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Synthesis and antifungal evaluation of 7-arylamino-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates

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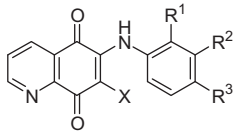
ABSTRACT

7-Arylamino-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates were synthesized and tested for in vitro antifungal activity against two pathogenic strains of fungi. Most of tested compounds showed good antifungal activity. The results suggest that those 5,8-dioxo-5,8-dihydroisoquinolines would be potent antifungal agents.

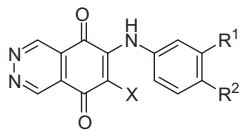
Heterocyclic quinonoid scaffolds represent an important class of biologically active molecules.[1] 5,8-Quinolinedione derivative, a heterocyclic quinone, inhibits cytochrome B-complex by the blockade of mitochondrial electron transport in *Saccaromyces cerevisiae*, which is different from commonly used antifungal drugs.[2] In our previous reports,[3,4] 6-arylamino-quinoline-5,8-diones **1** and 6-arylamino-phthalazine-5,8-diones **2** have demonstrated potent antifungal activity against pathogenic fungi (Fig. 1).

Structure–activity relationship studies from quinonoid compounds indicated that the position and number of nitrogen (N) atoms substituted in the heterocyclic ring with various substituents were considerably important factors to affect the biological activities.[4,5] We speculated that incorporation of nitrogen atom into the ring of the quinone skeleton with various substituents would change the physicochemical properties and lead to a new pharmacophore with a different biological profile from quinone compounds. The presence of arlyamino, arylthio or halo moiety to the quinones affects their antifungal activity.[6–8] Based on this speculation, 7-arylamino- and 7-arylthio-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinolines **3** which would be bioisosteres or analogues of quinones **1** and **2**, were synthesized and evaluated for their antifungal activity.

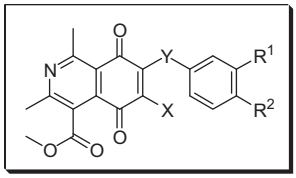
There have been a few reports[9,10] on 5,8-dioxo-5,8-dihydroisoquinolines exhibiting cytotoxic activity[10] against cancer cell lines. However, the antifungal activity of compounds **3** against pathogenic fungi has not been reported to the best of our knowledge. Therefore, 5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates **3** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity.



1 : R^1, R^2, R^3 = H, F, ..
X = Cl, Br or H



2 : R^1, R^2 = H, F, ..
X = Cl or H



3 : R^1, R^2 = H, F, ..
X = Cl, Br or H
Y = NH or S

Figure 1. Heterocyclic quinone derivatives.

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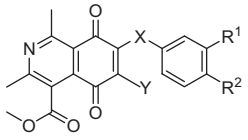
Table 1
Structures and in vitro antifungal activity for 5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates

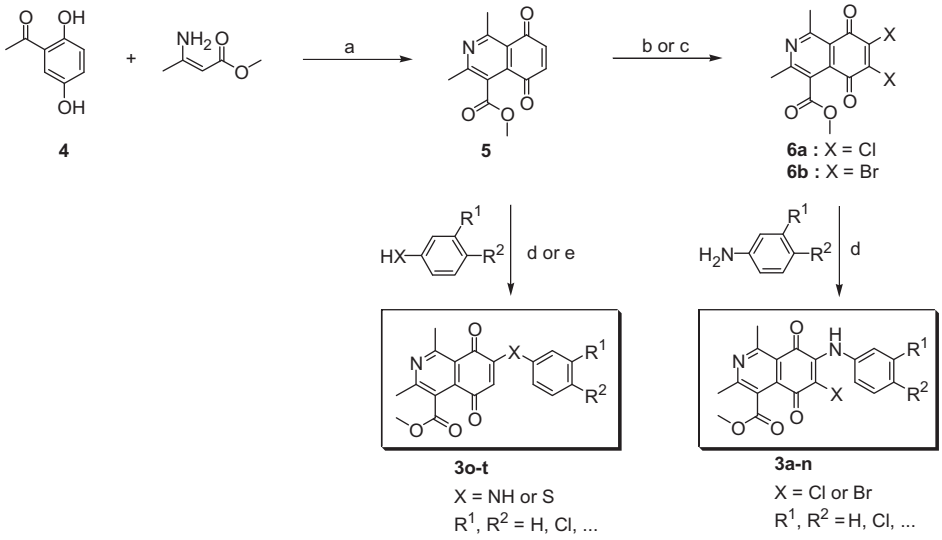


Compound	X	Y	R^1	R^2	MIC[a] (μg/mL)					
					C. albicans [b]	*C. tropicalis*	*C. krusei*	*C. neoformans*	*A. niger*	*A. flavus*
3a	NH	Cl	H	CH_3	1.6	0.8	3.2	6.3	1.6	25.0
3b	NH	Cl	H	CH_3O	1.6	12.5	12.5	3.2	3.2	6.3
3c	NH	Cl	H	F	1.6	1.6	6.3	6.3	1.6	6.3
3d	NH	Cl	H	CN	1.6	12.5	12.5	1.6	6.3	6.3
3e	NH	Cl	H	Cl	1.6	6.3	12.5	12.5	3.2	1.6
3f	NH	Cl	H	Br	1.6	12.5	6.3	3.2	1.6	6.3
3g	NH	Cl	H	I	6.3	6.3	12.5	3.2	1.6	25.0
3h	NH	Cl	F	F	1.6	25.0	6.3	12.5	1.6	6.3
3i	NH	Br	H	CH_3	6.3	1.6	6.3	6.3	6.3	25.0
3j	NH	Br	H	CH_3O	0.8	12.5	1.6	1.6	1.6	12.5
3k	NH	Br	H	F	6.3	6.3	12.5	6.3	6.3	12.5
3l	NH	Br	H	Cl	25.0	6.3	25.0	12.5	12.5	12.5
3m	NH	Br	H	Br	1.6	1.6	12.5	12.5	1.6	3.2
3n	NH	Br	F	F	6.3	1.6	25.0	12.5	6.3	12.5
3o	NH	H	H	CH_3	25.0	25.0	25.0	12.5	25.0	25.0
3p	NH	H	H	I	12.5	12.5	12.5	6.3	12.5	12.5
3q	NH	H	H	Br	12.5	12.5	12.5	12.5	6.3	12.5
3r	S	H	H	CH_3	6.3	6.3	12.5	1.6	6.3	3.2
3s	S	H	H	OH	25.0	25.0	6.3	3.2	6.3	6.3
3t	S	H	CH_3	CH_3	25.0	12.5	12.5	6.3	6.3	6.3
4	—	—	—	—	50.0	50.0	100.0	50.0	>100	100
5	—	—	—	—	25.0	12.5	50.0	50.0	>100	50
Fluconazole	—	—	—	—	50.0	6.3	25.0	6.3	25.0	25.0
5-FC[c]	—	—	—	—	3.2	3.2	6.3	12.5	6.3	6.3

[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 Lab.). 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



Scheme 1. Synthesis of 5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates. Reagents and conditions: (a) methyl 3-aminocrotonate (1 equiv)/$MgSO_4$/CH_2Cl_2/Ag_2O (4 equiv)/rt/3 h/72%; (b) **6a**: c-HCl/c-HNO_3/reflux/0.5 h/79%; (c) **6b**: NaAc/AcOH/Br_2/rt/24 h/85%; (d) arylamine (1 equiv)/EtOH/50 °C/30 min/49–91%; (e) arylthiol (1 equiv)/EtOH/24 h/rt/59–76%.

A method for the synthesis of 5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates **3a**–**t** (Table 1) is shown in Scheme 1. Methyl 1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (**5**) was prepared from commercially available 1-(2,5-dihydroxyphenyl)ethanone (**4**) and methyl 3-aminocrotonate through its cyclization to methyl 5,8-dihydroxy-1,3-dimethylisoquinoline-4-carboxylate followed by the oxidation with Ag_2O according to the reported method[10] with minor modification.

Methyl 6,7-dichloro-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (**6a**) was synthesized by chlorination of

compound **5** with HNO_3/HCl variation. Also, 6,7-dibromo-1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline **6b** was synthesized by bromination of compound **5** with Br_2/NaAc in AcOH.

7-Arylamino-6-halo-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates **3a**–**n** were synthesized by regioselective nucleophilic addition of compound **6a** or **6b** with appropriate arylamines and subsequent oxidation. When compound **6a** or **6b** with 1 equiv amount of arylamines in EtOH was refluxed for 4–12 h, compounds **3a**–**n** were formed. Most of these additions went as expected and had overall high yields of 49–91%.

In a similar manner, 7-arylamino-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates **3o**–**q** were synthesized by nucleophilic addition of compound **5** with arylamines in overall high yields of 63–86%.

To prepare 7-arylthio-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates **3r**–**t** by nucleophilic addition of arylthiol and to avoid a further nucleophilic addition of arylthiol to **5**, 1 equiv amount of appropriate arylthiol was treated with compound **5** in overall high yields of 59–76%.

The experimental details and data of representative compounds **3** were cited in Refs. 11–13.

The synthesized 5,8-dioxo-5,8-dihydroisoquinolines **3a**–**t** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the twofold broth dilution method.[14] The MIC (minimum inhibitory concentration) values were determined by comparison with fluconazole and 5-fluorocytosine as standard agents. 5,8-Dioxo-5,8-dihydroisoquinoline derivative **3** as a heterocyclic quinone could be an inhibitor of cytochrome B-complex by the blockade of mitochondrial electron transport in fungi. However, 5-fluorocytosine is an inhibitor of biosynthesis of both RNA and DNA, incorporation of 5-fluorouracil into RNA, inhibition of ribosomal protein synthesis.[15]

As indicated in the Table 1, most of 5,8-dioxo-5,8-dihydroisoquinolines **3a**–**t** generally showed potent antifungal activity against all tested fungi. The compounds **3a**–**t** completely inhibited the growth of all fungal species tested at the MIC level of 0.8–25 μg/mL and were superior or comparable to those of fluconazole against all tested fungi.

Actually, the activity of compounds **3a**, **3c** and **3m** was superior or comparable to those of 5-fluorocytosine against all tested fungi. The compounds **3a, 3c** and **3m** completely inhibited the growth of *Candida albicans* and *Aspergillus niger* at the MIC level of 1.6 μg/mL.

In terms of structure–activity relationship, the 7-arylamino-6-halo-5,8-dioxo-5,8-dihydroisoquinolines **3a**–**n** showed, in general, a more potent antifungal activity than the other 7-arylamino-5,8-dioxo-5,8-dihydroisoquinolines **3o**–**q**. The activity of 7-arylthio-5,8-dioxo-5,8-dihydroisoquinolines **3r**–**t** was comparable to that of 7-arylamino-5,8-dioxo-5,8-dihydroisoquinolines **3a**–**q**. Thus, the 7-arylamino- and 7-arylthio-moiety of compounds **3a**–**t** appear to be important factor to affect their antifungal activity. The 7-arylamino-compounds **3a**–**n** exhibited good activity, indicating a correlation that may offer insight into the mode of action of these compounds. The substituents (R^1, R^2: H, F, Cl, etc.) for the 7-arylamino and 7-arylthio moieties of compounds **3a**–**t** may contribute partially toward biological potency.

In addition, 1-(2,5-dihydroxyphenyl)ethanone (**4**) and methyl 1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (**5**) exhibited no or poor, if any, antifungal activity. The 5,8-dioxo-5,8-dihydroisoquinolines **3** showed, in general, more potent antifungal activity than compounds **4** and **5**. Thus, the 7-arylamino- and 7-arylthio-moiety in compound **3** could be important factor for the activity, for example, as compound **4** lost their activity.

In conclusion, 7-arylamino-5,8-dioxo-5,8-dihydroisoquinolines **3a**–**q** were synthesized by nucleophilic substitution of 5,8-dioxo-5,8-dihydroisoquinolinate **5**, 6,7-dichloro-5,8-dioxo-5,8-dihydroisoquinoline (**6a**) and 6,7-dibromo-5,8-dioxo-5,8-dihydroisoquinoline (**6b**) with equivalent of appropriate arylamines. 7-Arylthio-5,8-dioxo-5,8-dihydroisoquinolines **3r**–**t** were synthesized by nucleophilic substitution on 5,8-dioxo-5,8-dihydroisoquinolinate **5** with equivalent of arylthiols. All tested compounds **3a**–**t** showed potent antifungal activity against tested fungi. These 5,8-dioxo-5,8-dihydroisoquinoline scaffolds may thus be a promising lead for the development of antifungal agents. Moreover, the results would encourage the synthesis of 5,8-dioxo-5,8-dihydroisoquinoline analogs for improving antifungal properties.

Acknowledgment

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11. *Experimental:* All melting points were measured with Büchi melting point B-545 and were uncorrected. ^{1}H NMR spectra or ^{13}NMR spectra were recorded on Varian Unity INOVA 400 MHz FT-NMR spectrometer using $CDCl_3$ with TMS. HRMS spectra were recorded with a Agilent 6220 accurate-mass TOF/LC-MS equipped with an electrospray ionisation ion source used. Mass spectra were taken with Jeol JMS AX505 WA. Methyl 1,3-dimethyl-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylate (**5**) was prepared from commercially available 1-(2,5-dihydroxyphenyl)ethanone (**4**) and methyl 3-aminocrotonate according to the reported method [10].
12. *Synthesis of methyl 6,7-dichloro-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**6a**):* 2.5 mL of c-HNO_3 was added dropwise to compound **5** (0.50 g, 1.9 mmol) dissolved in 50 mL of c-HCl at 80–90 °C for 30 min. The solution was extracted with 100 mL of CH_2Cl_2 for three times. The organic layer was concentrated in vacuo. The residue was purified by column chromatography (silica gel, EtOAc/hexane = 1:5) to afford the compound **6a**: pale yellow crystal (50%); mp 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 3.03 (s, 3H, CH_3), 3.04 (s, 3H, CH_3), 4.02 (s, 3H, OCH_3); HRMS Calcd for chemical formula: $C_{13}H_9Cl_2NO_4$ 313.9988; Found: 313.9986 [$(M+H)^+$]. *Synthesis of methyl 6,7-dibromo-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**6a**)*: Compound **5** (0.25 g, 1 mmol) and 0.66 g of NaOAc were dissolved in 8 mL of acetic acid and stirred at rt for 10 min. The mixture was treated with 0.1 mL of bromine and stirred at rt for 24 h. 250 mL of water was added to the reaction mixture. After 5 min, the solid residue was filtered and purified by column chromatography (silica gel, EtOAc/$CHCl_3$ = 1:2.5) to afford compound **6a**: yellow powder (91%); mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 3.03 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 4.06 (s, 3H, OCH_3); HRMS Calcd for chemical formula: $C_{13}H_9BrNO_4$ 401.8977; Found: 401.8979 [$(M+H)^+$].
13. *General procedure for synthesis of 7-arylamino-6-halo-5,8-dioxo-5,8-dihydroisoquinoline-4-carboxylates **3**:* appropriate arylamine (1.39 mmol) was added to a solution of compound **6a** or **6b** (0.05 mol) in EtOH (100 mL). The mixture was stirred at 50 °C for 30 min. The mixture was evaporated in vacuo and the products **3** were purified by silica gel column chromatography and crystallized from EtOH. *Methyl 7-(p-tolylamino)-6-chloro-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**3a**):* purple powder (78%); mp 163–164 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 4.03 (s, 3H, OCH_3), 7.00 (d, 1H, J = 8.4), 7.00 (d, 1H, J = 8.4), 7.18 (d, 1H, J = 8.0), 7.18 (d, 1H, J = 8.0), 7.83 (s, 1H, NH); HRMS Calcd for chemical formula: $C_{20}H_{18}ClN_2O_4$ 385.0956; Found: 385.0958 [$(M+H)^+$]. *Methyl 7-(4-methoxyphenylamino)-6-chloro-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**3b**):* purple powder (76%); mp 122–123 °C; ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.90 (d, 1H, J = 8.8), 6.90 (d, 1H, J = 8.8), 7.05 (d, 1H, J = 8.4), 7.05 (d, 1H, J = 8.4), 7.81 (s, 1H, NH); HRMS Calcd for chemical formula: $C_{20}H_{18}ClN_2O_5$ 401.0905; Found: 401.0907 [$(M+H)^+$]. *Methyl 7-(4-fluorophenylamino)-6-chloro-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**3c**):* red powder (52%); mp 181–

182 °C; ^{1}H NMR ($CDCl_3$) δ 3.00 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 4.03 (s, 3H, OCH_3), 5.30 (s, 1H, NH), 7.08 (d, 1H, *J* = 8.8), 7.08 (d, 1H, *J* = 8.8), 7.78 (d, 1H, *J* = 8.4), 7.78 (d, 1H, *J* = 8.4); HRMS Calcd for chemical formula: $C_{19}H_{15}ClFN_2O_4$ 389.0705; Found: 389.0706 [$(M+H)^+$]. *Methyl 7-(p-tolylamino)-6-bromo-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**3i**):* red powder (80%); mp 153–156 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 4.56 (s, 1H, NH), 7.00 (d, 1H, *J* = 8.4), 7.00 (d, 1H, *J* = 8.4), 7.17 (d, 1H, *J* = 8.0), 7.17 (d, 1H, *J* = 8.0); HRMS Calcd for chemical formula: $C_{20}H_{18}BrN_2O_4$ 429.0451; Found: 429.0450 [$(M+H)^+$]. *Methyl 7-(4-methoxyphenylamino)-6-bromo-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**3j**):* red powder (83%); mp 136–138 °C; ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.89 (d, 1H, *J* = 8.8), 6.89 (d, 1H, *J* = 8.8), 7.06 (d, 1H, *J* = 8.4), 7.06 (d, 1H, *J* = 8.4), 7.92 (s, 1H, NH); HRMS Calcd for chemical formula: $C_{20}H_{18}BrN_2O_5$ 445.0400; Found: 445.0403 [$(M+H)^+$]. *Methyl 7-(4-bromophenylamino)-6-bromo-5,8-dihydro-1,3-dimethyl-5,8-dioxoisoquinoline-4-carboxylate (**3m**):* red powder (78%); mp 185–186 °C; ^{1}H NMR ($CDCl_3$) δ 3.00 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 6.99 (m, 1H), 6.99 (m, 1H), 7.50 (m, 1H), 7.50 (m, 1H), 7.84 (s, 1H, NH); HRMS Calcd for chemical formula: $C_{19}H_{15}Br_2N_2O_4$ 492.9399; Found: 492.9397 [$(M+H)^+$].

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