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Synthesis and antifungal activity of 6,7-bis(arylthio)-quinazoline-5,8-diones and furo[2,3-*f*]quinazolin-5-ols

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ABSTRACT

6,7-Bis(arylthio)-quinazoline-5,8-dione and furo[2,3-*f*]quinazolin-5-ol derivatives were synthesized and tested for in vitro antifungal activity against *Candida*, *Aspergillus* species, and *Cryptococcus neoformans*. Among them tested, many of furo[2,3-*f*]quinazolin-5-ols and 6,7-bis(arylthio)-quinazoline-5,8-diones showed good antifungal activity. The compounds **4a** and **4e** completely inhibited the growth of all against *Candida* and *Aspergillus* species tested at the MIC level of 12.5 μg/mL. The results suggest that furo[2,3-*f*]quinazolin-5-ols and 6,7-bis(arylthio)-quinazoline-5,8-diones would be promising antifungal agents.

A benzofuran derivative **1**, a novel myristoyltransferase inhibitor, has been reported as antifungal agent[1,2] as well as antibacterial agent[3,4] (Fig. 1). N-Myristoyltransferase has been proven to be essential for the viability of fungi, including medically important pathogenic fungi, *Candida albicans*[5] and *Cryptococcus neoformans*[6] making it a possible target for the development of antifungal agents with a novel mode of action.

In our previous Letters,[7] benzofuran-5-ol **2** and furo[2,3-*f*]quinolin-5-ol **3** scaffolds have demonstrated potent antifungal activity against pathogenic fungi (Fig. 1). Structure–activity relationship studies from heterocyclic quinonoid compounds indicated that the number and position of nitrogen (N) atoms substituted in the heterocyclic ring were considerably important factors to affect the biological activities.[5,6] Generally, increasing the number of substituent nitrogen atoms in the ring enhances the activities. We speculated that incorporation of a nitrogen atom into the ring of the skeleton in compounds **3** would change the physicochemical properties, and lead to a new pharmacophore furo[2,3-*f*]quinazolin-5-ols **4** with a different biological profile from scaffolds **3**. Furo[2,3-*f*]quinazolin-5-ols **4** which would be one of the bioisosteres of compounds **3**, could metabolize to 5,8-quinolinedione derivatives with a quinonoid structure in fungi (Fig. 1). Quinonoid compounds display potent biological properties including antifungal, antimalarial, and antibacterial activity.[8] We assumed that furo[2,3-*f*]quinazolin-5-ols **4** could have similar biological activities with those of quinonoid compounds.

There have not been any reports on furo[2,3-*f*]quinazolin-5-ols **4** to the best of our knowledge. The presence of aryl, thio, amino group, or halogen atoms on quinonoid compounds significantly affects their antifungal activity.[9] A variety of furo[2,3-*f*]quinazo-

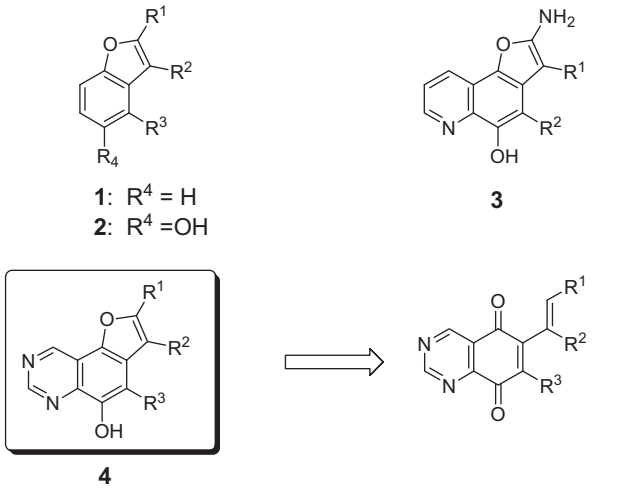

Figure 1. Benzofuran, furo[2,3-*f*]quinolin-5-ol and furo[2,3-*f*]quinazolin-5-ol scaffolds.

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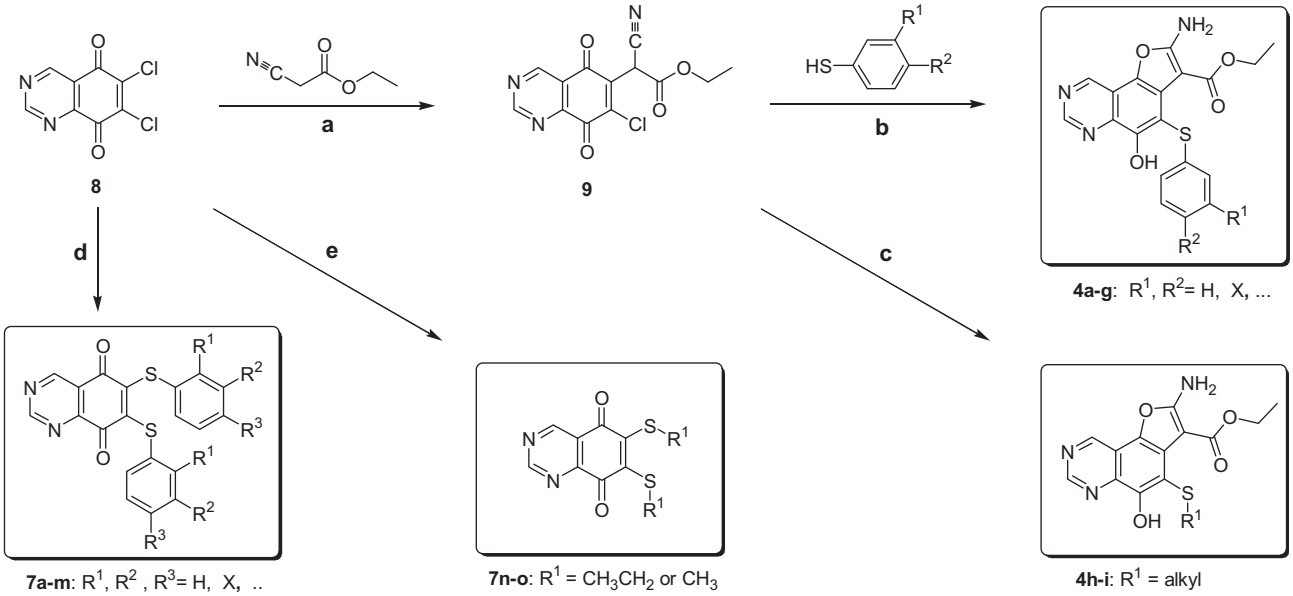

Scheme 1. Synthesis of furo[2,3-*f*]quinazolin-5-ols and 6,7-bis(arylthio)-quinazoline-5,8-dione derivatives. Reagents and conditions: (a) ethyl cyanoacetate/NH_4OH/$CeCl_3$ (0.1 equiv)/EtOH/rt/10 min/51%; (b) arylthiol (1 equiv)/EtOH/reflux/5 h/47–78%; (c) alkylthiol (1 equiv)/EtOH/reflux/5 h/52–66%; (d) arylthiol (2 equiv)/EtOH/reflux/15 h/69–87%; (e) alkylthiol (2 equiv)/EtOH/reflux/10 h/81–86%.

lin-5-ols **4** with different substituents could exhibit the biological activities through different actions and sometimes improve upon the activities.

Based on this speculation, furo[2,3-*f*]quinazolin-5-ols **4a–i** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity (Scheme 1).

Heterocyclic quinone compounds represent an important class of biologically active molecules[8] and exhibits antifungal activites.[9] In our previous reports,[10,11] 6,7-bis(arylthio)-quinoline-5,8-diones **5** and 6,7-bis(arylthio)-phthalazine-5,8-diones **6** have demonstrated potent antifungal activity against pathogenic fungi (Fig. 2). The presence of arylthio moiety to the quinones was considerably important factor to affect their antifungal activity.[11,12] We synthesized 6,7-bis(arylthio)-quinazoline-5,8-diones **7** which could be bioisosteres of quinones **5** and **6** to evaluate their antifungal activity (Scheme 1).

The in vitro antifungal activity of new compounds **4** and **7** against pathogenic fungi was determined by the two-fold broth dilution method. Additional data for antifungal activity are provided.

Methods for the synthesis of furo[2,3-*f*]quinazolin-5-ols **4a–i** are shown in Scheme 1 and Table 1. The 6,7-dichloro-quinazoline-5,8-dione (**8**) was prepared from 5,8-dimethoxyquinazoline according to the known method[13] with minor modification. Consequently, ethyl 2-(7-halo-5,8-dioxo-5,8-dihydroquinazolin-6-yl)-2-cyanoacetate (**9**) was synthesized by regioselective nucleophilic substitution of compound **8** with 1 equiv of ethyl cyanoacetate and 0.1 equiv of $CeCl_3$ in the presence of NH_4OH according to the reported method[7] with minor modification. 2-Amino-4-arylthio-5-hydroxyfuro[2,3-*f*]quinazolines **4a–g** were synthesized by nucleophilic substitution and cyclization of the compound **9** with appropriate arylthiols in EtOH. To a solution of the compound **9** in EtOH, 1 equiv 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–g**. Most of these reactions went as expected and had overall high yields.

In a similar manner, 4-alkylthio-5-hydroxyfuro[2,3-*f*]quinazolines **4h–i** were synthesized by cyclization of compound **9** with 1 equiv of ethylthiol or 2-mercaptoethanol in EtOH.

The mechanism for the formation of compound **9** involves Michael-type addition of the anion of ethyl cyanoacetate to quinone **8** followed by subsequent dechlorination.[7] The substitution of compound **9** with nucleophilic thiols resulted in the formation of aromatic hydroquinone system as intermediates and subsequent cyclization to compounds **4**. The substitution was similar to the formation of stable aromatic hydroquinone system by the substitution of thiols on quinones.[14]

6,7-Bis(arylthio)-quinazoline-5,8-diones **7a–o** were synthesized by nucleophilic substitution on compound **8** with 2 equiv of appropriate arylthiols or alkylthiols according to the known method.[11,12] Most of the substitutions went as expected and had an overall yield of 69–87%.

The experimental data of representative compounds **4a**, **7b**, **7n**, and **8** were cited in Ref. 15.

The synthesized furo[2,3-*f*]quinazolin-5-ols **4a–i**, 6,7-bis(arylthio)-quinazoline-5,8-diones **7a–o**, and 5,8-dimethoxyquinazoline (**10**) were tested in vitro for their growth inhibitory activity against pathogenic fungi using the standard method.[16] The MIC (minimum inhibitory concentration) values were determined by comparison with fluconazole[16] and 5-fluorocytosine as standard agents.

As indicated in Table 1, many of furo[2,3-*f*]quinazolin-5-ols **4a–i** and 6,7-bis(arylthio)-quinazoline-5,8-diones **7a–o** showed

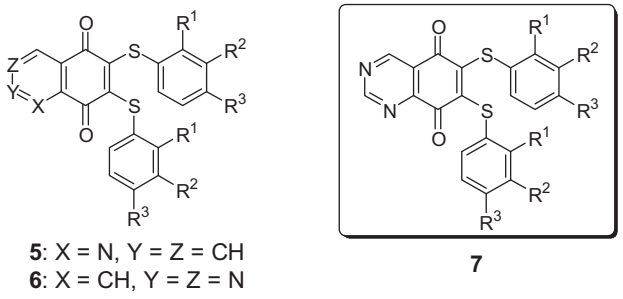

Figure 2. 6,7-Bis(arylthio)-quinones and 6,7-bis(arylthio)-quinazoline-5,8-dione derivatives.

Table 1
Structures and antifungal activity for furo[2,3-*f*]quinazolin-5-ols and 6,7-bis(arylthio)-quinazoline-5,8-diones

Compound	R^1	R^2	R^3	MIC[a] (μg/mL)					
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*C. neoformans*	*A. niger*	*A. flavus*
4a	H	CH_3	—	3.2	6.3	6.3	3.2	6.3	6.3
4b	H	CH_3O	—	12.5	25.0	50.0	12.5	12.5	12.5
4c	H	Cl	—	25.0	50.0	50.0	6.3	3.2	6.3
4d	H	Br	—	12.5	25.0	25.0	3.2	6.3	50.0
4e	F	F	—	3.2	3.2	6.3	6.3	12.5	3.2
4f	H	F	—	6.3	3.2	6.3	3.2	50.0	12.5
4g	Cl	H	—	50.0	25.0	50.0	12.5	25.0	12.5
4h	CH_3	—	—	50.0	25.0	12.5	12.5	6.3	6.3
4i	CH_3CH_2	—	—	50.0	25.0	25.0	12.5	3.2	6.3
7a	H	H	F	1.6	6.3	12.5	1.6	3.2	3.2
7b	H	H	CH_3O	25.0	25.0	3.2	6.3	3.2	0.8
7c	H	H	Cl	6.3	12.5	3.2	6.3	1.6	3.2
7d	H	H	Br	6.3	50.0	6.3	12.5	3.2	6.3
7e	H	H	CH_3	12.5	6.3	6.3	50.0	3.2	3.2
7f	H	CH_3	CH_3	12.5	12.5	12.5	3.2	25.0	12.5
7g	H	F	H	6.3	25.0	12.5	1.6	25.0	25.0
7h	H	CH_3	H	3.2	12.5	25.0	0.8	1.6	6.3
7i	H	H	H	6.3	6.3	6.3	25.0	6.3	3.2
7j	H	F	F	12.5	6.3	12.5	6.3	12.5	1.6
7k	F	H	F	6.3	6.3	12.5	1.6	12.5	6.3
7l	F	H	H	6.3	6.3	12.5	1.6	12.5	12.5
7m	H	H	OH	3.2	3.2	25.0	12.5	25.0	12.5
7n	CH_3CH_2	—	—	12.5	12.5	6.3	6.3	3.2	6.3
7o	CH_2CH_2OH	—	—	25.0	>50.0	12.5	6.3	25.0	25.0
10	—	—	—	>50.0	>50.0	50.0	50.0	25.0	50.0
Fluconazole	—	—	—	50.0	6.3	25.0	6.3	25.0	25.0
5-Fluorocytosine	—	—	—	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 day for *Candida* species and *Cryptococcus neoformans*, and 2 days 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.

potent antifungal activity against all tested fungi. Actually, the activity of compounds **4a** and **4e** was superior or comparable to those of 5-fluorocytosine against fungi. The compounds **4a** and **4e** completely inhibited the growth of all against *Candida* and *Aspergillus* species tested at the MIC level of 12.5 μg/mL. Many of compounds **4a–i** and **7a–o** also was comparable to those of 5-fluorocytosine against *Candida krusei*, *Cryptococcus neoformans*, and *Aspergillus* species. Actually, the activity of compounds **7a** or **7h** was superior to those of 5-fluorocytosine against *C. neoformans* and *Aspergillus flavus*.

Generally, the 4-arylthio-furo[2,3-*f*]quinazolin-5-ols scaffolds **4a–g** exhibited potent activity, indicating a correlation that may offer insight into the mode of action of these compounds. In contrast, 4-alkylthio-furo[2,3-*f*]quinazolin-5-ols scaffolds **4h–i** did not show significant antifungal activity against tested fungi, although they exhibited good activity against *C. neoformans* and *A. flavus*. 4-Alkylthio-moieties of compounds **4h–i** did not improve significantly their antifungal activity in comparison to 4-arylthio-compounds **4a–g**. 6,7-Bis(arylthio)-quinazoline-5,8-diones **7a–o** exhibited slightly more potent activity than furo[2,3-*f*]quinazolin-5-ols scaffolds **4a–i**. The structure–activity relationship may not exist between properties of substituents (R^1, R^2, R^3: H, X, Me) for the 4-arylthio moieties of compounds **4a–g** and **7a–m** and alkyl substituents for the compounds **4h–i** and **7n–o**. In addition, the nonquinonoid 5,8-dimethoxyquinazoline (**10**) exhibited no or poor, if any, antifungal activity. Thus, furo[2,3-*f*]quinazolin-5-ol and 6,7-bis(arylthio)quinazoline-5,8-dione moiety could be important for the antifungal activity.

In conclusion, ethyl 2-(7-halo-5,8-dioxo-5,8-dihydroquinazolin-6-yl)-2-cyanoacetate (**9**) was synthesized by regioselective substitution of 6,7-dichloro-quinazoline-5,8-dione (**8**) with 1 equiv of ethyl cyanoacetate and 0.1 equiv of $CeCl_3$ in the presence of NH_4OH. Furo[2,3-*f*]quinazolin-5-ols scaffolds **4a–i** were synthesized by cyclization of compound **9** with appropriate arylthiols or alkylthiols in EtOH. 6,7-Bis(arylthio)-quinazoline-5,8-diones **7a–o** were synthesized by nucleophilic substitution on compound **8** with 2 equiv of appropriate arylthiols or alkylthiols. Most of these reactions went as expected and had overall high yields. We have identified a lead compound that has antifungal activity by screening of our furo[2,3-*f*]quinazolin-5-ols **4a–i** and 6,7-bis(arylthio)-quinazoline-5,8-diones **7a–o**. Among them tested, many of furo[2,3-*f*]quinazolin-5-ols and 6,7-bis(arylthio)-quinazoline-5,8-diones showed potent antifungal activity. The results suggest that furo[2,3-*f*]quinazolin-5-ol and 6,7-bis(arylthio)-quinazoline-5,8-dione scaffolds would be promising leads for the development of antifungal agents. Moreover, the results should encourage the synthesis of furo[2,3-*f*]quinazolin-5-ols and 6,7-bis(arylthio)-quinazoline-5,8-dione analogs for improving antifungal properties.

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15. *Experimental*: All melting points were measured with Büchi melting point B-545 and were uncorrected. ^{1}H NMR spectra were recorded on Varian Unity INOVA 400 MHz FT-NMR spectrometer with TMS. Mass spectra were taken with Jeol JMS AX505 WA. *Ethyl 2-amino-5-hydroxy-4-(p-tolylthio)furo[2,3-f]quinazoline-3-carboxylate* (**4a**): violet powder (61%); mp 112–113 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, 3H, J = 7.2, CH_3), 2.39 (s, 3H, CH_3), 4.57 (q, 2H, J = 7.2, CH_2), 7.13–7.16 (m, 2H), 7.33–7.43 (m, 2H), 7.56 (s, 2H, NH_2), 9.43 (s, 1H), 9.51 (s, 1H), 9.69 (s, 1H, OH); MS (m/z) 395 (M^+). *6,7-Bis(4-methoxyphenylthio)quinazoline-5,8-dione* (**7b**): yellow green powder (89%); mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ 7.48 (d, 6H, OCH_3), 6.69–6.76 (m, 4H), 7.13–7.17 (m, 4H), 9.32 (s, 1H), 9.74 (s, 1H); MS (m/z) 436 (M^+). 6,7-*Bis(ethylthio)quinazoline-5,8-dione* (**7n**): red powder (76%); mp 117–119 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 6H, J = 7.2, CH_3), 3,.28 (q, 4H, J = 7.2, CH_2) 9.35 (s, 1H), 9.59 (s, 1H); MS (m/z) 280 (M^+). *6,7-Dichloro-5,8-quinazolinedione* (**8**)[13]: light yellow crystal (32%); mp 134–135 °C; ^{1}H NMR ($CDCl_3$) δ 9.63 (s, 1H), 9.74 (s, 1H; MS) (m/z) 229 (M^+).
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