthio-2,5-dimethyl-4,7-dioxobenzothiazoles 3a–3e

A solution of compound **10** (0.193 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylthiol (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4∼10 h. After the reaction mixture was kept overnight, the precipitate was collected by the filtration. The crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH. Crystallization from aq EtOH afforded 5-arylthio-2,5-dimethyl-4,7-dioxobenzothiazoles **3a**–**3e** (Table 1).

6-[*S*-(4-Methylphenyl)thio]-2,5-dimethyl-4,7-dioxobenzothiazole (3a). Dark orange powder (92%); mp: 88–90 °C; IR (KBr): ν 3088 (m), 1667 (s, C=O), 1438-1514, 1364 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.3 (d, J = 8 Hz, 2H), 7.1 (d, J = 8 Hz, 2H), 2.8 (s, 3H, CH_3), 2.2 (s, 3H, CH_3); HRMS Anal. calcd for $C_{16}H_{13}NO_2S_2$ 315.0388, Found: 315.0381.

6-[*S*-(4-Chlorophenyl)thio]-2,5-dimethyl-4,7-dioxobenzothiazole (3b). Shining dark red powder (80%); mp: 153–154 °C; IR (KBr): ν 3083 (m), 1658 (s, C=O), 1427–1516, 1364 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.4 (d, J = 8.8 Hz, 2H), 7.3 (d, J = 8.8 Hz, 2H), 2.8 (s, 3H, CH_3), 2.3 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{10}O_2N_2ClS_2$ 334.9841, Found: 334.9844.

6-[*S*-(4-Florophenyl)thio]-2,5-dimethyl-4,7-dioxobenzothiazole (3c). Dark pink powder (66%); mp: 101–104 °C; IR (KBr): ν 3061 (w), 1659 (s, C=O), 1428–1516, 1367 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.4 (d, 2H), 7.2 (d, 2H), 2.8 (s, 3H, CH_3), 2.3 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{10}FNO_2S_2$ 319.0137, Found: 319.0138.

6-[*S*-(2,4-Difluorophenyl)thio]-2,5-dimethyl-4,7-dioxobenzothiazole (3d). Red powder (45%); mp: 122–126 °C; IR (KBr): ν 3081 (m), 1658 (s, C=O), 1417-1516, 1365 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.6 (m, 1H), 7.4 (td, 1H), 7.1 (td, 1H), 2.8 (s, 3H, CH_3), 2.3 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_9F_2NO_2S_2$ 337.0043, Found: 337.0043.

6-[*S*-(2-Chlorophenyl)thio]-2,5-dimethyl-4,7-dioxobenzothiazole (3e). Blight pink powder (61%); mp: 196–199 °C; IR (KBr): ν 3061 (m), 1663 (s, C=O), 1451–1516, 1252 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.5 (m, 1H), 7.1 (t, 2H), 6.4 (m, 1H), 2.8 (s, 3H, CH_3), 2.2 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{10}ClNO_2S$ 303.0121, Found: 303.0122.

General procedure for synthesis of 6-arylthio-5-methoxy-2-methyl-4,7-dioxobenzothiazoles 4a–4e

A solution of compound **11**[8] (0.209 g, 0.1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylthiol (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4–10 h. After the reaction mixture was kept overnight, the precipitate was collected by the filtration. The crude product was purified through silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH. Crystallization from aq EtOH afforded 6-arylthio-5-methoxy-2-methyl-4,7-dioxobenzothiazoles **4a**–**4e** (Table 1).

6-[*S*-(4-Methoxyphenyl)thio]-5-methoxy-2-methyl-4,7-dioxobenzothiazole (4a). Dark purple powder (36%); mp: 94–96 °C; IR (KBr): ν 3231 (s), 1680 (s, C=O), 1491–1590, 1330 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.2 (d, J = 8.4 Hz, 2H), 6.8 (d, J = 8.4 Hz, 2H), 3.9 (s, 3H, OCH_3), 3.7 (s, 3H, OCH_3), 2.8 (s, 3H, OCH_3); HRMS Anal. calcd for $C_{16}H_{13}NO_4S_2$ 347.0286, Found: 347.0286.

6-[*S*-(4-Methylphenyl)thio]-5-methoxy-2-methyl-4,7-dioxobenzothiazole (4b). Dark purple powder (50%); mp: 102–103 °C; IR (KBr): ν 3014 (s), 1685 (s, C=O), 1475–1555, 1314 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.4 (d, J = 8 Hz, 2H), 7.2 (d, J = 8 Hz, 2H), 4.0 (s, 3H, OCH_3), 2.8 (s, 3H, CH_3), 2.3 (s, 3H, CH_3); HRMS Anal. calcd for $C_{16}H_{13}NO_3S_2$ 331.0337, Found: 331.0336.

6-[*S*-(Phenyl)thio]-5-methoxy-2-methyl-4,7-dioxobenzothiazole (4c). Violet powder (41%); mp: 158–159 °C; IR (KBr): ν 3233 (s), 1663 (s, C=O), 1459-1540, 1308 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.0 (m, 1H), 7.3 (m, 3H), 4.0 (s, 3H, OCH_3), 2.8 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}NO_3S_2$ 317.0181, Found: 317.0182.

6-[*S*-(3-Fluorophenyl)thio]-5-methoxy-2-methyl-4,7-dioxobenzothiazole (4d). Brown powder (40%); mp: 163–164 °C; IR (KBr): ν 3249 (s), 1674 (s, C=O),

1495–1545, 1303 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.2 (t, 1H), 7.0 (d, 1H), 6.9 (s, 1H), 6.8 (d, 1H), 3.8 (s, 3H, OCH_3), 2.8 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{10}FNO_3S_2$ 335.0086, Found: 335.0086.

6-[*S*-(2-Naphthyl)thio]-5-methoxy-2-methyl-4,7-dioxobenzothiazole (4e). Dark red needle (47%); mp: 114–116 °C; IR (KBr): ν 3056 (s), 1692 (s, C=O), 1491–1567, 1301 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.8 (m, 4H, naphthalene), 7.5 (m, 3H, naphthalene), 4.0 (s, 3H, OCH_3), 2.8 (s, 3H, CH_3); HRMS Anal. calcd for $C_{19}H_{13}NO_3S_2$ 367.0337, Found: 367.0337.

General procedure for synthesis of 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 5a–5l

A solution of compound **10** (0.193 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylamine (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4∼6 h. After the reaction mixture was kept overnight, the precipitate was collected by the filtration. The crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH. Crystallization from aq EtOH afforded 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **5a–5l** (Table 2).

6-[*N*-(4-Methoxyphenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5a). Dark violet powder (53%); mp: 195–196 °C; IR (KBr): ν 3243 (w, NH), 3016 (w), 1694 (s, C=O), 1486–1554 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.0 (d, J = 8.8 Hz, 2H), 6.9 (d, J = 8.8 Hz, 2H), 3.7 (s, 3H, OCH_3), 2.8 (s, 3H, CH_3), 1.5 (s, 3H, CH_3); HRMS Anal. calcd for $C_{16}H_{14}N_2O_3S$ 314.0725, Found: 314.0725.

6-[*N*-(4-Ethoxyphenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5b). Black powder (52%); mp: 202–203 °C; IR (KBr): ν 3253 (s, NH), 3073 (w), 1667 (s, C=O), 1470–1554, 1324 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.0 (d, J = 8.8 Hz, 2H), 6.9 (d, J = 8.8 Hz, 2H), 4.0 (q, J = 7.8 Hz, 2H, CH_2), 2.8 (s, 3H, CH_3), 1.5 (s, 3H, CH_3), 1.4 (t, J = 7.8 Hz, 3H, CH_3); HRMS Anal. calcd for $C_{17}H_{16}N_2O_3S$ 328.0882, Found: 328.0881.

6-[*N*-(4-Methylphenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5c). Dark violet flake (56%); mp: 190–192 °C; IR (KBr): ν 3314 (s, NH), 3029 (w), 1660 (s, C=O), 1454–1554 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.1 (d, J = 8.4 Hz, 2H), 6.9 (d, J = 8.4 Hz, 2H), 2.8 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{16}H_{14}N_2O_2S$ 298.0776, Found: 298.0776.

6-[*N*-(4-Fluorophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5d). Black powder (76%); mp: 171–172 °C; IR (KBr): ν 3320 (s, NH), 3061 (w), 1653 (s, C=O), 1455-1591 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.1 (d, 2H), 6.9 (d, 2H), 2.8 (s, 3H, CH_3), 1.5 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}FN_2O_2S$ 302.0525, Found: 302.0526.

6-[*N*-(2-Fluorophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5e). Black powder (54%); mp: 142–143 °C; IR (KBr): ν 3357 (s, NH), 3072 (w), 1660 (s, C=O), 1459–1515 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.1–7.2 (m, 4H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}FN_2O_2S$ 302.0525, Found: 302.0526.

6-[*N*-(2,4-Difluorophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5f). Dark violet powder (72%); mp: 161–162 °C; IR (KBr): ν 3329 (s, NH), 3063 (w), 1670 (s, C=O), 1454–1515 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.0 (s, 1H, NH), 7.0–7.2 (m, 2H), 6.9 (d, 1H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{10}F_2N_2O_2S$ 320.0431, Found: 320.0430.

6-[*N*-(4-Chlorophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5g). Black powder (86%); mp: 207–208 °C; IR (KBr): ν 3348 (s, NH), 3056 (w), 1658 (s, C=O), 1491–1599, 1369 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.0 (m, J = 8.8 Hz, 2H), 6.9 (d, J = 8.8 Hz, 2H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}ClN_2O_2S$ 318.0230, Found: 318.0227.

6-[*N*-(3,4-Dichlorophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5h). Dark violet powder (90%); mp: 210–213 °C; IR (KBr): ν 3331 (s, NH), 3066 (w), 1660 (s, C=O), 1469–1568, 1384 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.5 (m, J = 8.8 Hz, 2H), 7.2 (s, 1H), 6.9 (dd, J = 8.8 Hz, 1H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{10}Cl_2N_2O_2S$ 351.9840, Found: 351.9841.

6-[*N*-(4-Iodophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5i). Black powder (76%); mp: 202–204 °C; IR (KBr): ν 3354 (s, NH), 3056 (w), 1658 (s, C=O), 1497–1588, 1271 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.6 (d, J = 8.4 Hz, 2H), 6.8 (d, 2H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}IN_2O_2S$ 409.9586, Found: 409.9587.

6-[*N*-(4-Bromophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5j). Shining dark black powder (80%); mp: 215–217 °C; IR (KBr): ν 3352 (s, NH), 3088 (w), 1647 (s, C=O), 1493–1599, 1369 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.4 (d, J = 8.4 Hz, 2H), 6.9 (d, J = 8.4 Hz, 2H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}BrN_2O_2S$ 363.9705, Found: 363.9730.

6-[*N*-(3-Bromophenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5k). Shining black powder (88%); mp: 194–198 °C; IR (KBr): ν 3313 (s, NH), 3077 (w), 1661 (s, C=O), 1454–1567, 1310 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.2 (m, 2H), 7.12 (d, 1H), 7.0 (d, 1H), 2.8 (s, 3H, CH_3), 1.6 (s, 3H, CH_3); HRMS Anal. calcd for $C_{15}H_{11}BrN_2O_2S$ 363.9705, Found: 363.9730.

6-[*N*-(4-Butylphenyl)amino]-2,5-dimethyl-4,7-dioxobenzothiazole (5l). Black plate (51%); mp: 118–120 °C; IR (KBr): ν 3323 (s, NH), 2927 (w), 1658 (s, C=O), 1454–1554, 1308 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.1 (s, 1H, NH), 7.1 (d, 2H), 6.9 (d, 2H), 2.8 (s, 3H, CH_3), 2.7 (t, 2H, CH_2), 2.6 (s, 3H, CH_3), 2.5 (quintet, 3H, CH_3), 2.3 (sextet, 2H, CH_2), 0.9 (t, 3H, CH_3); HRMS Anal. calcd for $C_{19}H_20N_2O_2S$ 340.1246, Found: 340.1246.

General procedure for synthesis of 6-arylamino-4,7-dioxobenzothiazoles 6a–6e

A solution of compound **12**[5] (0.195 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylamine (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4–6 h. After the reaction mixture was kept overnight, the precipitate was collected by the filtration. The crude product was purified by silica gel column chromatography with $CHCl_3$ or crystallized from 95% EtOH. Crystallization from aq EtOH afforded 6-arylamino-4,7-dioxobenzothiazoles **6a–6e** (Table 2).

6-[*N*-(4-Fluorophenyl)amino]-4,7-dioxobenzothiazole (6a). Black powder (80%); mp: 242–243 °C; IR (KBr): 3270 (NH), 3050 (w), 1694 (s, C=O), 1590–1470, 1230 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.5 (s, 1H, H2), 9.3 (d, 1H, NH), 7.5–7.3 (m, 4H), 5.8 (s, 1H, H5); HRMS Anal. calcd for $C_{13}H_7FN_2O_2S$ 274.0212, Found: 274.0211.

6-[*N*-(3-Fluorophenyl)amino]-4,7-dioxobenzothiazole (6b). Violet powder (84%); mp: 236–238 °C; IR (KBr): 3270 (NH), 3050 (w), 1694 (s, C=O), 1590–1470, 1230 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.6 (s, 1H, H2), 9.4 (s, 1H, NH), 7.5–7.3 (m, 4H), 6.0 (s, 1H, H5); HRMS Anal. calcd for $C_{13}H_7FN_2O_2S$ 274.0212, Found: 274.0212.

6-[*N*-(Phenyl)amino]-4,7-dioxobenzothiazole (6c). Black powder (72%); mp: 219–221 °C; IR (KBr): 3270 (NH), 3050 (w), 1693 (s, C=O), 1553–1470 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.5 (s, 1H, H2), 9.4 (s, 1H, NH), 7.4–7.5 (m, 5H), 5.9 (s, 1H, H5); HRMS Anal. calcd for $C_{13}H_8N_2O_2S$ 256.0307, Found: 256.0308.

6-[*N*-(4-Chlorophenyl)amino]-4,7-dioxobenzothiazole (6d). Dark violet powder (85%); mp: 216–217 °C; IR (KBr): 3277 (NH), 3000 (w), 1694 (s, C=O), 1590–1470 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.5 (d, 1H, H2), 9.4 (s, 1H, NH), 7.5–7.4 (m, 4H), 6.2 (s, 1H, H5); HRMS Anal. calcd for $C_{13}H_7ClN_2O_2S$ 289.9917, Found: 289.9917.

6-[*N*-(4-Trifluoromethylphenyl)amino]-4,7-dioxobenzothiazole (6e). Purple powder (87%); mp: 227–228 °C; IR (KBr): 3270 (NH), 3000 (w), 1695 (s, C=O), 1590–1470, 1270, 1230 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.6 (s, 1H, H2), 9.4 (s, 1H, NH), 7.5–7.4 (m, 4H), 6.0 (s, 1H, H5); HRMS Anal. calcd for $C_{14}H_7F_3N_2O_2S$ 324.0180, Found: 324.0181.

Antifungal in vitro susceptibility testing

The MIC values of compounds **3–6** were determined by the standard broth dilution method.[10] The antifungal activities were tested in modified Sabouraud dextrose broth against the following fungal strains: *C. albicans* ATCC 10231, *C. krusei* ATCC 749, *C tropicalis* ATCC 28775 and *A. niger* KCTC 1231. Ketoconazole as an antifungal standard agent was used. The compounds were tested in the 0.1–100 μg/mL range. That was added to the modified Sabouraud dextrose broth (Difco Lab.) for fungi over a final concentration range of 0.1–100 μg/mL. The inoculum sizes contained approximately 1×10^5 CFU/mL. They were incubated at 37 °C for appropriate periods of time that sufficed to show clearly visible growth on drug-free control broths. The MIC value was defined, as the lowest concentration of the antifungal agent at which there showed optically clear. MIC values were read after 1 day for *Candida* species and 2 days for *A. niger* in 37 °C.

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12. Unpublished data; we also tested cytotoxic activity of compounds **3–6** against human tumor cell lines such as A 549, HL-60 and HepG2 according to the NCI protocols.[11] All of the tested compounds did not show significant cytotoxic activity.