Synthesis of Benzofuro[6,7-*d*]thiazoles, Benzofuro[7,6-*d*]thiazoles and 6-Arylaminobenzo[*d*]thiazole-4,7-diones as Antifungal Agent

Chung-Kyu Ryu,* Ji-Hee Nho, Guohua Jin, Sun Young Oh, and Soo Jung Choi

College of Pharmacy & Graduate School of Pharmaceutical Sciences, Ewha Womans University; 52 Ewhayeodae-gil, Seodaemun-ku, Seoul 120–750, Republic of Korea.

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Benzofuro[6,7-*d*]thiazoles, benzofuro[7,6-*d*]thiazoles and 6-arylaminobenzo[*d*]thiazole-4,7-diones were synthesized and tested for *in vitro* antifungal activity against *Candida*, *Aspergillus* species and *Cryptococcus neoformans*. Among them tested, many of synthesized compounds showed potent antifungal activity. The compounds 4d, 6e and 6h completely inhibited the growth of all *Candida* and *Aspergillus* species tested at the MIC level of 6.3 μg/mL. The results suggest that benzofuro[6,7-*d*]thiazoles and 6-arylaminobenzo[*d*]-thiazole-4,7-diones would be promising antifungal agents.

Key words benzofuro[6,7-*d*]thiazole; benzofuro[7,6-*d*]thiazole; benzo[*d*]thiazole-4,7-dione; antifungal; antimicrobial; fungi

The recent increase of fungal infections, especially among patients undergoing anticancer chemotherapy and AIDS patients, has generated a renewed interest in antifungal drugs, including development of new antifungal agents in the development of resistance to drugs.[1,2] In a program aimed at identifying novel antifungal agents, we focused on developing benzofuro[6,7-*d*]thiazoles, benzofuro[7,6-*d*]thiazoles and 6-arylaminobenzo[*d*]thiazole-4,7-diones with new mode of antifungal action.

N-Myristoyltransferase has been showed to be essential for the viability of fungi, including medically important pathogenic fungi, *Candida albicans*[3] and *Cryptococcus neoformans*.[4,5] It could be a target for the development of antifungal agents with a novel mode of action. A benzofuran derivative **1**, a novel myristoyltransferase inhibitor, has been reported as antifungal agent[6,7] as well as antibacterial agent[8,9] (Fig. 1). Benzofuran-5-ol **2** and 5-hydroxyfuro[2,3-*f*]quinoline **3** scaffolds[10] have demonstrated potent antifungal activity against pathogenic fungi.

Structure–activity relationship studies from heterocyclic quinonoid compounds indicated that the number and position of nitrogen (N) or sulfur (S) atoms substituted in the heterocyclic ring were considerably important factors to affect the biological activities.[10,11] Generally, increasing the number of substituent nitrogen or sulfur atoms in the ring enhances the activities. We speculated that incorporation of thiazole into the skeleton in compounds **2** or **3** would change the physicochemical properties, and lead to a new pharmacophore alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]thiazole-6-carboxylates **4** and alkyl 7-amino-4-hydroxy-2-methylbenzofuro[7,6-*d*]-thiazole-6-carboxylates **5** with a different biological profile from scaffolds **2** or **3**. The compounds **4** and **5** which would be bioisosteres of compound **3** could metabolize to benzo[*d*]-thiazole-4,7-dione derivatives with a quinonoid structure in fungi. Quinonoid benzo[*d*]thiazole-4,7-diones display potent biological properties including antifungal, antimalarial and antibacterial activity.[10] The thiazole moiety of quinonoid compounds could improve upon the biological activities. We

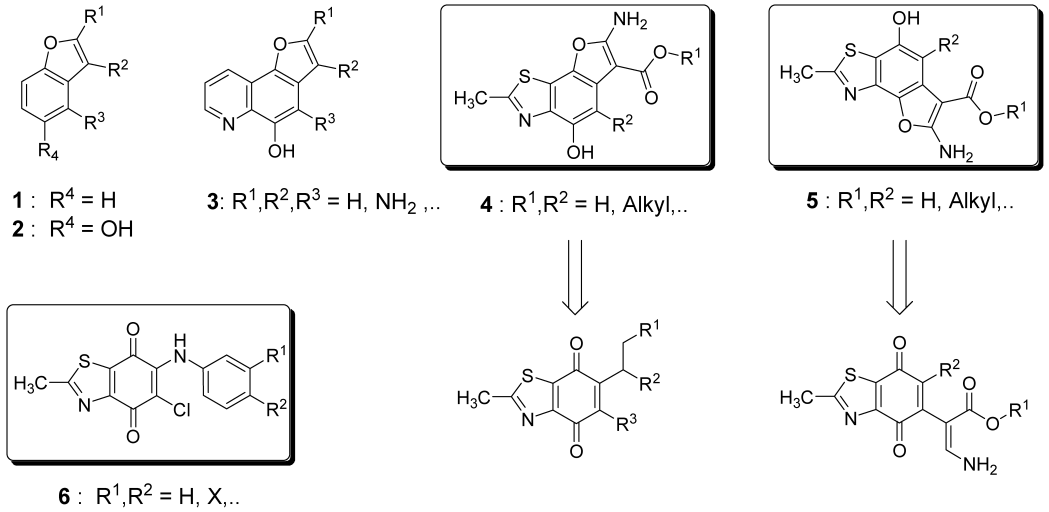

Fig. 1. Benzofuran, 4-Hydroxybenzofuro[6,7-*d*]thiazoles, 4-Hydroxybenzofuro[7,6-*d*]thiazoles and 6-Arylaminobenzo[*d*]thiazole-4,7-diones

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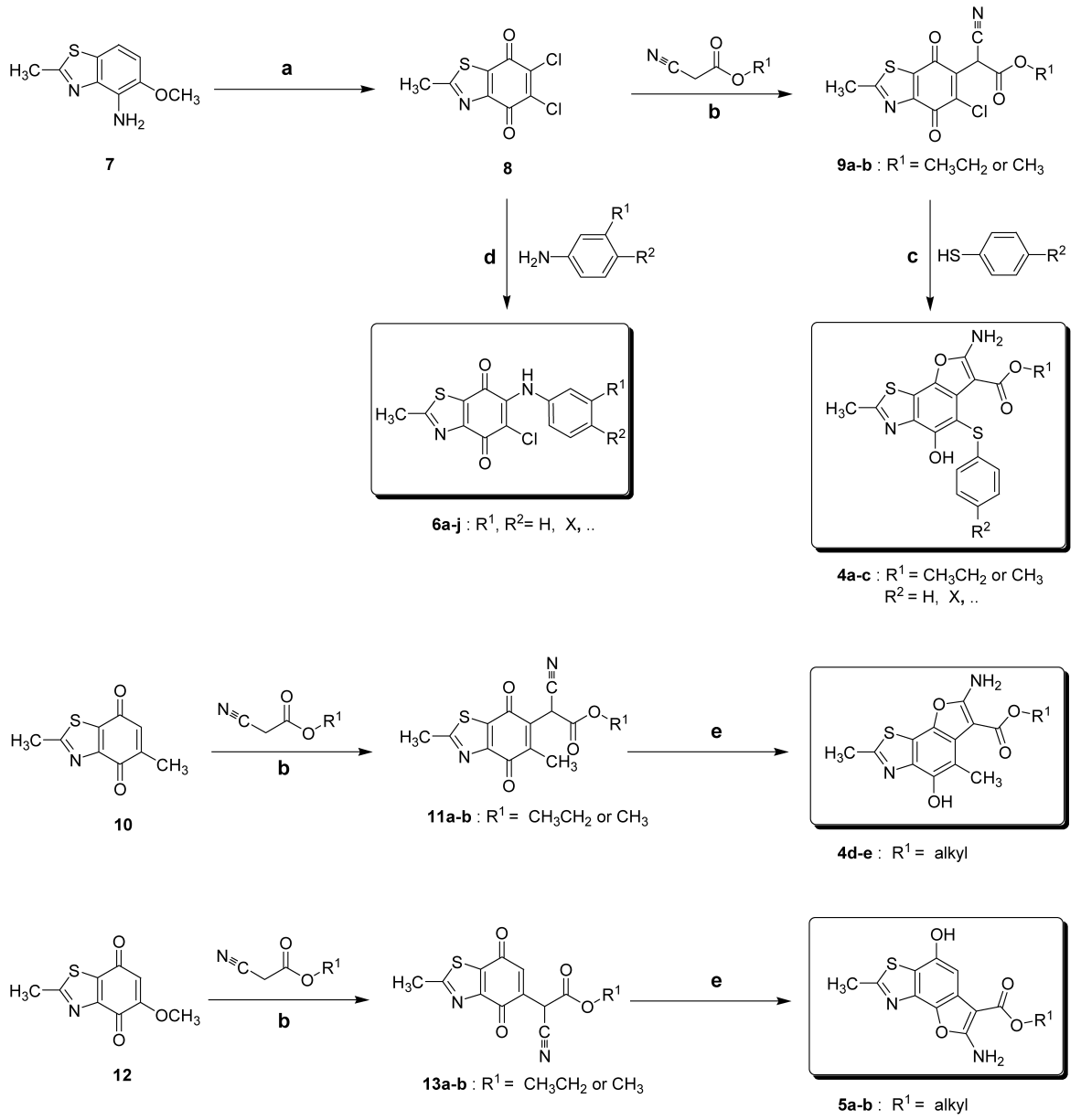
* To whom correspondence should be addressed. e-mail: ckryu@ewha.ac.kr



Reagents and conditions: (a) c-HCl/$NaClO_4$/rt/0.5 h, (b) alkyl cyanoacetate/NH_4OH/$CeCl_3$/EtOH/rt/30 min, (c) arylthiol (1 eq)/EtOH/hydrazine/reflux/5 h, (d) arylamine (1 eq)/$CeCl_3$/EtOH/rt/20 min, (e) hydrazine hydrate/EtOH/rt/2 h.

Chart 1. Synthesis of Alkyl 7-Amino-4-hydroxybenzofuro[6,7-*d*]thiazoles, 7-Amino-4-hydroxybenzofuro[7,6-*d*]thiazoles and 5-Chloro-6-arylamino-benzo[*d*]thiazole-4,7-diones

assumed that alkyl 4-hydroxybenzofuro[6,7-*d*]thiazoles **4** and 4-hydroxy-2-methylbenzofuro[7,6-*d*]thiazole **5** could have similar biological activities with those of benzo[*d*]thiazole-4,7-diones.

There have not been any reports on alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]thiazole **4** and 7-amino-4-hydroxybenzofuro[7,6-*d*]thiazole scaffolds **5** to the best of our knowledge. The presence of thio, alkyl, amino group or halogen atoms on quinonoid compounds such as benzo[*d*]-thiazole-4,7-diones,[12] 1,4-naphthoquinones[13] and 5-hydroxyfuro[2,3-*f*]quinoline **3** significantly affects their antifungal activity. Thus, a variety of alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]thiazoles **4a**–**e**, alkyl 7-amino-4-hydroxy-2-methylbenzofuro[7,6-*d*]thiazole-6-carboxylates **5a**–**b** and 5-chloro-6-arylaminobenzo[*d*]thiazole-4,7-diones **6a**–**j** with different substituents could exhibit the biological activities through different actions and sometimes improve upon the activities (Chart 1, Table 1).

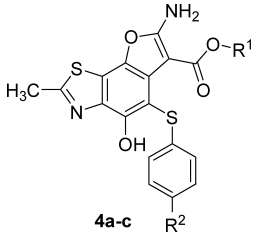
Based on this speculation, compounds **4a**–**e**, **5a**, **b** and **6a**–**j** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity.

The *in vitro* antifungal activity of new compounds **4a**–**e**, **5a**, **b** and **6a**–**j** against pathogenic fungi was determined by the twofold broth dilution method. Additional data for antifungal activity are provided.

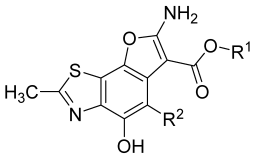
Results and Discussion

Chemistry Methods for the synthesis of compounds **4a**–**e**, **5a**, **b** and **6a**–**j** are shown in Chart 1 and Table 1. 6-Methoxy-7-aminobenzothiazole (**7**) was prepared from commercially available 6-methoxyobenzothiazole.[14] 5,6-Dichloro-2-methylbenzo[*d*]thiazole-4,7-dione (**8**) was synthesized by

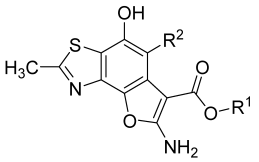
Table 1. Structures and Antifungal Activity for 7-Amino-4-hydroxybenzofuro[6,7-*d*]thiazoles, 7-Amino-4-hydroxybenzofuro[7,6-*d*]thiazoles and 5-Chloro-6-arylaminobenzo[*d*]thiazole-4,7-diones



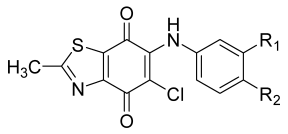
4a-c



4d-e



5a-b



6a-j

Compound	R^1	R^2	MIC[a] (μg/mL)					
			C. albicans [b]	*C. tropicalis*	*C. krusei*	*C. neoformans*	*A. niger*	*A. flavus*
4a	CH_3CH_2	CH_3	12.5	12.5	25.0	12.5	12.5	50.0
4b	CH_3CH_2	F	12.5	6.3	50.0	12.5	12.5	12.5
4c	CH_3CH_2	CH_3O	6.3	12.5	1.6	12.5	6.3	25.0
4d	CH_3CH_2	CH_3	3.2	6.3	6.3	3.2	6.3	3.2
4e	CH_3	CH_3	25.0	12.5	12.5	6.3	50.0	12.5
5a	CH_3CH_2	H	100.0	50.0	25.0	25.0	100.0	50.0
5b	CH_3	H	100.0	25.0	25.0	25.0	100.0	50.0
6a	H	CH_3	12.5	6.3	6.3	12.5	25.0	12.5
6b	H	H	12.5	6.3	6.3	12.5	25.0	12.5
6c	H	F	12.5	25.0	3.2	6.3	25.0	1.6
6d	H	Br	3.2	12.5	12.5	6.3	6.3	12.5
6e	H	CH_3O	1.6	6.3	6.3	6.3	1.6	1.6
6f	H	CN	3.2	25.0	12.5	12.5	3.2	6.3
6g	F	H	12.5	6.3	100.0	12.5	6.3	25.0
6h	CH_3	CH_3	3.2	3.2	6.3	6.3	1.6	6.3
6i	H	I	3.2	25.0	12.5	12.5	3.2	6.3
6j	H	Cl	50.0	12.5	50.0	1.6	25.0	50.0
7	—	—	100.0	50.0	100.0	>100.0	>100.0	>100.0
Fluconazole	—	—	25.0	6.3	25.0	6.3	25.0	25.0
5-FC[c]	—	—	3.2	3.2	6.3	12.5	6.3	6.3

a) The MIC value was defined as the lowest concentration of the antifungal agent. MIC values were read after 1 d for *Candida* species and *Cryptococcus neoformans*, and 2 d for *Aspergillus* species in 37°C. The inoculum sizes contained approximately 1×10^5 cells/mL. Culture media tested were the modified Sabouraud dextrose broth (Difco Lab.). The final concentration of antifungal agents was between 0.2 and 50.0 μg/mL. *b*) Fungi tested: *Candida albicans* Berkout KCCM 50235, *C. tropicalis* Berkout KCCM 50662, *C. krusei* Berkout KCCM 11655, *Cryptococcus neoformans* KCCM 50564, *Aspergillus niger* KCTC 1231 and *Aspergillus flavus* KCCM 11899. *c*) 5-FC: 5-fluorocytosine.

chlorooxidizing compound **7** with $HCl/NaClO_4$ resulting in 70% yields. Consequently, alkyl 2-(5-chloro-2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]thiazol-6-yl)-2-cyanoacetates **9a**, **b** were synthesized by nucleophilic substitution of compound **8** with 1 eq of alkyl cyanoacetate in the presence of NH_4OH and $CeCl_3$. Alkyl 7-amino-5-arylthio-4-hydroxy-2-methylbenzofuro[6,7-*d*]thiazole-6-carboxylates **4a**–**c** were synthesized by nucleophilic substitution and cyclization of the compound **9a** or **9b** with appropriate arylthiols in EtOH. To a solution of the compound **9a** or **9b** in EtOH, 1 eq of arylthiol was added. The mixture was refluxed for 5 h and concentrated *in vacuo*. Purification of residual crude product by column chromatography yielded compounds **4a**–**c**. Most of these reactions went as expected and had overall low yields.

2,5-Dimethylbenzo[*d*]thiazole-4,7-dione (**10**) was prepared according to previously reported method.[12] Consequently, alkyl 2-cyano-2-(2,5-dimethyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-6-yl)acetates **11a**, **b** were synthesized by nucleophilic substitution of compound **10** with 1 eq of alkyl cyanoacetate in the presence of NH_4OH. Alkyl 7-amino-4-hydroxy-2,5-dimethylbenzofuro[6,7-*d*]thiazole-6-carboxylates **4d**, **e** were synthesized by cyclization of compounds **11a**, **b** with hydrazine hydrate in EtOH. To a solution of the compounds **11a**, **b** in EtOH, 1 eq of hydrazine was added.

In a similar manner, alkyl 7-amino-4-hydroxy-2-methylbenzofuro[7,6-*d*]thiazole-6-carboxylates **5a**, **b** were synthesized from 5-methoxy-2-methylbenzo[*d*]thiazole-4,7-dione (**12**). Alkyl 2-cyano-2-(2-methyl-4,7-dioxo-4,7-dihydrobenzo-[*d*]thiazol-5-yl)acetates **13a**, **b** were synthesized by nucleophilic substitution of compound **12** with 1 eq of alkyl cyanoacetate in the presence of NH_4OH. Alkyl 2-cyano-2-(2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]thiazol-5-yl)acetates **5a**, **b** were synthesized by cyclization of the compounds **13a**, **b** with hydrazine hydrate in EtOH. 6-Arylamino-5-chloro-2-methylbenzo[*d*]thiazole-4,7-diones **6a**–**j** were prepared from the compound **8**. The compounds **6a**–**j** were formed by regioselective nucleophilic substitution of the compound **8** with the appropriate arylamines in the presence of $CeCl_3$. The substitutions gave exclusively or mainly 6-arylamino-substituted compounds **6a**–**j** along with traces of 7-arylamino-substituted compounds as regioisomer. Compounds **6a**–**j** were purified by column chromatography. Most of these substitutions went as expected and had overall high yields.

Antifungal Evaluation The synthesized alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]thiazoles **4a**–**e**, 7-amino-4-hydroxybenzofuro[7,6-*d*]thiazoles **5a**, **b**, 5-chloro-6-arylaminobenzo[*d*]thiazole-4,7-diones **6a**–**j** and **7** were tested *in vitro* for their growth inhibitory activity against pathogenic fungi using

the standard method.[15] The minimum inhibitory concentration (MIC) values were determined by comparison with fluconazole and 5-fluorocytosine as standard agents.

As indicated in Table 1, many of compounds **4a**–**e**, **5a**, **b** and **6a**–**j** showed potent antifungal activity against tested fungi. Actually, the activity of compounds **4d**, **6e** and **6h** was superior or comparable to that of fluconazole against fungi. The compounds **4d**, **6e** and **6h** completely inhibited the growth of all against *Candida* and *Aspergillus* species tested at the MIC level of 6.3 μg/mL. The activity of many compounds among them tested was comparable to that of 5-fluorocytosine against some strain of fungi. Many of compounds **4a**–**e**, **5a**, **b** and **6a**–**j** also were comparable to those of fluconazole against *Candida* species, *Cryptococcus neoformans*, and *Aspergillus* species. Actually, the activity of compounds **6c** and **6f** was superior to that of 5-fluorocytosine against *C. neoformans* and *A. flavus*. The alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]-thiazoles **4a**–**e**, showed also antifungal activity. In contrast, 7-amino-4-hydroxybenzofuro[7,6-*d*]thiazoles **5a**, **b** did not show significant antifungal activity, although some compounds of them exhibited good activity against *C. krusei* and *C. neoformans*.

In terms of structure–activity relationship, the 5-chloro-6-arylaminobenzo[*d*]thiazole-4,7-diones **6a**–**j** showed, in general, a more potent antifungal activity than the other alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]thiazoles **4a**–**e** or 7-amino4-hydroxybenzofuro[7,6-*d*]thiazoles **5a**, **b**. The activity of alkyl 7-amino-4-hydroxybenzofuro[6,7-*d*]thiazoles **4a**–**e** was better than that of 7-amino-4-hydroxybenzofuro[7,6-*d*]-thiazoles **5a**, **b**. Thus, the benzo[*d*]thiazole-4,7-dione skeleton of compounds **6a**–**j** appear to be important factor to affect their antifungal activity. The benzo[*d*]thiazole-4,7-dione skeleton **6a**–**j** exhibited good activity, indicating a correlation that may offer insight into the mode of action of these compounds.

In addition, 6-methoxy-7-aminobenzothiazole (**7**) exhibited no or poor, if any, antifungal activity. In contrast, compounds **4a**–**e**, **5a**, **b** and **6a**–**j** showed more potent antifungal activity than 6-methoxy-7-aminobenzothiazole (**7**). The quinonoid 4-hydroxybenzofuro[6,7-*d*]thiazole, 4-hydroxybenzofuro[7,6-*d*]thiazole or benzo[*d*]thiazole-4,7-dione moiety in compounds **4**, **5** and **6** should be essential for the activity, for example, as nonquinonoid compound **7** lost the activity, indicating a correlation that may offer insight into the mode of action of these compounds. The substituents (R^1, R^2, R^3: H, X, Me, *etc*.) for the compounds **4**, **5** and **6** may not contribute partially toward biological potency. Thus, the substituents appear to be not an important factor to affect their antifungal activity.

Conclusion

Alkyl 2-(5-chloro-2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-6-yl)-2-cyanoacetates **9a**, **b** were synthesized by nucleophilic substitution of 5,6-dichloro-2-methylbenzo[*d*]-thiazole-4,7-dione (**8**) with 1 eq of alkyl cyanoacetate in the presence of NH_4OH. Alkyl 7-amino-5-arylthio-4-hydroxy-2-methylbenzofuro[6,7-*d*]thiazole-6-carboxylates **4a**–**c** were synthesized by nucleophilic substitution and cyclization of compound **9a** or **9b** with 1 eq of appropriate arylthiols in EtOH. Alkyl 2-cyano-2-(2,5-dimethyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-6-yl)acetates **11a**, **b** were synthesized by nucleophilic substitution of 2,5-dimethylbenzo[*d*]thiazole-4,7-dione (**10**) with 1 eq of alkyl cyanoacetate. Alkyl 7-amino-4-hydroxy-2,5-dimethylbenzofuro[6,7-*d*]thiazole-6-carboxylates **4d**–**e** were synthesized by cyclization of the compounds **11a**, **b** with hydrazine in EtOH. 6-Arylamino-5-chloro-2-methylbenzo[*d*]-thiazole-4,7-diones **6a**–**j** were prepared by regioselective nucleophilic substitution of the compound **8** with the appropriate arylamines in the presence of $CeCl_3$. Most of these substitutions went as expected and had overall high yields. We have identified a lead compound that has antifungal activity by screening of our compounds **4a**–**e**, **5a**, **b** and **6a**–**j**. Among them tested, most of 5-chloro-6-arylaminobenzo[*d*]-thiazole-4,7-diones showed potent antifungal activity. The results suggest that benzofuro[6,7-*d*]thiazole and 5-chloro-6-arylaminobenzo[*d*]thiazole-4,7-dione scaffolds would be promising leads for the development of antifungal agents.

Experimental

All melting points were measured with Büchi melting point B-545 and were uncorrected. ^{1}H-NMR spectra, ^{13}C-NMR and ^{19}F-NMR spectra were recorded on Varian Unity INOVA 400 MHz FT-NMR spectrometer with tetramethylsilane (TMS) or $CFCl_3$. High resolution (HR)-MS spectra were recorded with a Agilent 6220 Accurate-Mass time-of-flight (TOF)/LC-MS equipped with an electrospray ionisation ion source used. Mass spectra were taken with Jeol JMS AX505 WA. The IR spectra were taken from PerkinElmer, Inc. 1420r IR spectrometer with KBr pellets or Nujol.

6-Methoxy-7-aminobenzothiazole (**7**) was prepared from commercially available 6-methoxybenzothiazole. 5,6-Dichloro-2-methylbenzo[*d*]thiazole-4,7-dione (**8**) was synthesized by chlorooxidizing compound **7** with $HCl/NaClO_4$. Dimethylbenzo[*d*]-thiazole-4,7-dione (**10**)[14] and 5-methoxy-2-methylbenzo[*d*]-thiazole-4,7-dione (**12**)[16] were prepared according to previously reported method.

The products were separated by silica gel column chromatography. Purity of products was determined both by to TLC and HPLC. The results showed that a single compound was contained in each product. TLC was performed on precoated silica gel (60G 254, Merck) using *n*-hexane/EtOAc for solvent. The compounds were detected under UV light (254 nm).

General Procedure for Synthesis of Alkyl 7-Amino-4-hydroxy-2,5-dimethylbenzofuro[6,7-*d*]thiazole-6-carboxylates 4 To a solution of compound **8** (2.66 mmol) and ethylcyanoacetate or methylcyanoacetate (2.66 mmol) in 100 mL of EtOH, 2 mL of NH_4OH solution was added dropwise. The mixture was stirred at room temperature for 10 min, 5 mL of *d*-HCl was then added. The mixture was then extracted several times with CH_2Cl_2, and the organic layer was washed with water, dried with anhydrous $MgSO_4$, and concentrated *in vacuo*. The product **9a** or **9b** was seperated by silica gel column chromatography (eluted from *n*-hexane : EtOAc=1 : 1 to 1 : 2).

Ethyl 2-(5-Chloro-2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-6-yl)-2-cyanoacetate (**9a**): Brown oil (87%). IR (Nujol) cm^{-1}: 2262 (CN), 1697 (s, C=O), 1377, 1227 (s). ^{1}H-NMR ($CDCl_3$) δ: 4.38 (s, 1H, CH), 4.21 (q, 2H, J=7.2 Hz, CH_2), 2.81 (s, 3H, CH_3), 1.31 (t, 3H, J=7.2 Hz, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 187.4, 176.2, 171.9, 168.5, 148.9, 148.1, 143.8, 142.7, 62.9, 23.1, 18.5, 14.4. HR-MS *m/z*: 325.0009 [$(M+H)^+$] (Calcd for $C_{13}H_{10}ClN_2O_4S$: 325.0005).

Methyl 2-(5-Chloro-2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-6-yl)-2-cyanoacetate (**9b**): Brown oil (73%). IR (Nujol) cm^{-1}: 2257 (CN), 1692 (s, C=O), 1390, 1224 (s). ^{1}H-NMR

($CDCl_3$) δ: 4.30 (s, 1H, CH), 4.10 (s, 3H, CH_3), 2.80 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 188.1, 176.4, 172.3, 168.4, 148.7, 147.9, 143.5, 142.3, 61.7, 22.9, 18.9. HR-MS *m/z*: 310.9891 [(M+H)$^+$] (Calcd for $C_{12}H_8ClN_2O_4S$: 310.9893).

To the solution of compound **9a** or **9b** (0.45 mmol) in 100 mL of 95% EtOH, appropriate arylthiol (0.45 mmol) was added and the mixture was stirred at room temperature or refluxed for 4–5 h. To the reaction mixture, 1 eq of hydrazine was added. The mixture was refluxed further for 5 h and concentrated *in vacuo*. The products **4a–c** were purified by silica gel column chromatography (eluted from *n*-hexane : EtOAc=2 : 1 to 1 : 2), and crystalized from 95% EtOH.

Ethyl 7-Amino-4-hydroxy-2-methyl-5-(*p*-tolylthio)benzofuro[6,7-*d*]thiazole-6-carboxylate (**4a**): Brown powder (54%). mp 219–221°C. IR (KBr) cm^{-1}: 3515–3270 (s, OH), 3315 (m, NH_2), 1692 (s, C=O), 1620 (w), 1228 (s). ^{1}H-NMR (DMSO-d_6) δ: 7.28 (d, 2H, J=8.8 Hz), 6.86 (d, 2H, J=8.8 Hz), 6.42 (s, 2H) 5.10 (s, 1H), 4.08 (q, 2H, J=7.2 Hz), 2.72 (s, 3H), 2.58 (s, 3H), 1.10 (t, 3H, J=7.2 Hz). ^{13}C-NMR (DMSO-d_6) δ: 166.1, 158.9, 151.6, 150.2, 145.6, 139.9, 132.8, 131.4, 130.2, 129.5, 127.2, 122.5, 115.3, 110.1, 61.3, 21.1, 14.7, 13.3. HR-MS *m/z*: 415.0783 [(M+H)$^+$] (Calcd for $C_{20}H_{19}N_2O_4S_2$: 415.0786).

Ethyl 7-Amino-5-((4-fluorophenyl)thio)-4-hydroxy-2-methylbenzofuro[6,7-*d*]thiazole-6-carboxylate (**4b**): Brown powder (45%). mp 204–209°C. IR (KBr) cm^{-1}: 3496–3242 (s, OH), 3285 (m, NH_2), 1695 (s, C=O), 1625 (w), 1220 (s). ^{1}H-NMR (DMSO-d_6) δ: 7.32–6.78 (m, 4H) 6.42 (s, 2H), 4.11 (q, 2H, J=7.2 Hz), 2.71 (s, 3H), 2.55 (s, 3H), 1.11 (t, 3H, J=7.2 Hz). ^{13}C-NMR (DMSO-d_6) δ: 166.4, 161.4, 158.5, 151.7, 150.7, 145.3, 139.4, 132.3, 131.7, 129.2, 127.4, 122.4, 115.3, 110.9, 60.3, 14.9, 13.2. ^{19}F-NMR (376.3 MHz, $CDCl_3$) δ: −116.3. HR-MS *m/z*: 419.0537 [(M+H)$^+$] (Calcd for $C_{19}H_{16}FN_2O_4S_2$: 419.0535).

Ethyl 7-Amino-4-hydroxy-5-((4-methoxyphenyl)thio)-2-methylbenzofuro[6,7-*d*]thiazole-6-carboxylate (**4c**): Brown powder (67%). mp 221–225°C. IR (KBr) cm^{-1}: 3490–3234 (s, OH), 3247 (m, NH_2), 1690 (s, C=O), 1616 (w), 1228 (s). ^{1}H-NMR (DMSO-d_6) δ: 7.48 (d, 2H, J=8.8 Hz), 7.29 (d, 2H, J=8.8 Hz), 6.71 (s, 2H), 5.10 (s, 1H), 4.08 (q, 2H, J=7.2 Hz), 2.72 (s, 3H), 2.58 (s, 3H), 1.10 (t, 3H, J=7.2 Hz). ^{13}C-NMR (DMSO-d_6) δ: 166.4, 159.4, 158.5, 151.7, 150.7, 145.3, 139.4, 132.3, 131.7, 129.2 127.4, 122.4, 115.3, 110.9, 60.3, 55,8, 14.9, 13.2. HR-MS *m/z*: 431.0733 [(M+H)$^+$] (Calcd for $C_{20}H_{19}N_2O_5S_2$: 431.0735).

General Procedure for Synthesis of Alkyl 7-Amino-4-hydroxy-2,5-dimethylbenzofuro[6,7-*d*]thiazole-6-carboxylate 4d and 4e To a solution of compound **10** (2.66 mmol) and ethylcyanoacetate or methylcyanoacetate (2.66 mmol) in 100 mL of EtOH, 2 mL of NH_4OH solution was added dropwise. The mixture was stirred at room temperature for 20 min, 5 mL of *d*-HCl was then added. The mixture was then extracted several times with CH_2Cl_2, and the organic layer was washed with water, dried with anhydrous $MgSO_4$, and concentrated *in vacuo*. The product **11a** or **11b** was seperated by silica gel column chromatography (eluted from *n*-hexane : EtOAc=1 : 1 to 1 : 3).

Ethyl 2-Cyano-2-(4,7-dihydro-2,5-dimethyl-4,7-dioxobenzo-[*d*]thiazol-6-yl)acetate (**11a**): Brown oil (85%). IR (Nujol) cm^{-1}: 2246 (CN), 1703 (s, C=O), 1385, 1214 (s). ^{1}H-NMR ($CDCl_3$) δ: 5.38 (s, 1H, CH), 4.33 (q, 2H, J=7.2 Hz, CH_2), 2.32 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 1.34 (t, 3H, J=7.2 Hz, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 189.4, 177.2, 171.2, 167.4, 148.3, 148.3, 143.9, 142.2, 63.5, 23.3, 19.1, 14.7, 11.7. HR-MS *m/z*: 305.0598 [(M+H)$^+$] (Calcd for $C_{14}H_{13}N_2O_4S$: 305.0596).

Methyl 2-Cyano-2-(4,7-dihydro-2,5-dimethyl-4,7-dioxobenzo[*d*]thiazol-6-yl)acetate (**11b**): Brown oil (80%). IR (Nujol) cm^{-1}: 2259 (CN), 1698 (s, C=O), 1373, 1220 (s). ^{1}H-NMR ($CDCl_3$) δ: 5.39 (s, 1H, CH), 3.83 (s, 3H, OCH_3), 2.85 (s, 3H, CH_3), 2.19 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 189.1, 176.7, 172.0, 167.1, 148.9, 147.8, 143.4, 142.2, 62.1, 23.5, 19.3, 11.5. HR-MS *m/z*: 291.0437 [(M+H)$^+$] (Calcd for $C_{13}H_{11}N_2O_4S$: 291.0439).

One equivalent of hydrazine (0.45 mmol) was added to the solution of compound **11a** or **11b** (0.45 mmol) in 100 mL 95% EtOH. The mixture was stirred further at rt for 24 h and concentrated *in vacuo*. The products **4d–e** were purified by silica gel column chromatography (eluted from *n*-hexane : EtOAc= 2 : 1 to 1 : 2), and crystalized from 95% EtOH.

Ethyl 7-Amino-4-hydroxy-2,5-dimethylbenzofuro[6,7-*d*]-thiazole-6-carboxylate (**4d**): Brown powder (73%). mp 212–213°C. IR (KBr) cm^{-1}: 3452–3231 (s, OH), 3278 (m, NH_2), 1699 (s, C=O), 1215 (s). ^{1}H-NMR (DMSO-d_6) δ: 9.35 (s, 1H, OH), 7.71 (s, 2H, NH_2), 4.25 (q, 2H, J=7.2 Hz, CH_2), 2.78 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 1.31 (t, 3H, J=7.2 Hz, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 166.5, 158.4, 152.6, 150.4, 146.3, 131.4, 127.7, 122.1, 116.3, 111.1, 60.2, 18.1, 14.3, 13.7. HR-MS *m/z*: 307.0755 [(M+H)$^+$] (Calcd for $C_{14}H_{15}N_2O_4S$: 307.0753).

Methyl 7-Amino-4-hydroxy-2,5-dimethylbenzofuro[6,7-*d*]-thiazole-6-carboxylate (**4e**): Brown powder (68%). mp 205–206°C. IR (KBr) cm^{-1}: 3427–3250 (s, OH), 3305 (m, NH_2), 1708 (s, C=O), 1221 (s). ^{1}H-NMR (400 MHz) δ: 9.35 (s, 1H, OH), 7.75 (s, 2H, NH_2), 3.75 (s, 3H, OCH_3), 2.78 (s, 3H, CH_3), 2.54 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 166.9, 157.6, 152.9, 151.4, 147.1, 131.9, 127.1, 123.5, 115.7, 112.5, 61.0, 19.2, 14.1. HR-MS *m/z*: 293.0597 [(M+H)$^+$] (Calcd for $C_{13}H_{13}N_2O_4S$: 293.0596).

General Procedure for Synthesis of Alkyl 7-Amino-4-hydroxy-2-methylbenzofuro[7,6-*d*]thiazole-6-carboxylates 5 To a solution of compound **12** (2.5 mmol) and ethylcyanoacetate or methylcyanoacetate (2.5 mmol) in 100 mL of EtOH, 2 mL of NH_4OH solution was added dropwise. The mixture was stirred at rt for 20 min, 5 mL *d*-HCl was then added. The mixture was then extracted several times with CH_2Cl_2. The combined organic layer was washed with water, dried with anhydrous $MgSO_4$, and concentrated. The product **13a** or **13b** was seperated by silica gel column chromatography (eluted from *n*-hexane : EtOAc=2 : 1 to 1 : 1).

Ethyl 2-Cyano-2-(2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-5-yl)acetate (**13a**): Yellow oil (86%). IR (Nujol) cm^{-1}: 2237 (CN), 1706 (s, C=O), 1372, 1214 (s). ^{1}H-NMR (DMSO-d_6) δ: 6.90 (s, 1H), 4.22 (q, 2H, J=7.3 Hz, CH_2), 4.10 (s, H), 2.79 (s, 3H, CH_3), 1.30 (t, 3H, J=7.3 Hz, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 188.8, 177.8, 171.5 168.1, 148.1, 147.8, 143.5, 142.5, 63.7, 23.1, 18.7, 14.4. HR-MS *m/z*: 291.3027 [(M+H)$^+$] (Calcd for $C_{13}H_{11}N_2O_4S$: 291.3024).

Methyl 2-Cyano-2-(2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]-thiazol-5-yl)acetate (**13b**): Yellow oil (72%). IR (Nujol) cm^{-1}: 2260 (CN), 1705 (s, C=O), 1381, 1220 (s). ^{1}H-NMR (DMSO-d_6) δ: 6.84 (s, 1H), 4.30 (s, 3H), 4.15 (s, H), 2.81 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 188.3, 177.4, 172.6, 167.8, 148.5, 148.1, 143.2, 143.1, 62.9, 23.4, 18.9. HR-MS *m/z*: 277.2755 [(M+H)$^+$] (Calcd for $C_{12}H_9N_2O_4S$: 277.2759).

One equivalent of hydrazine (0.45 mmol) was added to the

solution of compound **13a** or **13b** (0.45 mmol) in 100 mL of 95% EtOH. The mixture was refluxed further for 5 h and concentrated *in vacuo*. The products **4d**, **e** were purified by silica gel column chromatography (eluted from *n*-hexane : EtOAc = 2 : 1 to 1 : 1) and crystalized from 95% EtOH.

Ethyl 7-Amino-4-hydroxy-2-methylbenzofuro[7,6-*d*]thiazole-6-carboxylate (**5a**): Pale brown powder (35%). mp 300–304°C. IR (KBr) cm^{-1}: 3432–3259 (s, OH), 3297 (m, NH_2), 1703 (s, C=O), 1228 (s). ^{1}H-NMR (DMSO-d_6) δ: 10.11 (s, 1H, OH), 7.68 (s, 2H, NH_2), 7.13 (s, 1H, quinone), 4.25 (q, 2H, J=7.20 Hz, CH_2), 2.78 (s, 3H, CH_3), 1.34 (t, 3H, J=7.20 Hz, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 167.3, 158.8, 151.3, 150.7, 147.3, 132.1, 129.5, 122.4, 114.2, 112.1, 61.2, 17.9, 13.9. HR-MS *m/z*: 293.0594 [(M+H)$^+$] (Calcd for $C_{13}H_{13}N_2O_4S$: 293.0596).

Methyl 7-Amino-4-hydroxy-2-methylbenzofuro[7,6-*d*]thiazole-6-carboxylate (**5b**): Pale brown powder (30%). mp 245–258°C. IR (KBr) cm^{-1}: 3430–3246 (s, OH), 3273 (m, NH_2), 1695 (s, C=O), 1236 (s). ^{1}H-NMR (DMSO-d_6) δ: 10.12 (s, 1H, OH), 7.73 (s, 2H, NH_2), 7.11 (s, 1H, quinone), 3.78 (s, 3H, OCH_3), 2.78 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 166.4, 155.7, 153.2, 151.0, 149.2, 131.3, 128.7, 124.2, 113.7, 111.2, 60.8, 18.8. HR-MS *m/z*: 279.0437 [(M+H)$^+$] (Calcd for $C_{12}H_{11}N_2O_4S$: 279.0439).

General Procedure for Synthesis of 6-Arylamino-5-chloro-2-methylbenzo[*d*]thiazole-4,7-diones 6 To a solution of compound **8** (100 mg, 0.40 mmol) in 100 mL of EtOH in the presence of $CeCl_3$ (0.02 mmol), appropriate arylamine (0.4 mmol) was added and the mixture was stirred at room temperature for 20–60 min. The solvent was evaporated off and the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc : MeOH = 15 : 1) to give compound **6**.

5-Chloro-2-methyl-6-(*p*-tolylamino)benzothiazole-4,7-dione (**6a**): Black powder (70%). IR (KBr) cm^{-1}: 3315 (s, NH), 3031 (w), 1662 (s, C=O), 1452–1558. ^{1}H-NMR (CD_3OD) δ: 7.34 (d, 2H, J=8.0 Hz), 7.02 (d, 2H, J=8.0 Hz), 2.55 (s, 3H), 2.44 (s, 3H). ^{13}C-NMR (CD_3OD) δ: 189.2, 175.2, 168.5, 152.7, 149.2, 148.8, 133.7, 130.2, 116.4, 116.1, 111.9, 22.0, 19.4. HR-MS *m/z*: 319.0307 [(M+H)$^+$] (Calcd for $C_{15}H_{12}ClN_2O_2S$: 319.0308).

5-Chloro-2-methyl-6-phenylaminobenzothiazole-4,7-dione (**6b**): Black powder (65%). IR (KBr) cm^{-1}: 3275 (NH), 3050 (w), 1690 (s, C=O), 1550–1470. ^{1}H-NMR ($CDCl_3$) δ: 7.55 (d, 1H, J=6.8 Hz), 7.48 (d, 1H, J=7.6 Hz), 7.35 (d, 1H, J=6.8 Hz), 7.33–7.31 (m, 1H), 6.76 (d, 1H, J=7.6 Hz), 2.59 (s, 3H). ^{13}C-NMR ($CDCl_3$) δ: 187.2, 173.5, 169.7, 151.5, 149.8, 147.3, 135.2, 128.8, 114.2, 113.9, 110.3, 21.7. δ: HR-MS *m/z*: 305.0152 [(M+H)$^+$] (Calcd for $C_{14}H_{10}ClN_2O_2S$: 305.0151).

5-Chloro-6-((4-fluorophenyl)amino)-2-methylbenzothiazole-4,7-dione (**6c**): Black purple powder (72%). IR (KBr) cm^{-1}: 3275 (NH), 3045 (w), 1695 (s, C=O), 1590–1475, 1230. ^{1}H-NMR (CD_3OD) δ: 7.25–7.20 (m, 2H), 7.02–7.01 (m, 2H) 2.57 (s, 3H). ^{13}C-NMR (CD_3OD) δ: 187.2, 177.6, 169.1, 155.9, 152.1, 147.9, 145.0, 139.2, 116.8, 113.3, 111.8, 21.0. ^{19}F-NMR (376.3 MHz, $CDCl_3$) δ: −116.9. HR-MS *m/z*: 323.0059 [(M+H)$^+$] (Calcd for $C_{14}H_9ClFN_2O_2S$: 323.0057).

6-((4-Bromophenyl)amino)-5-chloro-2-methylbenzothiazole-4,7-dione (**6d**): Black powder (56%). IR (KBr) cm^{-1}: 3350 (s, NH), 3080 (w), 1650 (s, C=O), 1495–1560, 1370. ^{1}H-NMR (CD_3OD) δ: 7.64 (d, 2H, J=8.4 Hz), 6.96 (d, 2H, J=8.4 Hz), 2.60 (s, 3H). ^{13}C-NMR (CD_3OD) δ: 185.4, 175.1, 168.3, 153.1, 149.4, 145.8, 133.6, 131.8, 117.2, 115.3, 113.8, 20.9. HR-MS *m/z*: 382.9257 [(M+H)$^+$] (Calcd for $C_{14}H_9BrClN_2O_2S$: 382.9256).

5-Chloro-6-((4-methoxyphenyl)amino)-2-methylbenzothiazole-4,7-dione (**6e**): Black powder (75%). IR (KBr) cm^{-1}: 3245 (w, NH), 3030 (w), 1695 (s, C=O), 1490–1555. ^{1}H-NMR ($CDCl_3$) δ: 7.20 (d, 2H, J=8.0 Hz), 7.08 (d, 2H, J=8.0 Hz), 3.88 (s, 3H), 2.55 (s, 3H). ^{13}C-NMR (CD_3OD) δ: 185.8, 177.2, 170.3, 157.0, 151.7, 147.1, 146.9, 137.8, 117.2, 114.9, 112.3, 57.3, 21.7. HR-MS *m/z*: 335.0257 [(M+H)$^+$] (Calcd for $C_{15}H_{12}ClN_2O_3S$: 335.0259).

4-((5-Chloro-2-methyl-4,7-dioxo-4,7-dihydrobenzo[*d*]thiazol-6-yl)amino)benzonitrile (**6f**): Black powder (47%). IR (KBr) cm^{-1}: 3240 (w, NH), 3036 (w), 2130 (CN), 1692 (s, C=O), 1496–1550. ^{1}H-NMR ($CDCl_3$) δ: 7.72 (d, 2H, J=8.0 Hz), 6.93 (d, 2H, J=8.0 Hz), 3.66 (s, 1H), 264 (s, 3H). ^{13}C-NMR ($CDCl_3$) δ: 187.2, 176.2, 168.7, 152.7, 149.6, 146.7, 133.6, 130.2, 121.9, 119.4. 116.1, 111.9, 19.2. HR-MS *m/z*: 330.0108 [(M+H)$^+$] (Calcd for $C_{15}H_9ClN_3O_2S$: 330.0104).

5-Chloro-6-((3-fluorophenyl)amino)-2-methylbenzothiazole-4,7-dione (**6g**): Black purple powder (54%). IR (KBr) cm^{-1}: 3275 (NH), 3040 (w), 1695 (s, C=O), 1595–1470, 1235. ^{1}H-NMR (CD_3OD) δ: 7.47–7.41 (m, 1H), 7.01 (td, 1H, J=6.4, 2.0 Hz), 6.67 (d, 1H, J=8.0 Hz), 6.63 (d, 1H, J=8.0 Hz), 2.61 (s, 3H). ^{13}C-NMR (CD_3OD) δ: 187.9, 175.8, 169.6, 157.2, 152.9, 148.2, 145.8, 135.2, 133.8, 115.1, 112.6, 111.2, 110.6, 20.2. ^{19}F-NMR (376.3 MHz, $CDCl_3$) δ: −114.2. HR-MS *m/z*: 323.0058 [(M+H)$^+$] (Calcd for $C_{14}H_9ClFN_2O_2S$: 323.0057).

5-Chloro-6-((3,4-dimethylphenyl)amino)-2-methylbenzo[*d*]-thiazole-4,7-dione (**6h**): Black powder (58%). IR (KBr) cm^{-1}: 3310 (s, NH), 3030 (w), 1665 (s, C=O), 1450–1560. ^{1}H-NMR ($CDCl_3$) δ: 7.22 (m, 1H), 6.75 (s, 1H), 6.71–6.70 (m, 1H), 4.01 (s, 1H), 2.58 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.30 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 189.8, 173.6, 163.8, 154.5, 148.7, 149.2, 134.2, 131.9, 119.2, 115.7, 112.5, 119.6, 118.5, 22.1. HR-MS *m/z*: 333.0469 [(M+H)$^+$] (Calcd for $C_{16}H_{14}ClN_2O_2S$: 333.0465).

5-Chloro-6-((4-iodophenyl)amino)-2-methylbenzothiazole-4,7-dione (**6i**): Black powder (64%). IR (KBr) cm^{-1}: 3355 (s, NH), 3052 (w), 1650 (s, C=O), 1495–1588, 1270. ^{1}H-NMR ($CDCl_3$) δ: 7.75 (d, 2H, J=8.0 Hz), 6.60 (d, 2H, J=8.0 Hz), 3.67 (s, 1H), 2.62 (s, 3H). ^{13}C-NMR ($CDCl_3$) δ: 187.2, 173.5, 169.7, 151.5, 149.8, 147.3, 135.2, 128.8, 114.2, 113.9, 83.2, 21.7. HR-MS *m/z*: 430.9120 [(M+H)$^+$] (Calcd for $C_{14}H_9ClIN_2O_2S$: 430.9118).

5-Chloro-6-((4-chlorophenyl)amino)-2-methylbenzothiazole-4,7-dione (**6j**): Black powder (56%). IR (KBr) cm^{-1}: 3275 (NH), 3010 (w), 1695 (s, C=O), 1594–1475. ^{1}H-NMR (CD_3OD) δ: 7.43 (d, 2H, J=8.8 Hz), 6.82 (d, 2H, J=8.8 Hz), 3.64 (s, 1H), 2.62 (s, 3H). ^{13}C-NMR (CD_3OD) δ: 188.3, 176.2, 168.4, 152.2, 146.4, 144.2, 136.3, 126.2, 113.7, 112.6, 111.0, 21.2. HR-MS *m/z*: 338.9764 [(M+H)$^+$] (Calcd for $C_{14}H_9C_{12}N_2O_2S$: 338.9762).

Antifungal *in Vitro* Susceptibility Testing The MIC values of compounds **4**–**7** were determined by the standard broth dilution method.[15] The antifungal activities were tested in modified Sabouraud dextrose broth against the following fungal strains: *Candida albicans* ATCC 10231, *C. glabrata* ATCC 2001, *C. krusei* ATCC 749, *C tropicalis* ATCC 28775 and *Aspergillus niger* KCTC 1231. Fluconazole and 5-fluorocytosine as standard agents were 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 to 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 d for *Candida* species and 2 d for *A. niger* in 37°C.

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