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Synthesis and antifungal activity of noble 5-arylamino- and 6-arylthio-4,7-dioxobenzoselenazoles

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Abstract—5-Arylamino- and 6-arylthio-4,7-dioxobenzoselenazoles **4** and **5** were synthesized and tested for in vitro antifungal activity against *Candida* and *Aspergillus* species. 5-Arylamino-4,7-dioxobenzoselenazoles **4** showed, in general, more potent antifungal activity than 6-arylthio-4,7-dioxobenzoselenazoles **5**. The results suggest that 5-arylamino-4,7-dioxobenzoselenazoles **4** would be potent antifungal agents.

1. Introduction

Heterocyclic quinone compounds represent an important class of biologically active molecules.[1] The quinones such as 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT, **1**) block mitochondrial electron transport in *Saccaromyces cerevisiae*.[2] UHDBT (**1**) was reported as an inhibitor of mitochondrial cytochrome complex in yeast,[3,4] malaria,[5] bacteria[6] and mammals.[7] In our previous reports,[8] 5-arylamino- and 6-arylthio-4,7-dioxobenzothiazoles **2** and **3**, which have 1′-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 1′-sulfur by selenium (Se). The quinone analogues containing selenium such as 5-arylamino-4,7-dioxobenzoselenazoles **4** and 6-arylthio-4,7-dioxobenzoselenazoles **5** could have similar activity as compounds **2** and **3** since selenium is isoelectronic with sulfur.

A variety of heterocyclic quinones with different substituents could exhibit the activities through different action and sometimes improve the activities. The presence of arlyamino, arylthio, alkyl group or hetero atoms substituted in quinones was a considerably important factor to affect their antifungal activity.[8–10] Based on these considerations, the 4,7-dioxobenzoselenazoles **4** and **5** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity (Scheme 1).

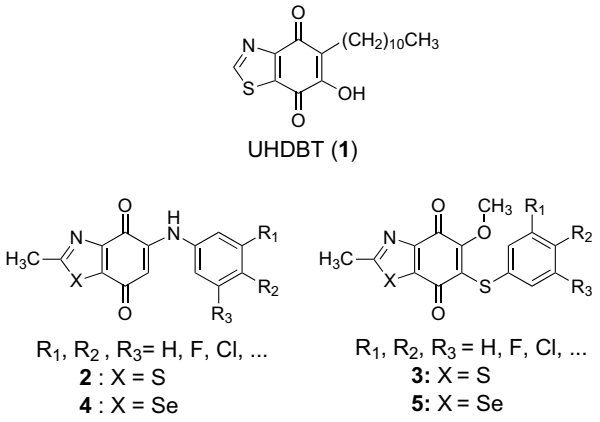

Figure 1. Antifungal 4,7-dioxobenzoselenazole derivatives.

The in vitro antifungal activity of the 4,7-dioxobenzoselenazoles **4** and **5** against pathogenic fungi was determined by the twofold broth dilution method. Additional data for properties and antifungal activity of 4,7-dioxobenzoselenazoles are provided.

Keywords: 4,7-Dioxobenzoselenazole; Antimicrobial compounds; Antifungal; Fungi; Substitution effects.
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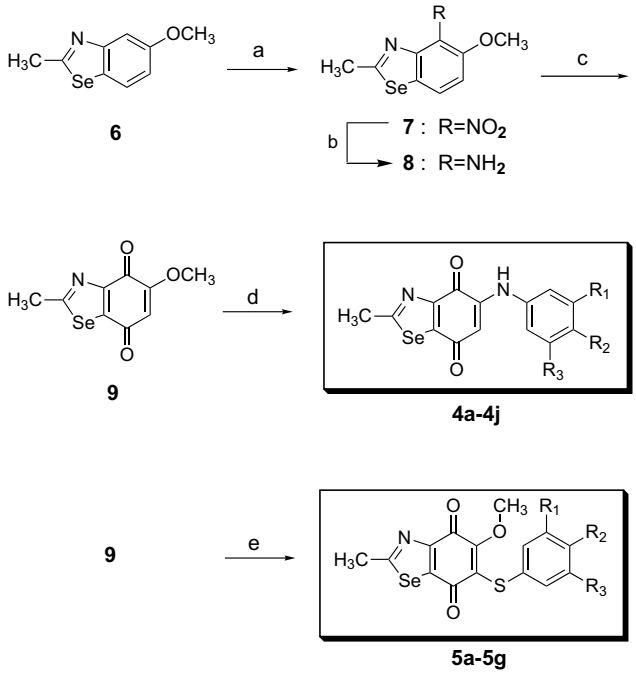

Scheme 1. Synthesis of 4,7-dioxobenzoselenazoles **4** and **5**. Reagents and conditions: (a) HNO_3/H_2SO_4/rt/5 h; (b) $SnCl_2$/HCl/60 °C/1 h; (c) Fremy's salt (2 equiv) in 0.3 M KH_2PO_4/H_2O/rt/5 h; (d) arylamine (1 equiv)/EtOH/reflux/4–6 h; (e) arylthiol (1 equiv)/EtOH/reflux/4–10 h.

2. Chemistry

A method for the synthesis of 5-arylamino-/6-arylthio-4,7-dioxobenzoselenazoles **4a–j**, and **5a–g** (Table 1) is shown in Scheme 1. Nitration of 5-methoxy-2-methyl-benzoselenazole (**6**) by HNO_3/H_2SO_4 afforded 5-methoxy-2-methyl-4-nitro-benzoselenazole (**7**) in about 92% yield. 4-Amino-5-methoxy-2-methyl-benzoselenazole (**8**) was prepared by reduction of the compound **7** with $SnCl_2$/HCl variation in about 70% yield. 5-Methoxy-2-methyl-4,7-dioxobenzoselenazole (**9**) was synthesized by oxidizing the compound **8** with Fremy's salt (potassium nitrosodisulfonate) in 59% yield. 5-Arylamino-2-methyl-4,7-dioxobenzoselenazoles **4a–j** were synthesized by regioselective nucleophilic substitution of compound **9** with appropriate arylamines. It is well known that the displacement of the methoxy group in quinones with the arylamine produces regioselective arylaminated quinones.[8] The substitution was similar to the regioselective arylamination on 6-position of 6-methoxy-4,7-dioxobenzothiazole in previously reported papers.[8,10]

6-Arylthio-5-methoxy-4,7-dioxobenzoselenazoles **5a–g** were synthesized by reaction of compound **9** with appropriate arylthiols. In the reactions, 6-arylthio compounds **5a–g** were exclusively formed. The regioselective reaction was similar to the substitution of arylthiols on 6-position of 5-methoxy-2-methyl-4,7-dioxobenzothiazole in the reported paper.[10]

Experimental details and data for this procedure are cited in References and notes.[11,12]

3. Antifungal activity

The synthesized 4,7-dioxobenzoselenazoles **4** and **5** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard method.[13] The MIC (minimum inhibitory concentration) values were determined by comparison with 5-fluorocytosine as a standard

Table 1. Structures and in vitro antifungal activity for 4,7-dioxobenzoselenazoles **4** and **5**

Compds	R_1	R_2	R_3	MIC[a] (μg/mL)				
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*A. niger*	*A. flavus*
4a	H	H	H	6.3	6.3	6.3	6.3	6.3
4b	H	F	H	6.3	6.3	6.3	6.3	12.5
4c	H	Cl	H	12.5	12.5	25.0	12.5	12.5
4d	H	Br	H	12.5	50.0	50.0	25.0	50.0
4e	H	I	H	25.0	25.0	25.0	25.0	25.0
4f	H	CH_3	H	6.3	25.0	25.0	6.3	12.5
4g	H	OCH_3	H	12.5	12.5	12.5	12.5	12.5
4h	H	CF_3	H	25.0	3.2	3.2	12.5	25.0
4i	F	H	H	12.5	3.2	6.3	12.5	12.5
4j	Cl	H	Cl	1.6	3.2	25.0	12.5	12.5
5a	H	H	H	6.3	6.3	6.3	12.5	12.5
5b	H	F	H	25.0	3.2	25.0	50.0	50.0
5c	H	Cl	H	25.0	12.5	25.0	25.0	25.0
5d	H	CH_3	H	12.5	6.3	25.0	25.0	50.0
5e	H	OCH_3	H	25.0	12.5	12.5	12.5	25.0
5f	F	F	H	12.5	6.3	6.3	25.0	25.0
5g	H	NO_2	H	3.2	1.6	25.0	50.0	12.5
9				50.0	12.5	25.0	25.0	50.0
5-Fluorocytosine				12.5	12.5	50.0	12.5	50.0

[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 CFU/mL. Culture media tested were the modified Sabouraud dextrose broth (Difco Lab.). The final concentration of antifungal agents was between 0.4 and 100 μg/mL.

[b] Fungi tested: *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 28775, *C. krusei* ATCC 749, *Aspergillus niger* KCTC 1231 and *A. flavus* KCCM 11899.

agent. As represented in Table 1, most of the 5-arylamino-4,7-dioxobenzoselenazoles **4a**–**j** and **5a**–**g** showed potent antifungal activity against all tested fungal species. The more active potential among the 4,7-dioxobenzoselenazole series **4**–**5** was found for the 5-arylamino-4,7-dioxobenzoselenazoles **4**, which generally showed good activity against all tested *Candida* and *Aspergillus* species. Also, many of 6-arylthio-4,7-dioxobenzoselenazoles **5a**–**g** exhibited good activity. Most of compounds **4** and **5** had more potent antifungal activity against *C. krusei* than 5-fluorocytosine. Actually, the activities of compounds **4a**, **4b** and **5a** were superior to those of 5-fluorocytosine against all tested fungi. The 4,7-dioxobenzoselenazoles **4a** and **4b** completely inhibited the growth of all fungal species tested at the MIC level of 12.5 μg/mL.

The cytotoxic potential of compounds **4** and **5** has also been determined in human cancer cells according to the NCI protocols.[14] All of the tested compounds did not show significant cytotoxic activity but showed selectivity, in that they possess potent antifungal activities without cytotoxicity in mammalian cells.[15]

In terms of structure–activity relationship, the 5-arylamino-4,7-dioxobenzoselenazole series **4a**–**j** showed, in general, more potent antifungal activity than 6-arylthio-4,7-dioxobenzoselenazole series **5a**–**g**. The 5-arylamino-substituted compounds **4** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds. In contrast, 6-arylthio-moieties of compounds **5** did not improve their antifungal activity in comparison to 5-arylamino-4,7-dioxobenzoselenazoles **4** significantly.

In addition, the 4,7-dioxobenzoselenazole **9** without the 5-arylamino- or 6-arylthio-group exhibited poor antifungal activity. Thus, the 5-arylamino- and 6-arylthio-moiety of 4,7-dioxobenzoselenazoles **4** and **5** partially improves the antifungal activity. The structure–activity relationship may not exist between properties of substituents (R: F, Cl, Br,...) for the 5-arylamino moieties of the 4,7-dioxobenzoselenazoles **4**.

4. Conclusion

The 5-arylamino-dioxobenzoselenazoles **4** and 6-arylthio-4,7-dioxobenzoselenazoles **5** were synthesized by regioselective substitution of 5-methoxy-2-methyl-4,7-dioxobenzoselenazole (**9**) with the appropriate arylamines or arylthiols. Most of these substitutions went as expected and had overall high yields.

5-Arylamino-4,7-dioxobenzoselenazoles **4** generally showed more potent antifungal activity than 6-arylthio-4,7-dioxobenzoselenazoles **5**. The 5-arylamino-moieties of compounds **4** improved their antifungal activity significantly. The results of this study suggested that 5-arylamino-4,7-dioxobenzoselenazoles would be potent antifungal agents. Moreover, the results should encourage the synthesis of 4,7-dioxobenzoselenazoles analogues for improving antifungal properties.

Acknowledgements

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11. *Experimental*: all melting points were measured with Büchi melting point B-545 and were uncorrected. ^{1}H NMR spectra were recorded on Brucker DPX 250 MHz spectrometer using $CDCl_3$ with TMS. High-resolution mass spectra (HRMS EI) were taken with Jeol JMS AX505 WA. 5-Methoxy-2-methyl-benzoselenazole (**6**) and other reagents were purchased from Aldrich Chemical Co.
12. *5-Methoxy-2-methyl-4-nitro-benzoselenazole* (**7**): 5-methoxy-2-methyl-benzoselenazole (**6**, 5 g, 22.1 mmol) in 13 mL of concd H_2SO_4 and 13 mL of concd HNO_3 was stirred at rt for 5 h. The precipitate was filtered and crystallized from CH_2Cl_2. 5-Methoxy-2-methyl-4-nitro-benzoselenazole (**7**) was obtained (5.9 g, 92%): pale yellow powder; mp 157–158 °C; ^{1}H NMR ($CDCl_3$): δ 7.8 (s, 1H, CH), 7.0 (s, 1H, CH), 4.0 (s, H, CH_3), 2.9 (s, 3H, CH_3); HRMS Anal. Calcd for $C_9H_8N_2O_2Se$: 271.9700. Found: 271.9702.
4-Amino-5-methoxy-2-methyl-benzoselenazole (**8**): to 16 g (71 mmol) of $SnCl_2$ in 30 mL of concd HCl was added 2.72 g (9.6 mmol) of compound **7**. The mixture was stirred at 60 °C for 1.5 h and was neutralized by K_2CO_3. Then it was extracted twice with CH_2Cl_2. The extract was evaporated and crystallized from CH_2Cl_2. 4-Amino-5-methoxy-2-methyl-benzoselenazole (**8**) was obtained (1.2 g, 70%): pale yellow plate; mp 122–123 °C; ^{1}H NMR ($CDCl_3$): δ 7.1 (s, 1H, CH), 6.5 (s, 1H, CH), 4.6 (s, 2H, NH_2), 3.9 (s, H, CH_3), 2.8 (s, 3H, CH_3); HRMS Anal. Calcd for $C_9H_{10}N_2OSe$: 241.9958. Found: 241.9959.
5-Methoxy-2-methyl-4,7-dioxobenzoselenazole (**9**): to a solution of compound **8** (0.68 g, 2.8 mmol) in 100 mL of KH_2PO_4 buffer (0.3 M, 200 mL) was added a solution of potassium nitrosodisulfonate (1.5 g, 5.60 mmol) in the KH_2PO_4 buffer. The mixture was stirred at rt for 5 h and was extracted twice with CH_2Cl_2. The extract was

evaporated and purified by column chromatography with $CHCl_3$. 5-Methoxy-2-methyl-4,7-dioxobenzoselenazole (**9**) was obtained (0.32 g, 59%): yellow powder; mp 258–260 °C; ^1H NMR ($CDCl_3$): δ 6.0 (s, 1H, CH), 3.9 (s, H, CH_3), 2.9 (s, 3H, CH_3); HRMS Anal. Calcd for $C_9H_7NO_3Se$: 256.9591. Found: 256.9592.

General procedure for synthesis of 5-arylamino-2-methyl-4,7-dioxobenzoselenazoles **4a**–**j**: a solution of compound **9** (0.256 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylamine (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4–6 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 EtOAc/*n*-hexane (1:2) or crystallized from 95% EtOH. Crystallization from aq EtOH afforded 5-arylamino-2-methyl-4,7-dioxobenzoselenazoles **4a**–**j** (Table 1).

General procedure for synthesis of 6-arylthio-5-methoxy-2-methyl-4,7-dioxobenzoselenazoles **5a**–**g**: a solution of compound **10** (0.256 g, 1 mmol) in 30 mL of 95% EtOH was added to a solution of the arylthiol (1.1 mmol) in 10 mL of 95% EtOH 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 EtOAc/*n*-hexane (1:2) or crystallized from 95% EtOH. Crystallization from aq EtOH afforded 6-arylthio-5-methoxy-2-methyl-4,7-dioxobenzoselenazoles **5a**–**g** (Table 1).

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15. Unpublished data; we also tested cytotoxic activity of compounds **3**–**6** against human tumour cell lines such as HL-60 and HepG2 according to the NCI protocols.[14] All of the tested compounds did not show significant cytotoxic activity.