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Synthesis and antifungal activity of 6-arylamino-phthalazine-5,8-diones and 6,7-bis(arylthio)-phthalazine-5,8-diones

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Abstract—6-Arylamino-phthalazine-5,8-diones and 6,7-bis(arylthio)-phthalazine-5,8-diones were synthesized and tested for in vitro antifungal activity against two pathogenic strains of fungi. Among those tested, many compounds showed good antifungal activity. The results suggest that phthalazine-5,8-diones would be potent antifungal agents.

Heterocyclic quinone compounds represent an important class of biologically active molecules.[1] 5,8-Quinolinedione derivative, a heterocyclic quinone, inhibits cytochrome B-complex by the blockade of mitochondrial electron transport in *Saccaromyces cerevisiae*, which is different from commonly used antifungal drugs.[2] In our previous reports,[3,4] 6-arylamino-quinoline-5,8-diones **1** and 6,7-bis(arylthio)-quinoline-5,8-diones **2** have demonstrated potent antifungal activity against pathogenic fungi (Fig. 1). Structure–activity relationship studies from 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 quinone skeleton in compounds **1** and **2** would change the physicochemical properties, and lead to a new pharmacophore with a different biological profile from compounds **1** and **2**. The presence of arylamino, arylthio or chloro moiety on the quinones was considerably important factor to affect their antifungal activity.[7] Based on this speculation, 6-arylamino-phthalazine-5,8-diones **3** and 6,7-bis(arylthio)-phthalazine-5,8-diones **4**, which would be bioisosteres of quinones **1** and **2**, were synthesized and evaluated for their antifungal activity.

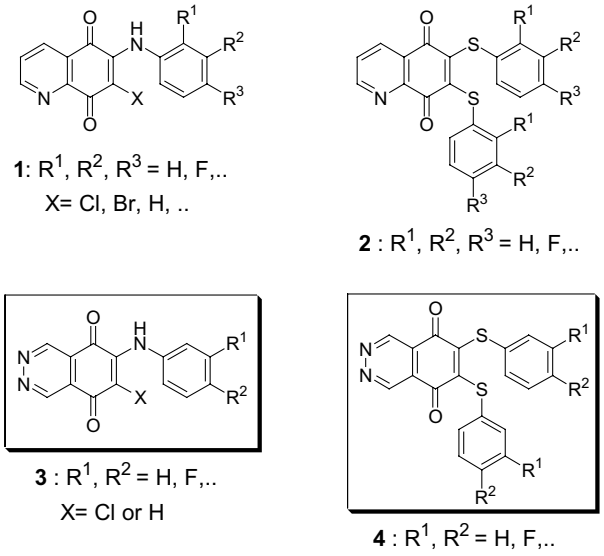

Figure 1. Quinoline-5,8-dione and phthalazine-5,8-dione derivatives.

There have been a few reports[5,8] on phthalazine-5,8-diones exhibiting cytotoxic activity[8] against cancer cell lines. However, the inhibitory activity of compounds **3** and **4** on the antifungal properties has not been reported to the best of our knowledge. Therefore, phthalazine-5,8-diones **3** and **4** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity.

A method for the synthesis of phthalazine-5,8-diones **3a**–**m** and **4a**–**i** (Table 1) is shown in Scheme 1.

Keywords: Phthalazine-5,8-dione; Antimicrobial compounds; Antifungal; Fungi; Substitution effects.
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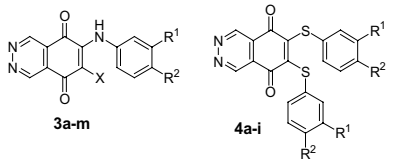
Table 1. Structures and in vitro antifungal activity for phthalazine-5,8-dione derivatives



Compound	X	R^1	R^2	MIC[a] (μg/mL)					
				Candida albicans[b]	*Candida tropicalis*	*Candida krusei*	*Cryptococcus neoformans*	*Aspergillus niger*	*Aspergillus flavus*
3a	Cl	H	H	50	25	25	3.2	100	25
3b	Cl	H	F	50	50	50	6.3	>100	>100
3c	Cl	H	Cl	12.5	12.5	6.3	100	>100	>100
3d	Cl	Cl	H	25	25	25	3.2	12.5	25
3e	Cl	H	Br	25	12.5	25	3.2	25	25
3f	Cl	H	I	12.5	12.5	12.5	100	>100	50
3g	Cl	F	F	25	3.2	>100	50	100	100
3h	Cl	CF_3	H	25	12.5	100	50	50	50
3i	H	H	F	50	25	25	6.3	>100	100
3j	H	H	Br	12.5	3.2	12.5	12.5	50	50
3k	H	H	CH_3O	12.5	6.3	12.5	6.3	25	25
3l	H	H	CH_3	6.3	12.5	12.5	6.3	50	25
3m	H	CH_3	CH_3	3.2	100	50	25	>100	>100
4a	—	H	H	12.5	>100	0.8	1.6	6.3	100
4b	—	H	F	6.3	6.3	100	>100	12.5	>100
4c	—	H	CH_3O	25	100	100	>100	12.5	>100
4d	—	Cl	H	12.5	100	>100	>100	6.3	>100
4e	—	CH_3	H	12.5	50	>100	>100	6.3	100
4f	—	F	F	3.2	12.5	>100	100	3.2	25
4g	—	H	H	50	100	>100	>100	6.3	>100
4h	—	H	OH	100	>100	>100	>100	>100	>100
4i	—	H	Br	50	50	>100	>100	>100	50
7	—	—	—	>100	>100	100	6.3	>100	100
9	—	—	—	>100	>100	100	25	>100	100
5-FC[c]	—	—	—	25	12.5	50	12.5	25	100

[a] The MIC value is defined as lowest concentration of the antifungal agent exhibiting no fungal growth. MIC values were read after 1 day for *Candida* species and *C. neoformans*, and 2 days for *A. niger*, *A. flavus* in 37 °C. The inoculum sizes contained approximately 1×10^5 cells/mL. Culture media tested were the modified Sabouraud dextrose broth (Difco Laboratory). The final concentration of antifungal agents was between 0.2 and 100 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 *A. flavus* KCCM 11899.

[c] 5-FC: 5-fluorocytosine.

8-Nitrophthalazine-5-amine (**6**)[9] was synthesized by the amination of 5-nitrophthalazine (**5**) with $HONH_2$ and KOH in EtOH in 87% yield according to the reported method[10] with minor modification.

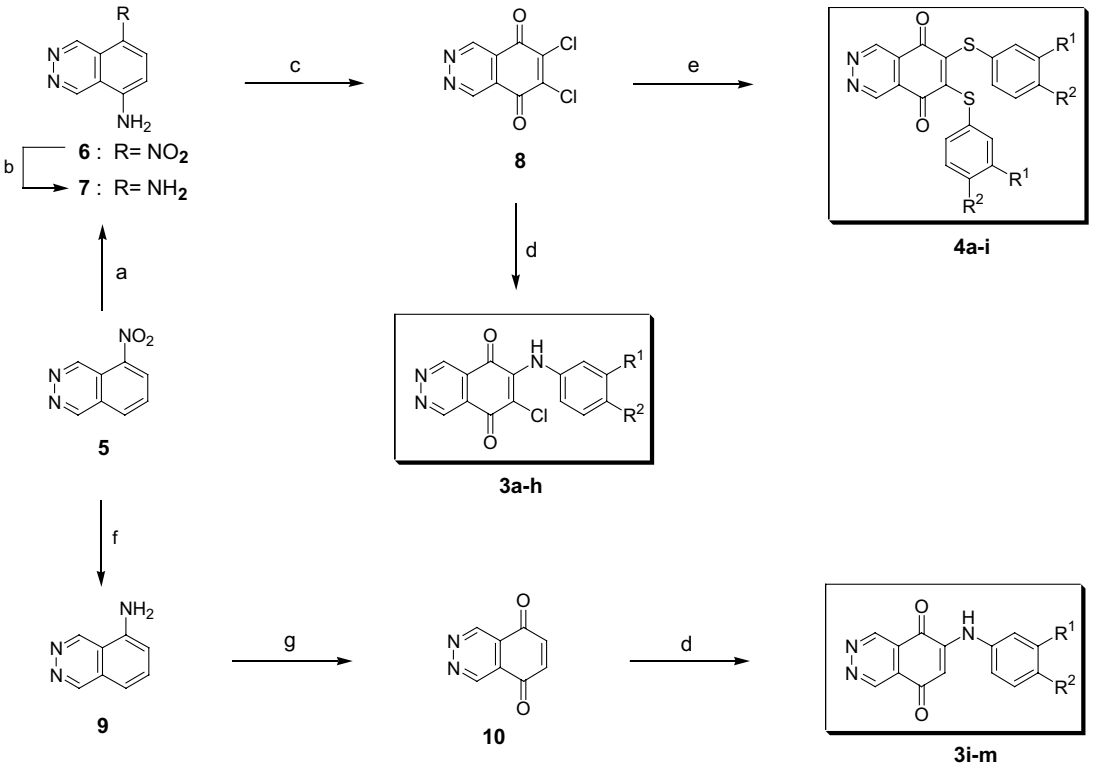
The compound **6** was reduced to phthalazine-5,8-diamine (**7**) by catalytic hydrogenation. The 6,7-dichloro-phthalazine-5,8-dione (**8**)[5] was synthesized by oxidizing compound **7** with the $NaClO_3$/HCl variation in 76% yield.

Phthalazine-5-amine (**9**) was prepared by catalytic hydrogenation of 5-nitrophthalazine (**5**) according to the reported method[11] with minor modification. The preparation of phthalazine-5,8-dione (**10**) by oxidation of compound **9** was carried out with $K_2Cr_2O_7$ in concd H_2SO_4.

6-Arylamino-phthalazine-5,8-diones **3a**–**m** were synthesized by nucleophilic substitution of compound **8** or **10** with appropriate arylamines. When compound **8** or **10** with equivalent amount of appropriate arylamines in EtOH was refluxed for 5 h, compounds **3a**–**m** were formed. Most of these substitutions went as expected and had overall high yields of 42–95%.

6,7-Bis(arylthio)-phthalazine-5,8-diones **4a**–**i** were synthesized by nucleophilic substitution on 6,7-dichloro-phthalazine-5,8-dione (**8**) with two equivalents of appropriate arylthiols. Most of the substitutions went as expected and had an overall yield of 55–86%.

The synthesized phthalazine-5,8-diones **3a**–**m** and **4a**–**i** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard twofold broth dilution method.[12] The MIC (minimum inhibitory concentration) values were determined by comparison with 5-fluorocytosine as a standard agent.[12] As indicated in Table 1, most of 6-arylamino-phthalazine-5,8-diones **3a**–**m** generally showed potent anti-



Scheme 1. Synthesis of phthalazine-5,8-dione derivatives. Reagents and conditions: (a) $HONH_2$/EtOH–MeOH/KOH/3 h/55 °C; (b) **6**/H_2/10% Pd/C/abs EtOH/3 psi/2 h/rt; (c) **7**/$NaClO_3$/HCl/1 h/65 °C; (d) arylamine (1 equiv)/EtOH/5 h/reflux/42–95%; (e) arylthiol (2 equiv)/EtOH/5 h/rt/55–86%; (f) H_2/10% Pd/C/EtOH/30 psi/4 h/rt; (g) $H_2SO_4/K_2Cr_2O_7$/2 h/0 °C.

fungal activity against *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Cryptococcus neoformans*. In contrast, 6,7-bis(arylthio)-phthalazine-5,8-diones **4a**–**i** did not show significant antifungal activity against *C. krusei* and*C. neoformans*, although many of compounds **4a**–**i** also showed potent antifungal activity against *C. albicans* and *Aspergillus niger*. Actually, the activity of compounds **3d**, **3e**, **3k**, and **3l** was superior or comparable to that of 5-fluorocytosine against all tested fungi. The compounds **3d**, **3e**, **3k**, and **3l** completely inhibited the growth of all fungal species tested at the MIC level of 3.2–25 μg/mL.

In terms of structure–activity relationship, the 6-arylamino-phthalazine-5,8-diones **3** showed, in general, a more potent antifungal activity than the other 5,6-bis(arylthio)-phthalazine-5,8-diones **4**. The 6-arylamino-compounds **3** exhibited good activity, indicating a correlation that may offer insight into the mode of action of these compounds. The activity of 6-arylamino-7-chloro-phthalazine-5,8-diones **3a**–**h** was comparable to that of 6-arylamino-phthalazine-5,8-diones **3i**–**m**. Thus, the 7-chloro moiety of compounds **3a**–**h** appears to be not important factor to affect their antifungal activity. The substituents (R^1, R^2: H, F, Cl, etc.) for the 6-arylamino and 6,7-bis(arylthio) moieties of compounds **3** and **4** may contribute partially toward biological potency.

In addition, phthalazine-5,8-diamine (**7**) and phthalazine-5-amine (**9**) exhibited no or poor, if any, antifungal activity. The phthalazine-5,8-diones **3** and **4** showed, in general, more potent antifungal activity than compounds **7** and **9**. Thus, the quinone moiety in phthalazine-5,8-diones **3** and **4** could be essential for the activity, for example, as nonquinonoid compounds **7** and **9** lost the activity.

In conclusion, phthalazine-5,8-diones **3a**–**m** and **4a**–**i** were synthesized by nucleophilic substitution of 6,7-dichlorophthalazine-5,8-dione (**8**) and phthalazine-5,8-dione (**10**) with equivalent of arylamine. 6,7-Bis(arylthio)-phthalazine-5,8-diones **4a**–**i** were synthesized by nucleophilic substitution on 6,7-dichloro-phthalazine-5,8-dione (**8**) with two equivalents of appropriate arylthiols. Among those tested, many of compounds **3a**–**m** showed potent antifungal activity against *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. neoformans.* These phthalazine-5,8-diones may thus be a promising lead for the development of antifungal agents. Moreover, the results should encourage the synthesis of phthalazine-5,8-dione analogs for improving antifungal properties.

Acknowledgment

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