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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1850–1853
Bioorganic & Medicinal Chemistry Letters

Synthesis and antifungal activity of 6-hydroxycinnolines

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Received 8 December 2005; revised 30 December 2005; accepted 4 January 2006
Available online 24 January 2006

Abstract—6-Hydroxycinnolines **2** and cyclohexa-2,5-diene-1,4-dione derivatives **6** were synthesized and tested for in vitro antifungal activity against *Candida* and *Aspergillus* species. 6-Hydroxycinnolines **2** showed, in general, more potent antifungal activity against *Candida* species than the other cyclohexa-2,5-diene-1,4-diones. The results suggest that 6-hydroxycinnolines would be potent antifungal agents.
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Cinnoline derivatives **1** are used in pharmaceuticals and mainly patented as bactericides[1] and fungicides[2] (Fig. 1). An interesting sub-group of cinnolines is 6-hydroxycinnolines **2** which tautomerize to cinnolin-6(2*H*)-ones with a quasi quinonoid structure.[3] The compounds **2** could, respectively, exist as a mixture of tautomers 6-hydroxycinnolines and cinnolin-6(2*H*)-one in equilibrium shown in Figure 1. Quinonoid compounds display potent biological properties including antimalarial, antibacterial, and antifungal activities.[4] We assumed that 6-hydroxycinnolines **2** could show biological activities similar to those of the quinonoid compounds. The antifungal activity of 6-hydroxycinnolines **2** against pathogenic fungi has not been reported to the best of our knowledge. A variety of quinonoid compounds with different substituents could exhibit the biological activities through different actions and sometimes improve upon the activities. The presence of arylthio, amino group or halogen atoms on quinonoid compounds was a considerably important factor to affect their antifungal activity.[5] Based on this speculation, 2-amino-7,8-dimethyl-6-hydroxycinnolines **2a**–**f** with various substituents were designed and synthesized to elucidate their contribution to the antifungal activity (Scheme 1). The in vitro antifungal activity of compounds **2a**–**f** against pathogenic fungi was determined by the twofold broth dilution method. Additional data for properties and antifungal activity of cyclohexa-2,5-diene-1,4-dione derivatives are provided.

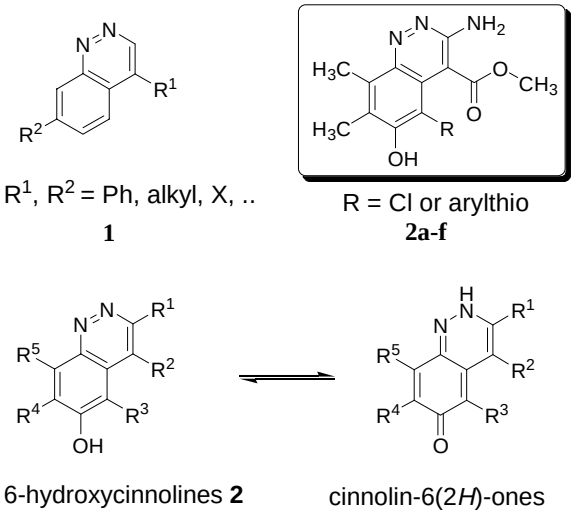

Figure 1. Cinnoline derivatives.

A method for the synthesis of 6-hydroxycinnolines **2a**–**f** (Table 1) is shown in Scheme 1. The 2,3-dichloro-5,6-dimethylcyclohexa-2,5-diene-1,4-dione (**4**)[6] was prepared by oxidizing 2,3-dimethylbenzene-1,4-diol (**3**) with HNO_3/HCl variation.

Methyl-2-(2-chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)-2-cyanoacetate (**5**) was synthesized by nucleophilic substitution of the compound **4** with equivalent of methyl cyanoacetate in EtOH in the presence of ammonia. When equivalent amounts of compound **5** and hydrazine hydrate[7] were mixed in EtOH and refluxed for 1 h, cinnoline **2a** was formed. Methyl-2-

Keywords: 6-Hydroxycinnoline; Antimicrobial compounds; Antifungal; Fungi; Substitution effects.
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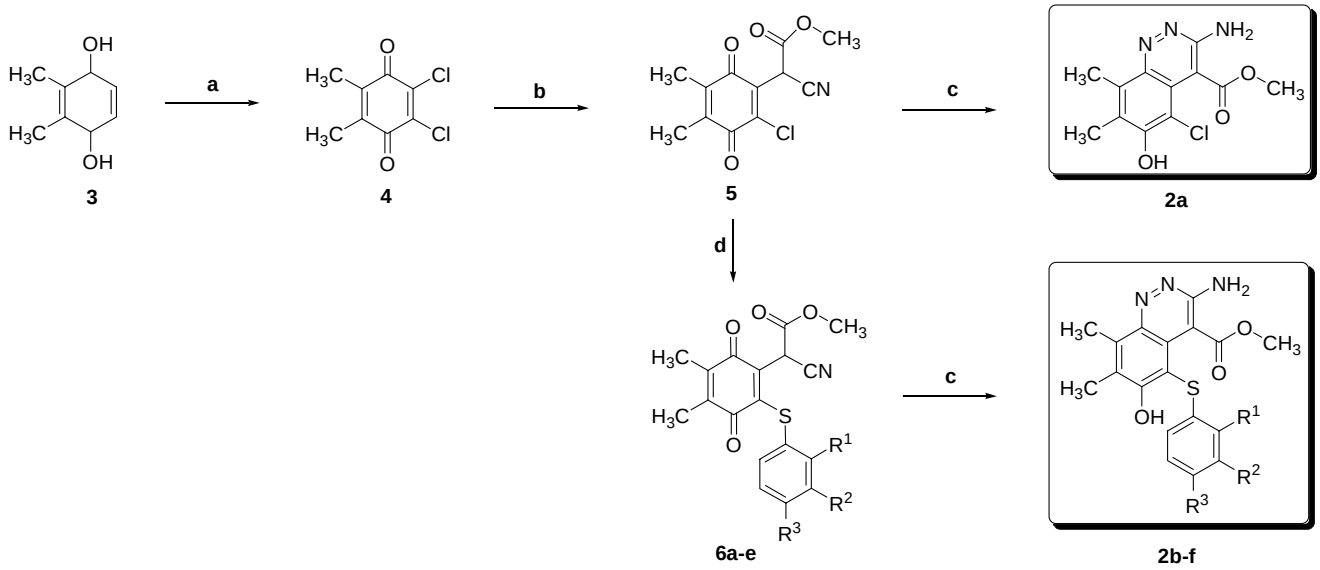
doi:10.1016/j.bmcl.2006.01.005



Scheme 1. Synthesis of 6-hydroxycinnolines. Reagents and conditions: (a) HNO_3/HCl/90 °C/10 min; (b) methyl cyanoacetate/EtOH/NH_4OH/rt/10 min; (c) hydrazine hydrate/EtOH/reflux/1 h; (d) arylthiol/EtOH/reflux/5 h.

Table 1. Structures and antifungal activity for 6-hydroxycinnolines and cyclohexa-2,5-diene-1,4-diones

Compound	R^1	R^2	R^3	MIC[a] (μg/mL)				
				C. albicans[b]	*C. tropicalis*	*C. krusei*	*C. neoformans*	*A. niger*
2a	—	—	—	>50	50	25	25	6.3
2b	H	H	H	>50	25	6.3	1.6	12.5
2c	H	H	CH_3	>50	25	3.2	6.3	25
2d	H	CH_3	CH_3	>50	25	3.2	1.6	12.5
2e	H	F	H	>50	50	25	6.3	1.6
2f	H	CH_3	H	>50	>50	>50	1.6	>50
6a	H	H	H	50	50	50	12.5	50
6b	H	H	CH_3	>50	>50	50	50	>50
6c	H	CH_3	CH_3	50	50	>50	>50	50
6d	H	F	H	50	50	25	50	50
6e	H	CH_3	H	>50	>50	1.6	50	>50
3				25	25	12.5	50	25.0
5				50	50	25	6.3	25.0
5-Fluorocytosine				6.3	12.5	6.3	12.5	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 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, and *Aspergillus niger* KCTC 1231.

cyano-2-(2-arylthio-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)acetates **6a**–**e** were synthesized by nucleophilic substitution of the compound **5** with appropriate arylthiols in boiling EtOH. When equivalent amounts of compounds **6a**–**e** and hydrazine hydrate were mixed in EtOH and refluxed for 1 h, cinnolines **2b**–**f** were formed. Experimental details and data for this procedure are cited in the References and notes.[8–12]

The synthesized 6-hydroxycinnolines **2a**–**f**, cyclohexa-2,5-diene-1,4-dione derivatives **3**, **5**, and **6a**–**e** were tested in vitro for their growth inhibitory activity against pathogenic fungi by the standard method.[13] The minimum inhibitory concentration (MIC) values were determined by comparison with 5-fluorocytosine as a standard agent.

As indicated in Table 1, most of the 2-amino-7,8-dimethyl-6-hydroxycinnolines **2a**–**f** showed potent antifungal activity against *C. krusei*, *C. neoformans*, and *A. niger*. The antifungal activity against *C. neoformans* was prominent. In contrast, the methyl-2-cyano-2-(2-arylthio-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)acetates **6a**–**e** did not show significant antifungal activity against all tested fungi, although some compounds of them exhibited poor activity. The 2-amino-7,8-dimethyl-6-hydroxycinnoline compounds **2a**–**f** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds. Actually, the activities of compounds **2b**–**d** were superior or comparable to those of 5-fluorocytosine against *C. krusei*, *C. neoformans*, and *A. niger*.

In addition, the 2,3-dimethylbenzene-1,4-diol (**3**) and methyl-2-(2-chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)-2-cyanoacetate (**5**) exhibited poor antifungal activity. Compounds **6a**–**e** exhibited no or poor, if any, antifungal activity. Thus, 6-hydroxycinnoline moiety is essential for the antifungal activity. The structure–activity relationship may not exist between properties of substituents (R^1, R^2, R^3: H, CH_3, F, ...) for the 5-arylthio moieties of the 6-hydroxycinnolines **2a**–**f**.

In conclusion, methyl-2-(2-chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)-2-cyanoacetate (**5**) was synthesized by nucleophilic substitution of 2,3-dichloro-5,6-dimethylcyclohexa-2,5-diene-1,4-dione (**4**) with equivalent of methylcyanoacetate in the presence of ammonia. Methyl-2-cyano-2-(2-arylthio-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)acetates **6a**–**e** were synthesized by nucleophilic substitution of the compound **5** with appropriate arylthiols. 2-Amino-7,8-dimethyl-6-hydroxycinnolines **2a**–**f** were synthesized by cyclization of compound **5** or **6a**–**e** with hydrazine hydrate. The 6-hydroxycinnolines **2a**–**f** showed, in general, a more potent antifungal activity than the other compounds **3**, **5**, and **6**. The results suggest that the 6-hydroxycinnolines would be potent antifungal agents. Moreover, the results should encourage the synthesis of 6-hydroxycinnoline analogs for improving antifungal properties.

Acknowledgment

This work was supported by Seoul Metropolitan Government Grant 2005.

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8. *Experimental*: All melting points were measured with Büchi melting point B-545 and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity INOVA 400 MHz FT-NMR spectrometer with TMS. High-resolution mass spectra (HRMS EI) were taken with Jeol JMS AX505 WA. 2,3-Dimethylbenzene-1,4-diol (**3**) and other reagents were purchased from Aldrich Chemical Co.
9. *Synthesis of 2,3-dichloro-5,6-dimethylcyclohexa-2,5-diene-1,4-dione* (**4**): 5 mL of concd HNO_3 was added over a period of 1 h to a stirred suspension of compound **3** (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 from EtOH afforded compound **4**: orange powder (25%); mp 248 °C[6]; ^{1}H NMR (DMSO-d_6) δ 2.03 (s, 6H, CH_3).
10. *Synthesis of methyl-2-(2-chloro-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)-2-cyanoacetate* (**5**): To a solution of compound **4** (1.295 g, 2.66 mmol) and methylcyanoacetate (0.26358 mL, 2.66 mmol) in 100 mL of EtOH, ammonia solution (2 mL) was added dropwise. The mixture was stirred at rt for 10 min, dil HCl was then added. The mixture was then extracted several times with CH_2Cl_2, and the organic layer was washed with water, dried with anhydrous $MgSO_4$, and concentrated. The product **5** was separated by silica gel column chromatography with *n*-hexane/EtOAc: brown oil (31%); ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 5.25 (s, 1H, CH_2). HRMS calcd for $C_{12}H_{10}ClNO_4$: 267.0299. Found: 267.0298.
11. *General procedure for synthesis of methyl-2-cyano-2-(-2-arylthio-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)acetates.* **6a**–**e**: arylthiol (0.448 mmol) was added to the solution of compound **5** (120 mg, 0.448 mmol) in 100 mL of 95% EtOH and the mixture was stirred at rt or refluxed for 4-5 h. The oily products were purified by silica gel column chromatography.
12. *General procedure for synthesis of methyl-3-amino-5-chloro-7,8-dimethyl-6-hydroxycinnoline-4-carboxylates.* **2a**–**f**: equivalent weight of hydrazine hydrate was added to the solution of compounds **5** or **6a**–**e** 300 mg in 100 mL of 95% EtOH and heated under reflux for 1 h. The product was purified by silica gel column chromatography and crystalized from 95% EtOH. *Methyl-3-amino-5-chloro-7,8-dimethyl-6-hydroxycinnoline-4-carboxylate.* (**2a**): beige needle (46%); mp 220–221 °C; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 5.81 (s, 1H, OH), 6.13 (s, 2H, NH); HRMS calcd for $C_{12}H_{12}ClN_3O_3$: 281.0567. Found: 281.0568. *Methyl-3-amino-7,8-dimethyl-6-hydroxy-5-(phenylthio)-cinnoline-4-carboxylate.* (**2b**): beige needle (80%); mp 154–155 °C; ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.54 (s, 3H, OCH_3), 7.02 (m, 2H, Ph), 7.07 (m, 1H, Ph), 7.18 (m, 2H, Ph), 7.38 (s, 1H, OH); HRMS calcd for $C_{18}H_{17}N_3O_3S$: 355.0991. Found: 355.0990. *Methyl-3-amino-7,8-dimethyl-6-hydroxy-5-(p-tolylthio)cinnoline-4-carboxylate.* (**2c**): beige needle (71%); mp 142–143 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.60 (s, 3H, OCH_3), 6.21 (s, 2H, NH), 6.94 (m, 2H, Ph), 6.99 (m, 2H, Ph), 7.38 (s, 1H, OH); HRMS calcd for $C_{19}H_{19}N_3O_3S$: 369.1147. Found: 369.1148. *Methyl-3-amino-7,8-dimethyl-5-(3,4-dimethylphenylthio)-6-hydroxycinnoline-4-carboxylate.* (**2d**): beige needle (68%); mp 155–156 °C; ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 6H, CH_3), 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 6.20 (s, 2H, NH), 6.74 (d, 1H, J = 8.0, Ph), 6.86 (m, 1H, Ph), 6.93 (d, 1H, J = 8.0, Ph), 7.39 (s, 1H, OH); HRMS calcd for $C_{20}H_{21}N_3O_3S$: 383.1304. Found: 383.1303. *Methyl-3-amino-7,8-dimethyl-5-(3-fluorophenylthio)-6-hydroxycinnoline-4-carboxylate.* (**2e**): beige needle (65%); mp 148 °C (dec); ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.55 (s, 3H, OCH_3), 6.18 (s, 2H, NH), 6.67 (m, 1H, Ph), 6.76 (m, 1H, Ph), 6.85 (m, 1H, Ph), 7.15 (m, 1H, Ph), 7.18 (s, 1H, OH); HRMS calcd for $C_{18}H_{16}FN_3O_3S$: 369.1147. Found: 369.1148. *Methyl-3-amino-7,8-dimethyl-6-hydroxy-5-(m-tolylthio)cinnoline-4-carboxylate.* (**2f**): beige needle (86%); mp 142–143 °C; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.32

(s, 3H, CH_3), 3.59 (s,3H, OCH_3), 6.29 (s, 2H, NH), 6.82 (m, 1H, Ph), 6.86 (d, 2H, $J = 8.0$, Ph), 6.89 (m, 1H, Ph), 7.06 (t, 1H, $J = 8.0$, Ph), 7.38 (s, 1H, OH); HRMS calcd for $C_{19}H_{19}N_3O_3S$: 369.1147. Found: 369.1149.

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