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Synthesis and antifungal activity of 5-arylamino-4,7-dioxobenzo[*b*]thiophenes

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Abstract—5-Arylamino-4,7-dioxobenzo[*b*]thiophenes **3**–**6** were synthesized and tested for in vitro antifungal activity against *Candida* and *Aspergillus* species. 5-Arylamino-6-chloro-2-(methoxycarbonyl)-4,7-dioxobenzo[*b*]thiophenes **5** showed, in general, more potent antifungal activity against *Candida* species than the other 4,7-dioxobenzo[*b*]thiophenes **3**, **4** and **6**. The results suggest that 5-arylamino-4,7-dioxobenzo[*b*]thiophenes would be potent antifungal agents.

1. Introduction

Heterocyclic quinone compounds are an attractive 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 *Saccharomyces cerevisiae*.[2] UHDBT (**1**) has been reported as an inhibitor of mitochondrial cytochrome complex in yeast[3] and bacteria.[4] In our previous report,[5] 5-arylamino-4,7-dioxobenzothiazoles **2**, which could be analogues of UHDBT, have demonstrated potent antifungal activity against pathogenic fungi (Fig. 1). A variety of heterocyclic quinones with different substituents could exhibit biological activities through different action and sometimes improve the activities. The presence of arylamino, arylthio, alkyl group or halogen atoms substituted in quinones was a considerably important factor to affect their antifungal activity.[5] Based on this speculation, we further extended to synthesize 5-arylamino-4,7-dioxobenzo[*b*]thiophenes **3**–**6**, which would be bioisosteres of quinones **2** and evaluated their antifungal activity.

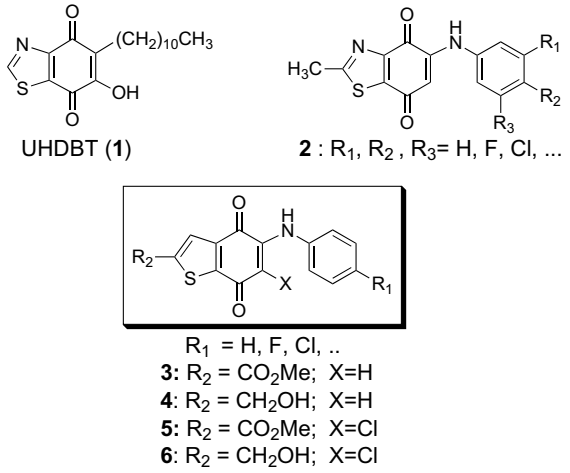

Figure 1. Antifungal 4,7-dioxobenzo[*b*]thiophene derivatives.

There have been a few reports[6,7] on 4,7-dioxobenzo[*b*]thiophene derivatives, exhibiting antiprotozoal activity against *Trypanosome cruzi* and strains of *Leishmania*. However, the inhibitory activity of 4,7-dioxobenzo[*b*]thiophenes on the antifungal properties has not been reported to the best of our knowledge. Therefore, the 4,7-dioxobenzo[*b*]thiophenes **3**–**6** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity. The in vitro antifungal activity of the 4,7-dioxobenzo[*b*]thiophenes **3**–**6** against pathogenic fungi was determined by the 2-fold broth dilution method. Additional data for

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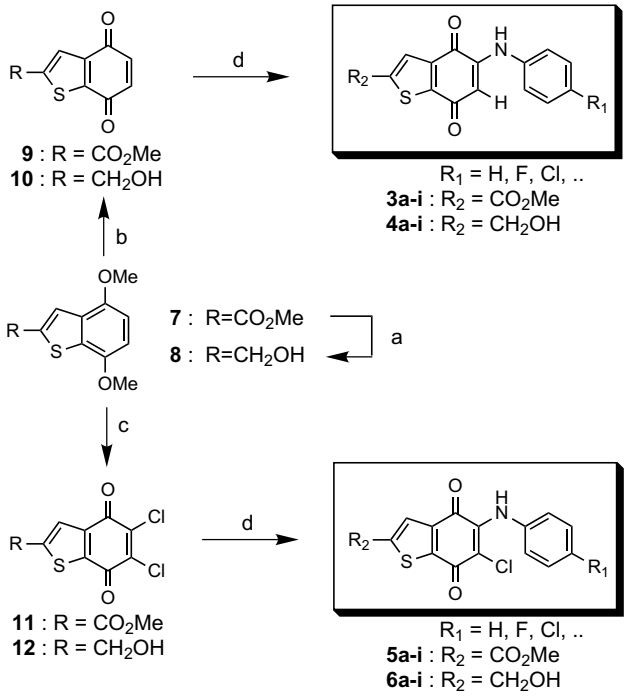


Scheme 1. Synthesis of 4,7-dioxobenzo[*b*]thiophenes **3**–**6**. Reagents and conditions: (a) $LiAlH_4$/ether/rt; (b) CAN/AcCN/H_2O/rt; (c) HNO_3/HCl/80 °C; (d) arylamine/$CeCl_3$/EtOH/reflux/10 h.

properties and antifungal activity of 4,7-dioxobenzo[*b*]-thiophenes are provided (Scheme 1).

2. Chemistry

A method for the synthesis of 5-arylamino-4,7-dioxobenzo[*b*]thiophenes **3**–**6** (Table 1) is shown in Scheme 1. 4,7-Dimethoxy-2-(methoxycarbonyl)-benzo[*b*]thiophene (**7**) was prepared according to a reported method[8] with minor modification. Cyclization of 2,5-dimethoxy-6-nitrobenzaldehyde[9] with methyl thioglycolate gave compound **7**. 4,7-Dimethoxy-2-(hydroxymethyl)benzo-[*b*]thiophene (**8**) was synthesized by reduction of compound **7** with $LiAlH_4$ in 57% yield. The preparation of 4,7-dioxobenzo[*b*]thiophenes **9** and **10** by oxidative demethylation of 4,7-dimethoxybenzo[*b*]thiophenes **7** and **8** was carried out with ammonium cerium(IV) nitrate (CAN) in AcCN/water solution.[8] 5,6-Dichloro-4,7-dioxobenzo[*b*]thiophenes **11** and **12** were synthesized by oxidizing compounds **7** and **8** with HNO_3/HCl variation. 5-Arylamino-4,7-dioxobenzo[*b*]thiophenes **3**–**6** were synthesized by nucleophilic substitution of compounds **9**–**12** with the appropriate arylamines. Most of these substitutions went as expected and had overall high yields of 76–94%. Experimental details and data for this procedure are cited in Refs. 10–14.

3. Antifungal activity

The synthesized 4,7-dioxobenzo[*b*]thiophenes **3**–**6** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard method.[15] The MIC (minimum inhibitory concentration) values were determined by comparison with 5-fluorocytosine as a standard agent. As indicated in Table 1, the more active potential among the 4,7-dioxobenzo[*b*]thiophene series **3**–**6** was found for 5-arylamino-6-chloro-2-(methoxycarbonyl)-4,7-dioxobenzo[*b*]thiophenes **5**, which showed generally good activity against all tested *Candida* species and *A. niger*. The 5-arylamino-4,7-dioxobenzo[*b*]thiophenes **3**, **4** and **6** did not show significant antifungal activity against *C. albicans* and *A. niger*, although many compounds of them exhibited good activity against *C. tropicalis* and *C. krusei*. Most of the 5-arylamino-2-hydroxymethyl-4,7-dioxobenzo-[*b*]thiophenes **4** showed potent antifungal activity against *C. tropicalis*, *C. krusei* and *A. niger*. Most of compounds **3**–**6** were superior or comparable to those of 5-fluorocytosine against *C. tropicalis*. Actually, the activity of 4,7-dioxobenzo-[*b*]thiophenes **5a** and **6a** was superior to those of 5-fluorocytosine against all tested fungi. Compounds **5a** and **6a** completely inhibited the growth of all fungal species tested at the MIC level of 12.5 μg/mL.

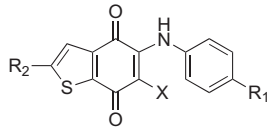
In terms of structure activity relationship, the 5-arylamino-6-chloro-2-(methoxycarbonyl)-4,7-dioxobenzo[*b*]-thiophenes **5** showed, in general, a more potent antifungal activity than the other 5-arylamino-4,7-dioxobenzo[*b*]thiophenes **3**, **4** and **6**. The 5-arylamino-6-chloro-2-(methoxycarbonyl)-compounds **5** exhibited the greatest activity, indicating a correlation that may offer an insight into the mode of action of these compounds. The 6-chloro- and 2-methoxycarbonyl moieties of compounds **5** appears to contribute partially towards biological potency. In contrast, 2-hydroxymethyl moiety of compounds **4** and **6** did not improve their antifungal activity in comparison to compounds **5** significantly.

In addition, the 4,7-dioxobenzo[*b*]thiophenes **9** and **10** without a 5-arylamino group exhibited no or poor, if any, antifungal activity. Thus, 5-arylamino moiety of 4,7-dioxobenzo[*b*]thiophenes **3**–**6** partially improves the antifungal activity. The structure activity relationship may not exist between properties of substituents (R_1: F, Cl, Br, ...) for the 5-arylamino moieties of the 4,7-dioxobenzo[*b*]thiophenes **3**–**6**.

4. Conclusion

The 5-arylamino-4,7-dioxobenzo[*b*]thiophenes **3**–**6** were synthesized by nucleophilic substitution of the 4,7-dioxobenzo[*b*]thiophenes **9**–**12** with the appropriate arylamines. 5-Arylamino-6-chloro-2-(methoxycarbonyl)-4,7-dioxobenzo[*b*]thiophenes **5** showed generally more potent antifungal activity than 4,7-dioxobenzo[*b*]thiophenes **3, 4** and **6**. The 6-chloro moiety of compounds **5** improved their antifungal activity significantly. The results suggest that the 4,7-dioxobenzo[*b*]thiophenes would be potent antifungal agents. Moreover, the results should encourage the synthesis of 4,7-dioxobenzo[*b*]thiophenes analogues for improving antifungal properties.

Table 1. Structures and in vitro antifungal activity for 4,7-dioxobenzo[*b*]thiophenes **3**–**6**



Compds	R_1	R_2	X	MIC[a] (μg/mL)			
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*A. niger*
3a	H	$COOCH_3$	H	50	6.3	12.5	50
3b	F	$COOCH_3$	H	>50	25	25	>50
3c	Cl	$COOCH_3$	H	50	6.3	25	50
3d	Br	$COOCH_3$	H	50	0.2	12.5	50
3e	I	$COOCH_3$	H	50	0.2	6.3	50
3f	CH_3	$COOCH_3$	H	50	3.2	6.3	50
3g	OCH_3	$COOCH_3$	H	>50	6.3	25	>50
3h	OH	$COOCH_3$	H	50	0.4	12.5	50
3i	CF_3	$COOCH_3$	H	50	12.5	1.6	50
4a	H	CH_2OH	H	50	0.8	6.3	0.8
4b	F	CH_2OH	H	50	12.5	>25	0.8
4c	Cl	CH_2OH	H	>50	12.5	>25	25
4d	Br	CH_2OH	H	50	12.5	3.2	3.2
4e	I	CH_2OH	H	50	1.6	25	3.2
4f	CH_3	CH_2OH	H	>50	25	25	3.2
4g	OCH_3	CH_2OH	H	3.2	0.8	25	3.2
4h	OH	CH_2OH	H	0.8	0.8	12.5	0.8
4i	CF_3	CH_2OH	H	0.8	0.8	12.5	0.8
5a	H	$COOCH_3$	Cl	0.8	3.2	6.3	12.5
5b	F	$COOCH_3$	Cl	12.5	6.3	25	12.5
5c	Cl	$COOCH_3$	Cl	12.5	6.3	25	25
5d	Br	$COOCH_3$	Cl	0.8	3.2	25	25
5e	I	$COOCH_3$	Cl	6.3	1.6	25	3.2
5f	CH_3	$COOCH_3$	Cl	0.8	12.5	25	50
5g	OCH_3	$COOCH_3$	Cl	3.2	6.3	3.2	50
5h	OH	$COOCH_3$	Cl	12.5	0.8	6.3	50
5i	CF_3	$COOCH_3$	Cl	12.5	1.6	25	50
6a	H	CH_2OH	Cl	6.3	3.2	1.6	12.5
6b	F	CH_2OH	Cl	12.5	0.4	1.6	50
6c	Cl	CH_2OH	Cl	12.5	0.4	1.6	>50
6d	Br	CH_2OH	Cl	50	0.8	0.8	50
6e	I	CH_2OH	Cl	50	1.6	1.6	50
6f	CH_3	CH_2OH	Cl	50	1.6	0.8	50
6g	OCH_3	CH_2OH	Cl	25	1.6	3.2	50
6h	OH	CH_2OH	Cl	>50	25	12.5	>50
6i	CF_3	CH_2OH	Cl	50	0.4	3.2	50
9				>50	25	>50	>50
10				>50	25	50	50
5-Fluorocytosine				6.3	12.5	6.3	12.5

[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 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 μg/mL and 100 μg/mL.

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

Acknowledgments

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10. *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 using $CDCl_3$ with TMS. High-resolution mass spectra (HRMS EI) were taken with Jeol JMS AX505 WA. 2,5-Dimethoxybenzaldehyde and other reagents were purchased from Aldrich Chemical Co.
11. *4,7-Dimethoxy-2-(hydroxymethyl)benzo[b]thiophene* (**8**): to a solution of $LiAlH_4$ (1.0 g, 26.4 mmol) in dry ether (10 mL) was added to a solution of 4,7-dimethoxy-2-(methoxycarbonyl)benzo[*b*]thiophene[8] (**7**, 0.20 g, 0.80 mmol) in dry ether (30 mL). The mixture was stirred for 3 h at rt. The resulting mixture was acidified with AcOH and then extracted with EtOAc. The extract was washed with aqueous Na_2CO_3, water, dried and evaporated. The residue was chromatographed on silica gel and eluted with EtOAc to afford pure alcohol **8** as a white needle (0.103 g, 57%): mp 132–136 °C; ^{1}H NMR ($CDCl_3$) δ 1.90 (br s, 1H, OH), 3.96 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.97 (s, 2H, CH_2), 6.72 (s, 2H), 7.43 (s, 1H); HRMS calcd for $C_{11}H_{12}O_3S$: 224.0507. Found: 224.0508.
12. *General procedure for synthesis of 4,7-dioxobenzo[b]thiophenes* (**9** and **10**): a solution of CAN (540 mg, 1.0 mmol) in AcCN/water (4:1, 5 mL) was added dropwise to compounds **7** or **8** (0.48 mmol) dissolved in AcCN (10 mL). The mixture was kept at rt for 10 min, diluted with water (70 mL) and extracted with EtOAc (3×15 mL). The extract was washed with brine, dried and evaporated. The residue was chromatographed on silica gel (1:1 EtOAc/petroleum ether) to afford pure quinones **9** and **10**, respectively.
2-(Methoxycarbonyl)-benzo[b]thiophene-4,7-dione (**9**): yellow powder (79%); mp 140–141 °C; ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H, OCH_3), 7.09 (d, 1H, $J = 9.2$), 7.28 (d, 1H, $J = 9.2$), 7.29 (s, 1H); HRMS calcd for $C_{10}H_6O_4S$: 221.9987. Found: 221.9988. *2-(Hydroxymethyl)benzo[b]-thiophene-4,7-dione* (**10**): yellow powder (61%); mp 106–108 °C; ^{1}H NMR ($CDCl_3$) δ 2.14 (t, 1H, OH, $J = 5$), 4.95 (d, 2H, CH_2, $J = 5$), 6.77 (d, 1H, $J = 10$), 6.85 (d, 1H, $J = 10$), 7.29 (d, 1H, $J = 5$); HRMS calcd for $C_9H_6O_3S$: 194.0038. Found: 194.00377.
13. *General procedure for synthesis of 5,6-dichlorobenzo[b]thiophene-4,7-diones* (**11** and **12**): 5 mL of concd HNO_3 was added over a period of 1 h to a stirred suspension of compounds **7** or **8** (10 mmol) in 15 mL of concd HCl at 80–90 °C. The mixture was stirred at rt for 2 h and was extracted twice with ether. The extract was evaporated and crystallization form EtOH afforded compounds **11** and **12**, respectively. *5,6-Dichloro-2-(methoxycarbonyl)benzo[b]thiophene-4,7-dione* (**11**): yellow needle (79%); mp 186–188 °C; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3H, OCH_3), 8.19 (s, 1H); HRMS calcd for $C_{10}H_4Cl_2O_4S$: 289.9207. Found: 289.9208. *5,6-Dichloro-2-(hydroxymethyl)benzo[b]thiophene-4,7-dione* (**12**): yellow powder (48%); mp 140–141 C; ^{1}H NMR ($CDCl_3$) δ 2.14 (t, 1H, OH, $J = 5$), 4.95 (d, 2H, CH_2, $J = 5$), 7.39 (s, 1H); HRMS calcd for $C_9H_4Cl_2O_3S$: 261.9258. Found: 261.9259.
14. *General procedure for synthesis of 5-arylamino-4,7-dioxobenzo[b]thiophenes* (**3**–**6**): a solution of compounds **9**–**11** or **12** (1 mmol) and $CeCl_3 \cdot 7H_2O$ (0.01 mmol) in 20 mL of EtOH was added to a solution of the arylamine (1.1 mmol) in 10 mL EtOH and stirred at rt for 2 h 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 $CHCl_3$/EtOAc or crystallized from EtOH afforded 5-arylamino-4,7-dioxobenzo[*b*]thiophenes **3**–**5** or **6**, respectively.
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