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Synthesis of chlorogenic acid derivatives with promising antifungal activity

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Abstract—Derivatives of chlorogenic acid or its analogues were synthesized by coupling protected chlorogenic acid or its analogues with *p*-octyloxyaniline and selected amino acids. Most of the compounds exhibited significant potency against *Cryptococcus neoformans* and *Candida* species with low toxicity to brine shrimps. The 4,5-dihydroxyl groups in the quinic acid moiety were necessary for the activity and introduction of a free amino group increased the inhibitory activity against *Aspergillus fumigatus*.

Life-threatening fungal infections have increased dramatically in recent years in immunocompromised patients such as those undergoing cancer chemotherapy, organ transplant, and patients with AIDS. As clinically used antifungal drugs have drawbacks with respect to side effects and development of drug resistance, discovery and development of new types of antifungal agents is of high importance.

Some natural products and their semisynthetic derivatives have been known as effective antifungal agents for decades. Nystatin and amphotericin B have long been used clinically for the treatment of fungal infections. Candin class of cyclic peptides have recently been developed as effective drugs to treat opportunistic and invasive fungal infections.[1] We have previously reported the synthesis of peptidomimetic analogues of echinocandin B.[2] In that report, we presumed that a suitable position of homotyrosine ring (a phenolic moiety), with respect to the lipophilic side chain, is the determining factor for the antifungal activity of echinocandins. This assumption was in agreement with that suggested by Zambias et al., with respect to structural requirement for 1,3-β-glucan synthase inhibitory activity of echinocandin-related analogues.[3]

To examine this hypothesis, we attempted determining the stereochemically optimized form as well as molecular dynamic of echinocandin B using Hyperchem-3™ software on an Octane-2 Silicon Graphic computer. After molecular optimization of echinocandin B, the overlay of a series of structurally related natural and synthetic analogues of homotyrosine on echinocandin B was performed. Among the phenolic compounds tried, chlorogenic acid linking with a long chain was found to have the most favorable spatial relationship of the essential groups (the phenolic moiety and the lipophilic side chain) with those of echinocandin B (unpublished data). Chlorogenic acid is a natural product existing widely in many vegetables and plants. Chlorogenic acid linking with one more caffeoyl group (dicaffeoylquinic acids) has been reported to have inhibitory activity on HIV integrase.[4] Methyl ester of chlorogenic acid from natural source has been shown to inhibit HIV protease.[5] Based on the above information we designed and synthesized chlorogenic acid analogues possessing a lipophilic chain, including an amino acid group, at the position 1. The synthesis of chlorogenic acid derivatives was carried out as depicted in Schemes 1–3. Chlorogenic acid was treated with dried acetone in the presence of catalytic amount of concentrated H_2SO_4 to afford its acetonide derivative, which was then condensed with 4-(octyloxy) aniline in the presence of

Keywords: Chlorogenic acid; *p*-Octyloxyaniline; *Candida albicans*; *Cryptococcus neoformans*; *Aspergillus fumigatus*; H2N-aa-4-(octyloxy) aniline; Brine shrimp lethality assay; Echinocandin B; 1,3-β-Glucan; ^{1}H NMR; ESI-MS; FAB-MS.
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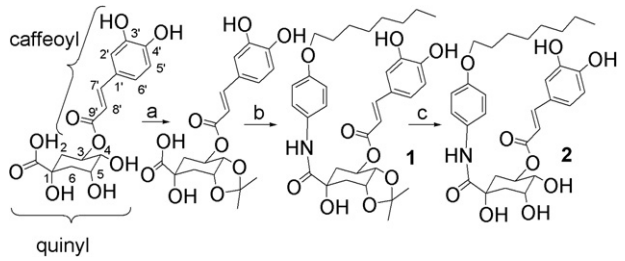
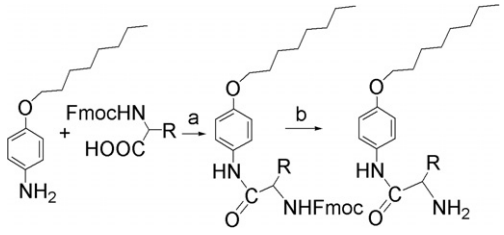
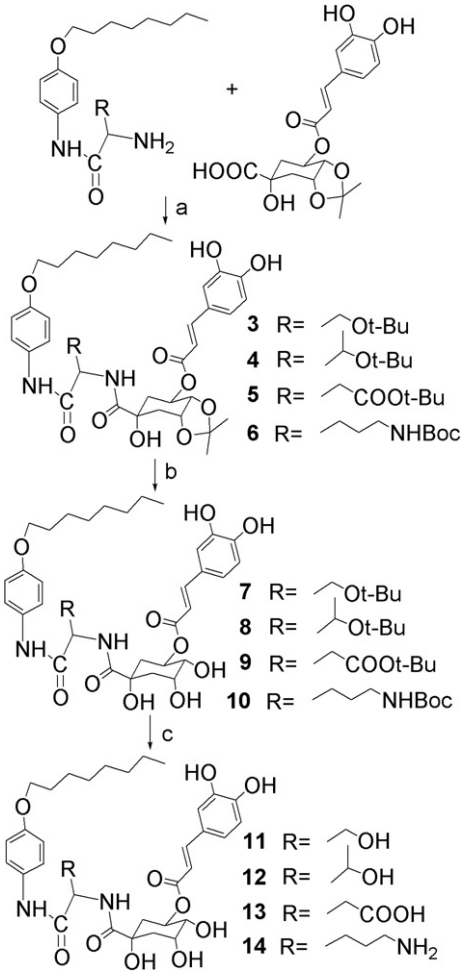


Scheme 1. Synthesis of chlorogenic acid derivatives **1** and **2**. Reagents and conditions: (a) dry acetone, concd H_2SO_4; (b) 4-(octyloxy) aniline, CMC, HOBT; (c) acetone-H_2O, 0.67 N HCl, rt, 1 h.



Scheme 2. Synthesis of H_2N-aa-4-(octyloxy) aniline. Reagents and conditions: (a) CMC, HOBT; (b) 10% $NHEt_2$, DMF, rt, 2 h.



Scheme 3. Synthesis of chlorogenic acid derivatives **3**–**14**. Reagents and conditions: (a) CMC, HOBT; (b) acetone-H_2O, 0.67 N HCl, rt, 1 h; (c) 90% TFA, rt, 30 min.

CMC and HOBT to obtain compound **1**. Deprotection of compound **1** under acidic condition yielded compound **2**.

Both compounds **1** and **2** showed antifungal activity (Table 1). To investigate the influence of physicochemical properties on the antifungal activity, selected amino acids were introduced to the above structures. For the synthesis of these compounds, protected amino acid derivatives (Fmoc-Thr(t-Bu)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Asp(OtBu)-OH or Fmoc-Orn(Boc)-OH) were condensed with 4-(octyloxy) aniline in the presence of CMC and HOBT to obtain the amino acid derivatives with lipophilic chains. These compounds were deprotected with $NHEt_2$ to obtain compounds with free amino groups, H_2N-aa-4-(octyloxy) aniline (Scheme 2). The H_2N-aa-4-(octyloxy) aniline was reacted with the acetonide of chlorogenic acid to obtain compounds **3**–**6**. Acid hydrolysis of compounds **3**–**6** was performed under controlled condition to obtain compounds **7**–**10**. Further de-protection of **7**–**10** with 90% TFA afforded compounds **11**–**14**. The acid concentration, hydrolysis time, and temperature were carefully controlled to obtain stepwised products and to avoid the hydrolysis of the ester bond between caffeoyl and quinic acid moieties.

To investigate the influence of the caffeoyl moiety on the bioactivity, compounds **15**–**19** were synthesized. Hydrogenation of compound **10** in EtOH containing Pd/C produced compound **15**. Part of compound **15** was deprotected with 90% TFA to obtain compound **16**.

Quinic acid bisacetonide was synthesized according to the reported method.[6] Condensation of acetylcoumaryl

Table 1. Antifungal activity of chlorogenic acid derivatives

Compound	MIC[a,b] (μg/ml)			% BL[c]
	C. albicans	*C. neoformans*	*A. fumigatus*	
1	16	2	>64	100.0
2	8	2	>64	50.0
3	32	2	>64	31.3
4	32	4	>64	6.2
5	>64	2	>64	56.2
6	>64	2	>64	12.5
7	4	2	>64	31.1
8	2	1	>64	50.0
9	2	2	>64	37.5
10	2	1	>64	25.0
11	>64	4	>64	18.8
12	8	2	>64	12.5
13	64	16	>64	6.3
14	4	1	16	37.5
15	>64	4	>64	21.1
16	16	8	64	10.5
17	>64	>64	>64	0
18	>64	>64	>64	31.6
19	8	4	32	100
Fluconazole	0.25	0.5	>64	

[a] The MIC value was determined by methods of NCCLS. The final concentration of antifungal agents was between 0.12 and 64 μg/ml. MICs were read at 80% inhibition.

[b] Tested organisms: *Candida albicans* ATCC90028, *Cryptococcus neoformans* ATCC32045, and *Aspergillus fumigatus* ATCC13073.

[c] BL: (%) brine shrimp lethality at 100 μg/ml.

chloride with the bisacetonide followed by deprotection yielded chlorogenic acid analogue **A** (Scheme 4).

Compound **A** was protected as acetonide using the same condition as depicted in Scheme 1. The acetonide derivative of **A** was used as a starting material to synthesize compounds **17**–**19** by the same method as described in Scheme 3. The structures of compounds **15**–**19** are shown in Figure 1.

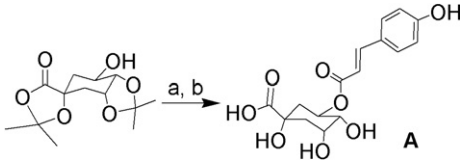
The synthesized compounds were evaluated for in vitro antifungal activity against *Candida albicans* ATCC90028, *Cryptococcus neoformans* ATCC32045, and *Aspergillus fumigatus* ATCC13073 according to the NCCLS guidlines.[7,8] Plates were incubated at 35 °C for 18–24 h for *C. albicans*, 48 h for *A. fumigatus*, and 48–72 h for *C. neoformans*. The MIC values were determined as the lowest concentration of the compound that inhibited growth up to 80%. The results are summarized in Table 1.

Overall, chlorogenic acid derivatives (**1**–**14**) showed better antifungal activity or less toxicity than those of chlorogenic acid analogues (**15**–**19**), suggesting that structural modification on the caffeoyl group, such as saturating the double bond (**16** vs **14**) or reducing the number of hydroxyl groups (**19** vs **14**), did not benefit the bioactivity profile. Significant antifungal activity was observed in most of the chlorogenic acid derivatives (**1**–**14**). The MICs on *C. neoformans* of nearly all these chlorogenic acid derivatives were as low as 1–4 μg/ml, except compound **13** with a free carboxylic acid group in its structure, which had MIC of 16 μg/ml. It has been reported that incorporation of an amino group (such as aminoproline residue) into the ring of echinocadin analogues led to improvement of its antifungal potency.[9] Similar effects were observed in the present chlorogenic acid derivatives. Compound **14** with a free amino group in its structure showed good activity against all the fungi tested, including *A. fumigatus* (MIC of 16 μg/ml). The MIC of the chlorogenic acid derivatives against *C. albicans* varied from 2 to >64 μg/ml. All the acetonide compounds (**1**, **3**–**6**) showed weaker inhibitory activity against *C. albicans* than the corresponding compounds with free hydroxyl groups (**2**, **7**–**10**), suggesting that the two hydroxyl groups in the quinic acid part are essential for the activity.

To test the general toxicity of these compounds, brine shrimp lethality assay was carried out according to the reported method.[10] Compounds **1** and **19** showed high toxicity toward the brine shrimps at 100 μg/ml. Compounds **2**, **5**, and **8** showed moderate toxicity, while other compounds exhibited low toxicity.

Compounds **8** and **12**, due to the existence of threonine in their molecular structures which make them structurally more resemble to the south-eastern component of echinocandin, were further tested for their inhibitory activity against two *Candida* species, including the drug (azole)-resistant fungus *C. krusei*, in comparison with the parent compound, chlorogenic acid. As shown in Table 2, both **8** and **12** showed much more potent inhibitory activity against the fungi but less toxicity on brine shrimp than chlorogenic acid.

With respect to mechanism(s) of action of this novel class of chlorogenic acid derivatives, based on their partial structural similarity with that of echinocandin B, we anticipated the same mode of action for these compounds as that of echinocandin B analogues, which is inhibition of 1,3-β-glucan synthase. This enzyme is essential for the biosynthesis of the glucan component of fungal cell wall. While *Candida* and *Aspergillus* species are sensitive toward candin class of antifungals, due to the presence of 1,3-β-glucan in their cell wall structure, *C. neoformans* does not exhibit sensitivity against this class of compounds. Indeed, the main glucan component in *C. neoformans* cell wall is 1,6-β-glucan with a biosynthetic



Scheme 4. Synthesis of chlorogenic analogue **A**: Reagents and conditions: (a) acetylcoumaryl chloride, CH_2Cl_2, pyridine; (b) 0.8 N HCl, THF-H_2O, rt, 48 h.

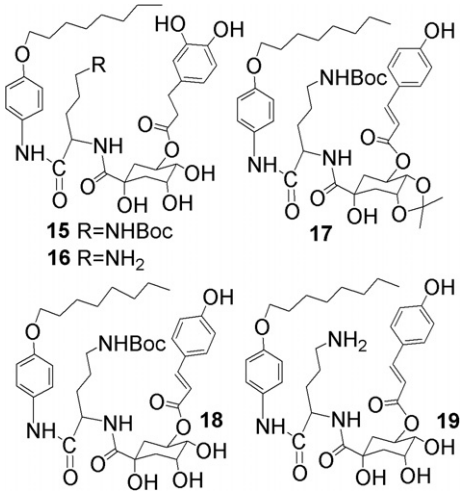


Figure 1. Structures of compounds **15**–**19**.

Table 2. Comparison of the bioactivity of **8**, **12** with chlorogenic acid

Compound	MIC[a,b] (μg/ml)		% BL[c]
	C. albicans	*C. krusei*	
8	4	2	50.0
12	4	2	12.5
Chlorogenic acid	>64	>64	100
Amphotericin B	0.5	1	

[a] The MIC value was determined by methods of NCCLS. The final concentration of antifungal agents was between 0.12 and 64 μg/ml. The MIC values are defined as the lowest concentration showing 80% inhibition of drug-free control growth.

[b] Tested organisms: *Candida albicans* ATCC90028, *Candida krusei ATCC6258.*

[c] BL: (%) brine shrimp lethality at 100 μg/ml.

pathway different from that of 1,3-β-glucan. *Candida* species possess both 1,3- and 1,6-β-glucan in their cell wall, while *Aspergillus* carries only 1,3-β-glucan.[11–15]

The in vitro biological assessment of the chlorogenic acid analogues synthesized in this study reveals a strong antifungal activity against *C. neofrmans*, a good to moderate activity against *C. albicans*, and weak to no activity against *A. fumigatus*. Considering the above biological activity results, as well as the differences in glucan structures in the tested fungi, we speculate that these chlorogenic acid derivatives may exhibit their antifungal effect via inhibition of 1,6-β-glucan synthase. Other possible mechanism is increasing the permeability of fungal cell wall via mimicking the action of bactericidal/permeability-increasing protein, a mechanism that has been reported for antifungal activity of some peptides that are structurally related to chlorogenic acid.[16]

In conclusion, we synthesized a novel series of lipochlorogenic acid derivatives. Most of the synthesized compounds exhibited potent antifungal activity against *Cryptococcus* and *Candida* species but lower toxicity to brine shrimps than the parent chlorogenic acid.

These novel compounds may serve as leads for the development of less toxic drugs with different structural feature from those of the currently utilized antifungal agents. The structure–activity relationships discussed above can provide useful information for further design and synthesis of compounds with optimized bioactivity profile. Further studies to optimize the structural feature as well as to explore the mechanism of action of this novel class of compounds is planned to start in our group in near future.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.07.038.

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