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Synthesis and antifungal activity of benzo[*d*]oxazole-4,7-diones

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ABSTRACT

Benzo[*d*]oxazole-4,7-diones were synthesized and tested for in vitro antifungal activity against fungi. Among them tested, many compounds showed good antifungal activity. The results suggest that benzo[*d*]oxazole-4,7-diones would be potent antifungal agents.

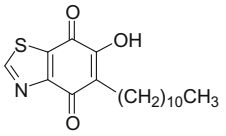
Heterocyclic quinone scaffolds represent often an important class of biologically active molecules.[1] The quinones such as 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT, **1**) blockade a mitochondrial electron transport in *Saccaromyces cerevisiae*.[2] UHDBT (**1**) has been reported as an inhibitor of mitochondrial cytochrome complex in yeast[3] and bacteria.[4]

In our previous reports,[5] 4,7-dioxobenzothiazoles **2** which have 3′-sulfur (S) as analogues of UHDBT, demonstrated potent antifungal activity against pathogenic fungi (Fig. 1). This fact prompted us to consider a bioisosteric substitution of the 3′-sulfur by oxygen (O). The quinone analogues containing oxygen such as benzo[*d*]oxazole-4,7-diones **3** could have similar activity as compounds **2** since oxygen is isoelectronic with sulfur.

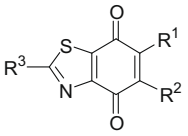
Structure–activity relationship studies from quinonoid compounds indicated that the number and position of nitrogen (N), sulfur (S) or oxygen atoms (O) substituted in the heterocyclic ring were considerably important factors to affect the biological activities.[5,6] We speculated that bioisosteric compounds **3** would be a new pharmacophore with a different biological profile from compounds **2**. Based on this information, we further extended to synthesize benzo[*d*]oxazole-4,7-diones **3** which would be bioisosteres of 4,7-dioxobenzothiazoles **2**, and evaluated their antifungal activity (Fig. 1).

There have been only a report on benzo[*d*]oxazole-4,7-diones as inhibitors of the dual specificity protein phosphatase CDC25C.[7] However, the antifungal activity of compounds **3** against fungi has not been reported to the best of our knowledge. The presence of arylamino, arylthio or halo moieties of quinones was considerably important factor to improve their antifungal activity.[5,6,8] Therefore, 5-arylamino-6-bromo-2-ethylbenzo[*d*]oxazole-4,7-diones **3a–m** and 5,6-bisarylthio-2-ethylbenzo[*d*]oxazole-4,7-diones **4a–e** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity (Scheme 1). The in vitro antifungal activity of compounds **3a–m** and **4a–e** against pathogenic fungi was determined by the twofold broth dilution method. Additional data for properties and antifungal activity of compound **6** is provided.

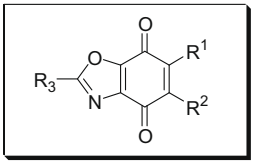
A convenient method for the synthesis of benzo[*d*]oxazole-4,7-diones **3a–m** is shown in Scheme 1 and Table 1.



UHDBT (**1**)



2 : R^1, R^2, R^3= H, X, amino, thio, Ph, ..



3 : R^1, R^2 = X, amino, R^3 = Et

4 : R^1 = R^2 = thio, R^3 = Et

Figure 1. Antifungal heterocyclic quinone compounds.

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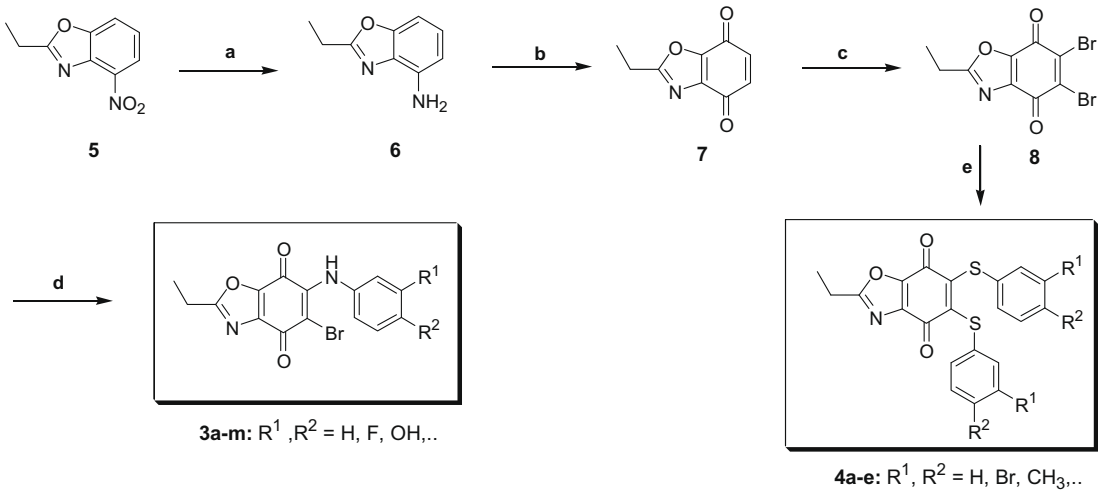
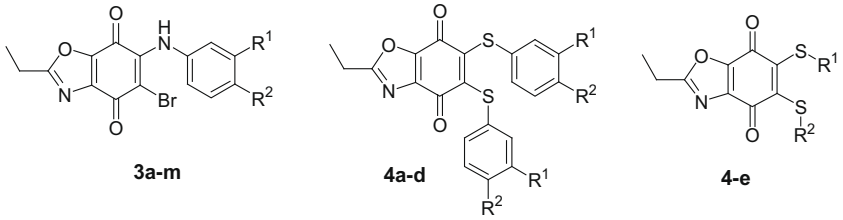


Scheme 1. Synthesis of benzo[*d*]oxazole-4,7-dione derivatives. Reagents and conditions: (a) H_2, PtO_2/EtOH/3 psi/rt/3 h/78%; (b) Fremy's salt (2 equiv) in 0.3 M KH_2PO_4/acetone/rt/4 h/ 62%; (c) Br_2, AcONa/AcOH/rt/8 h/89%; (d) arylamine (1 equiv)/EtOH/5 h/reflux/45–93%; (f) arylthiol (2 equiv)/EtOH/rt/5 h/56–85%.

Table 1
Structures and in vitro antifungal activity for benzo[*d*]oxazole-4,7-diones



Compound	R^1	R^2	MIC[a] (μg/mL)					
			C. albicans [b]	*C. tropicalis*	*C. krusei*	*C. neoformans*	*A. niger*	*A. flavus*
3a	H	H	1.6	3.2	3.2	1.6	1.6	3.2
3b	F	H	12.5	3.2	6.3	6.3	6.3	6.3
3c	H	F	12.5	6.3	6.3	6.3	6.3	3.2
3d	Cl	H	6.3	6.3	6.3	3.2	3.2	3.2
3e	H	Cl	6.3	25	6.3	6.3	3.2	3.2
3f	H	Br	0.8	3.2	3.2	1.6	0.8	1.6
3g	H	I	6.3	6.3	3.2	3.2	6.3	3.2
3h	OH	H	50	6.3	25	25	50	25
3i	H	OH	50	12.5	25	12.5	50	25
3j	CN	H	12.5	6.3	50	12.5	12.5	6.3
3k	H	CN	50	50	50	25	25	25
3l	H	OCH_3	6.35	12.5	1.6	3.2	3.2	6.3
3m	H	NO_2	50	50	50	25	25	25
4a	H	H	12.5	12.5	12.5	12.5	12.5	12.5
4b	Br	Br	50	50	12.5	12.5	25	25
4c	CH_3	CH_3	25	12.5	3.2	12.5	12.5	6.3
4d	CH_2CH_3	CH_2CH_3	12.5	25	50	25	25	25
4e	OCH_3	OCH_3	50	50	50	50	12.5	12.5
6		—	>100	50	100	100	>100	>100
5-Fluorocytosine		—	3.2	3.2	3.2	3.2	1.6	1.6

[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 *C. neoformans*, and 2 days for *A. niger* 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 100 μg/mL.

[b] Fungi tested: *Candida albicans* Berkout KCCM 50235, *Candida tropicalis* Berkout KCCM 50662, *Candida krusei* Berkout KCCM 11655, *Cryptococcus neoformans* KCCM 50564, *Aspergillus niger* KCTC 1231 and *Aspergillus flavus* KCCM 11899.

2-Ethyl-4-nitrobenzo[*d*]oxazole (**5**)[7] was synthesized by cyclization of commercially available 2-amino-3-nitrophenol with para toluene sulfonic acid (PTSA) and triethylorthopropionate in 87% yield according to the reported method[9] with minor modification.

The nitro group of compound **5** was reduced to 2-ethylbenzo[*d*]oxazol-4-amine (**6**) by catalytic hydrogenation with H_2 and PtO_2 in EtOH in 78% yield. The preparation of 2-ethylbenzo[*d*]oxazole-4,7-dione (**7**) by oxidation of compound **6** was

carried out with Fremy's salt [potassium nitrosodisulfonate, $(KO_3S)_2NO$] in 62% yield.

5,6-Dibromo-2-ethylbenzo[*d*]oxazole-4,7-dione (**8**) was synthesized by bromination of compound **7** with the Br_2, AcONa/AcOH variation in 89% yield.

5-Arylamino-6-bromo-2-ethylbenzo[*d*]oxazole-4,7-diones **3a–m** were synthesized by nucleophilic substitution of compound **8** with appropriate arylamines. When compound **8** with equivalent amount of appropriate arylamines in EtOH were refluxed for 5 h, compounds **3a–m** were formed. Most of these substitutions went as expected and had overall high yields of 45–93%.

In similar manner, 5,6-bisarylthio-2-ethyl benzo[*d*]oxazole-4,7-diones **4a–d** and 5,6-bisethylthio-2-ethylbenzo[*d*]oxazole-4,7-dione (**4e**) were synthesized by nucleophilic substitution on compound **8** with 2 equiv of appropriate aryl or alkylthiols in EtOH. Most of the substitutions went as expected and had an overall yield of 56–85%.

The synthesized compounds **3a–m** and **4a–e** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard method.[10] As indicated in Table 1, the MIC (minimum inhibitory concentration) values were determined by comparison with 5-fluorocytosine as standard agent.

Among tested 5-arylamino-benzo[*d*]oxazole-4,7-diones **3a–m**, many compounds generally showed potent antifungal activity against the tested pathogenic fungi. Actually, the activity of compounds **3a** and **3f** was superior or comparable to those of 5-fluorocytosine against all tested pathogenic fungi. The compounds **3a** and **3l** completely inhibited the growth of all fungal species tested at the MIC level of 0.8–3.2 μg/mL.

Many of 5,6-bisarylthio-benzo[*d*]oxazole-4,7-diones **4a–e** also showed potent antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. Actually, the activity of compound **4c** was comparable to those of 5-fluorocytosine against *Cryptococcus krusei*.

In terms of structure–activity relationship, 5-arylamino-benzo[*d*]oxazole-4,7-dione series **3a–m** showed, in general, more potent antifungal activity than 5,6-bisarylthio-benzo[*d*]oxazole-4,7-dione series **4a–e**. The 5-arylamino-substituted compounds **3** exhibited more potent activity, indicating a correlation that may offer insight into the mode of action of these compounds. In contrast, 5,6-bisarylthio-moieties of compounds **4** did not improve their antifungal activity in comparison to 5-arylamino-benzo[*d*]oxazole-4,7-dione **4** significantly.

In addition, 2-ethylbenzo[*d*]oxazol-4-amine (**6**) exhibited no or poor, if any, antifungal activity. Quinonoid benzo[*d*]oxazole-4,7-diones **3** and **4** showed, in general, more potent antifungal activity than compound **6**. Thus, the quinone moiety in compounds **3** and **4** could be essential for the activity, for example, as non-quinonoid compound **6** losts the activity. The structure–activity relationship may not exist between properties of subsistent (R^1 and R^2 and R^3) for the 5- or 6-aryl moieties of compounds **3** and **4**.

In conclusion, compound **3a–m** was synthesized by nucleophilic substitution of compound **8** with appropriate arylamines. Compounds **4a–e** were synthesized by nucleophilic substitution on compound **8** with 2 equiv of appropriate thiols in EtOH. Most of the substitutions went as expected and had an overall yield.

Among them tested, many of compounds **3a–m** and **4a–e** showed potent antifungal activity against pathogenic fungi. These benzo[*d*]oxazole-4,7-diones may thus be promising leads for the development of antifungal agents. Moreover, the results should encourage the synthesis of these analogs for improving antifungal properties.

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