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Synthesis and antifungal activity of benzofuran-5-ols

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ABSTRACT

Benzofuran-5-ol derivatives were synthesized and tested for in vitro antifungal activity against *Candida*, *Aspergillus* species, and *Cryptococcus neoformans*. Among them tested, many benzofuran-5-ols showed good antifungal activity. The results suggest that benzofuran-5-ols would be promising antifungal agents.

Benzofuran scaffolds **1** display potent biological properties including antifungal activity[1,2] as well as antibacterial,[3] angiogenesis inhibitory properties[4] (Fig. 1). A benzofuran derivative, a novel myristoyltransferase inhibitor, has been reported as antifungal agent.[1,2] *N*-Myristoyltransferase has been proven to be essential for the viability of fungi, including medically important pathogenic fungi, *Candida albicans*[5] and *Cryptococcus neoformans*[6,7] making it a possible target for the development of antifungal agents with a novel mode of action.

An interesting sub-group of benzofurans is benzofuran-5-ols **2** which could metabolize to benzoquinone 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 benzofuran-5-ols **2** could have similar biological activities with those of the quinonoid compounds. The antifungal activity of benzofuran-5-ols **2** against pathogenic fungi has not been reported to the best of our knowledge. A variety of benzofuran-5-ols or quinonoid compounds with different substituents could exhibit the biological activities through different actions and sometimes improve upon the activities. The presence of aryl, thio, amino group or halogen atoms on quinonoid compounds was considerably important factor to affect their antifungal activity.[9] Based on this speculation, benzofuran-5-ols **2a**–**n** 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 compounds **2a**–**n** against pathogenic fungi was determined by the twofold broth dilution method. Additional data for antifungal activity of cyclohexa-2,5-diene-1,4-dione derivatives **4**, **5d**, and **6** are provided.

A method for the synthesis of benzofuran-5-ols **2a**–**f** is shown in Scheme 1 and Table 1. The 2,3-dichloro-5,6-dimethylcyclohexa-2,5-diene-1,4-dione (**3**)[10] was prepared by oxidizing commercially available 2,3-dimethylbenzene-1,4-diol (**6**) with HNO_3/HCl variation.

Compound **4** was synthesized by nucleophilic substitution of the compound **3** with equivalent of methyl cyanoacetate in EtOH in the presence of NH_4OH according to the reported method[11] with minor modification. When the equivalent amount of compound **4** and hydrazine hydrate were mixed in EtOH and refluxed for 2 h, benzofuran-5-ol **2a** was formed. 2-Amino-4-arylthio-5-hydrox-

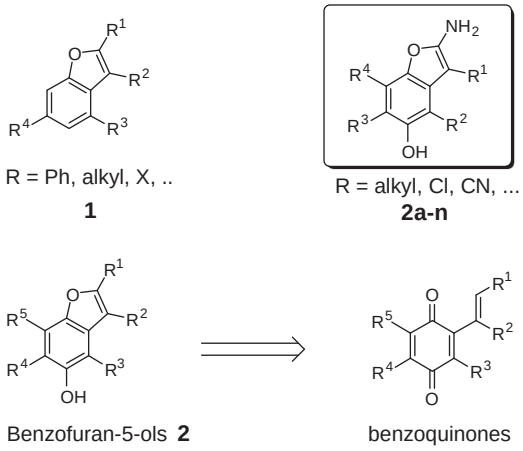

Figure 1. Benzofuran derivatives.

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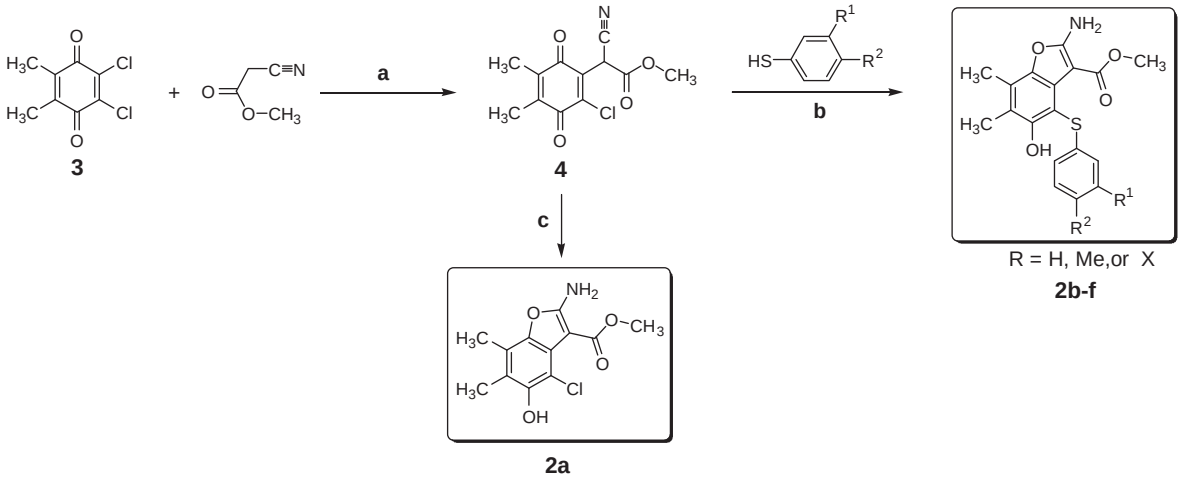
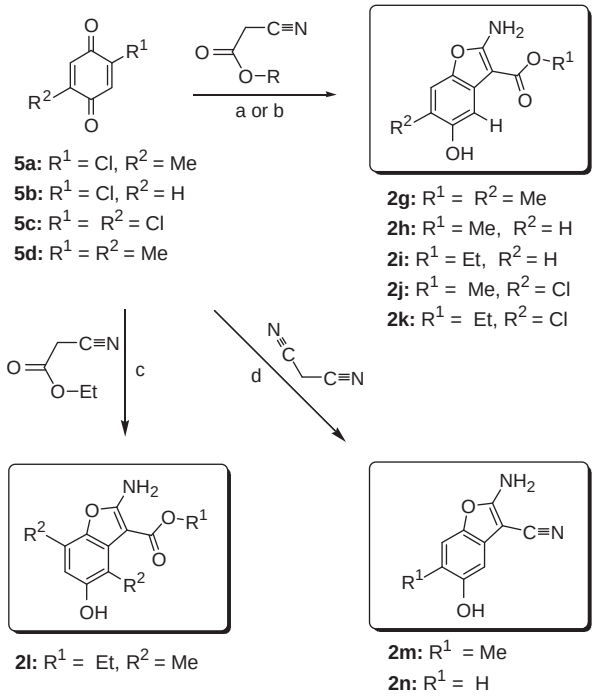


Scheme 1. Synthesis of benzofuran-5-ol derivatives. Reagents and conditions: (a) NH_4OH/EtOH/rt/10 min/45%; (b) arylthiol (1 equiv)/EtOH/reflux/5 h/65–90%; (c) hydrazine hydrate (1 equiv)/EtOH/reflux/2 h/78%.



Scheme 2. Synthesis of benzofuran-5-ol derivatives. Reagents and conditions: (a) **5a**, **5b**, or **5c**/methyl cyanoacetate (1 equiv)/K_2CO_3/EtOH/4 h/51–78%; (b) **5b** or **5c**/ethyl cyanoacetate (1 equiv)/K_2CO_3/EtOH/4 h; (c) **5d**/ethyl cyanoacetate (1 equiv)/K_2CO_3/EtOH/4 h/45%; (d) **5a** or **5b**/malononitrile (1 equiv)/K_2CO_3/EtOH/4 h/55–75%.

ybenzofurans **2b**–**f** were synthesized by nucleophilic substitution and cyclization of compound **4** with appropriate arylthiols in EtOH. To a solution of compound **4** in EtOH, equivalent of arylthiol was added. The mixture was refluxed for 5 h, and was concentrated in vacuo. Purification of residual crude product by column chromatography yielded compounds **2b**–**f**. Most of these reactions went as expected and had overall high yields of 65–90%. The compounds **2b**–**f** were identified by ^{1}H NMR, ^{13}C NMR, or Mass spectra. The mechanism of formation of compounds **2** were cited in Ref. 12.

A method for the synthesis of benzofuran-5-ols **2g**–**l** is shown in Scheme 2 and Table 1. Compounds **2g**–**l** were synthesized by nucleophilic substitution of commercially available benzoquinone **5a**, **5b**, **5c**, or **5d** with equivalent of alkyl cyanoacetate or malononitrile in EtOH in the presence of K_2CO_3 via one pot cyclization. To a suspension of compound **5a**, **5b**, **5c**, or **5d** in EtOH was added drop wise equivalent of alkyl cyanoacetate and K_2CO_3. The reaction mixture was stirred at rt for 4 h and monitored by TLC analysis. The mixture was then extracted with CH_2Cl_2, and the organic layer was washed with water, and concentrated. Purification of crude product by column chromatography yielded compounds **2g**–**k**. Most of these reactions went as expected and had overall high yields of 51–78%.

In a similar manner, 2-amino-5-hydroxybenzofuran-3-carbonitriles **2m**–**n** synthesized by cyclization of benzoquinone **5a** or **5b** with equivalent of malononitrile in the presence of K_2CO_3 in EtOH.

The experimental data of representative compounds **2a**, **2b**, **2g**, **2l**, **2m**, and **4** were cited in Ref. 14.

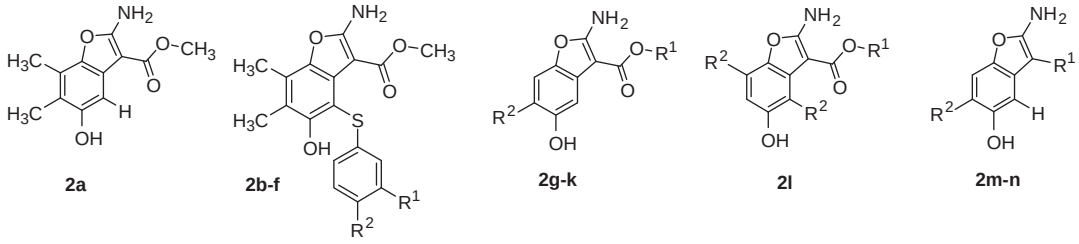
The synthesized benzofuran-5-ols **2a**–**n**, cyclohexa-2,5-diene-1,4-dione derivatives **4**, **5d**, and **6** were tested in vitro for their growth inhibitory activity against pathogenic fungi using the standard method.[15] The MIC (minimum inhibitory concentration) values were determined by comparison with fluconazole and 5-fluorocytosine as standard agents.

As indicated in the Table 1, most benzofuran-5-ols **2g**–**n** showed potent antifungal activity against all tested fungi. Actually, the activity of compounds **2h**, **2i**, and **2l** was superior or comparable to those of 5-fluorocytosine against fungi. The compounds **2h**, **2i**, and **2l** completely inhibited the growth of all fungal species tested at the MIC level of 1.6–12.5 μg/mL.

Many of 2-amino-4-arylthio-5-hydroxybenzofurans **2b**–**f** also showed potent antifungal activity against *Candida krusei*, *Cryptococcus neoformans*, and *Aspergillus niger*. Actually, the activity of compounds **2b**–**e** were superior to those of 5-fluorocytosine against *C. krusei* and *C. neoformans*.

In addition, compounds **4** and **5d** did not show significant antifungal activity against tested fungi, although they exhibited good activity against *C. neoformans* and *Aspergillus flavus*. The 2,3-dimethylbenzene-1,4-diol **6** exhibited no or poor, if any, antifungal activity. Thus, benzofuran-5-ols moiety is important for the antifungal activity. The structure activity relationship may not exist between properties of substituents (R^1, R^2: H, Me, Et, X, CN, ...) for the 4-arylthio moieties of benzofuran-5-ols **2b**–**f** and for the compounds **2g**–**l**. 4-Arylthio-moieties of compounds **2b**–**f** did not improve their antifungal activity in comparison to compounds **2g**–**l**. Generally, the benzofuran-5-ol scaffolds exhibited the

Table 1
Structures and antifungal activity for benzofuran-5-ols



Compound	R^1	R^2	MIC[a] (μg/mL)					
			C. albicans[b]	*C. tropicalis*	*C. krusei*	*C. neoformans*	*A. niger*	*A. flavus*
2a	—	—	50.0	50.0	25.0	25.0	6.3	25.0
2b	H	H	25.0	25.0	6.3	1.6	12.5	25.0
2c	H	CH_3	25.0	25.0	3.2	6.3	25.0	25.0
2d	CH_3	CH_3	25.0	25.0	3.2	1.6	12.5	3.2
2e	F	H	>50.0	50.0	25.0	6.3	1.6	3.2
2f	CH_3	H	>50.0	>50.0	>50.0	1.6	>50.0	>50.0
2g	CH_3	CH_3	25.0	25.0	3.2	12.5	12.5	25.0
2h	CH_3	H	1.6	3.2	6.3	12.5	3.2	12.5
2i	CH_3CH_2	H	1.6	3.2	12.5	12.5	3.2	6.3
2j	CH_3	Cl	25.0	12.5	25.0	12.5	6.3	6.3
2k	CH_3CH_2	Cl	12.5	12.5	25.0	50.0	25.0	12.5
2l	CH_3CH_2	CH_3	6.3	6.3	3.2	12.5	12.5	12.5
2m	CN	CH_3	50.0	>50.0	0.8	3.2	>50.0	12.5
2n	CN	H	12.5	6.3	25.0	12.5	6.3	12.5
4	—	—	50.0	50.0	25.0	6.3	25.0	12.5
5d	—	—	25.0	25.0	25.0	12.5	25.0	12.5
6	—	—	50.0	>50.0	25.0	12.5	50.0	12.5
Fluconazole			50.0	6.3	12.5	6.3	12.5	25.0
5-Fluorocytosine			6.3	6.3	12.5	12.5	12.5	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.

greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds.

In conclusion, the compound **4** were synthesized by nucleophilic substitution of 2,3-dichloro-5,6-dimethylcyclohexa-2,5-diene-1,4-dione (**3**) with equivalent of methyl cyanoacetate in the presence of NH_4OH. 2-Amino-4-arylthio-5-hydroxybenzofurans **2b**–**f** were synthesized by cyclization of compound **4** with appropriate arylthiols in overall high yields. Benzofuran-5-ol **2a** was synthesized by cyclization of compound **4** with hydrazine. 5-Benzofuran-5-ols **2g**–**m** were synthesized by one pot cyclization of benzoquinone **5a**, **5b**, **5c**, or **5d** with alkyl cyanoacetate or malononitrile in the presence of K_2CO_3. 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 benzofuran-5-ols **2a**–**n**. Among them tested, many benzofuran-5-ols showed potent antifungal activity. The results suggest that benzofuran-5-ol scaffolds would be promising leads for the development of antifungal agents. Moreover, the results should encourage the synthesis of benzofuran-5-ol analogs for improving antifungal properties.

Acknowledgment

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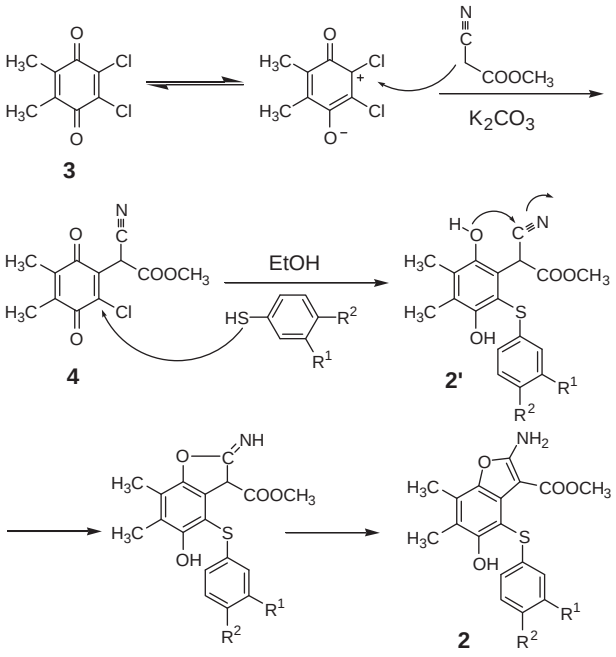
12. The mechanism of formation of compounds **2** from compound **3**: A 1,4-benzoquinone derivative **4** was synthesized by nucleophilic substitution of the compound **3** with methyl cyanoacetate in EtOH. The product **4** was formed by a Michael-type addition of methyl cyanoacetate to **3** and subsequent dechlorination.[11] The substitution of **4** with nucleophilic thiols resulted in the formation of aromatic hydroquinone system as intermediates **2′** and subsequent cyclization to compounds **2**. The substitution was similar to the formation of stable aromatic hydroquinone system by the substitution of thiols on 1,4-benzoquinones.[13]



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14. *Experimental*: All melting points were measured with Büchi melting point B-545 and were uncorrected. ^{1}H NMR spectra or ^{13}C NMR spectra were recorded on Varian Unity INOVA 400 MHz FT-NMR spectrometer with TMS. Mass spectra were taken with Jeol JMS AX505 WA. *2-Amino-5-hydroxy-6,7-dimethylbenzofuran-3-carboxylate* (**2a**): mp 221–223 °C; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 5.81 (s, 1H, OH), 6.13 (s, 2H, NH); ^{13}C NMR ($CDCl_3$) δ 12.5, 13.7, 61.5, 104.7, 117.2, 122.3, 124.4, 126.1, 147.4, 151.5, 169.4; MS (*m*/*z*) 235 (M^+). *Methyl 2-amino-5-hydroxy-6,7-dimethyl-4-(phenylthio)-benzofuran-3-carboxylate* (**2b**): mp 153–155 °C; ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.54 (s, 3H, OCH_3), 7.02 (m, 2H, Ph), 7.07 (m, 1H, Ph), 7.18 (m, 2H, Ph), 7.38 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 12.1, 12.9, 61.9, 109.7, 118.4, 122.6, 122.6, 128.7, 129.1, 132.8, 132.8, 132.8, 153.5, 153.5, 156.27, 167.1; MS (*m*/*z*) 343 (M^+). *Methyl 2-amino-5-hydroxy-6-methylbenzofuran-3-carboxylate* (**2g**): mp 191–192 °C; ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1H, OH), 7.79 (s, 1H), 6.23 (s, 2H, NH_2), 5.71 (s, 1H), 3.82 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 14.8, 61.3, 106.4,, 111.3, 116.3, 119.8, 126.2, 126.3, 140.8, 150.4, 167.9; MS (*m*/*z*) 221 (M^+). *Ethyl 2-amino-5-hydroxy-4,7-dimethylbenzofuran-3-carboxylate* (**2l**): mp 249–250 °C; ^{1}H NMR (DMSO-d_6) δ 9.73 (s, 1H, OH), 6.42 (s, 1H), 6.07 (s, 2H, NH_2), 4.21 (q, 2H, J = 7.2, CH_2), 2.25 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.29 (t, 3H, J = 7.2, CH_2CH_3); MS (*m*/*z*) 249 (M^+). *2-Amino-5-hydroxy-6-methylbenzofuran-3-carbonitrile* (**2m**): Black powder; mp 157–159 °C; ^{1}H NMR (DMSO-d_6) δ 8.7 (s, 1H), 7.56 (s, 2H, NH_2), 6.35 (s, 1H), 4.21 (q, 2H, J = 7.2, CH_2CH_3), 2.38 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 1.29 (t, 3H, J = 7.2, CH_2CH_3); MS (*m*/*z*) 188 (M^+). *Methyl-2-(2-chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)-2-cyanoacetate* (**4**): ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 5.25 (s, 1H, CH_2); MS (*m*/*z*) 267 (M^+).
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