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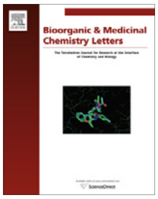

Synthesis and antifungal activity of a novel series of 13-(4-isopropylbenzyl)berberine derivatives

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ABSTRACT

By replacing the methyl group of 13-(4-isopropylbenzyl)berberine **2** with various acyl, alkyl, and benzyl groups via the demethylated intermediate, 13-(4-isopropylbenzyl)berberrubine **4**, a novel series of 9-*O*-alkyl-13-(4-isopropylbenzyl)berberine derivatives was synthesized and examined for antifungal activities against various human pathogenic fungi. The introduction of various alkyl groups led to enhanced antifungal activity but that of acyl groups resulted in decrease of the activity. Among them, 9-*O*-butyl-13-(4-isopropylbenzyl)berberine **6d** exhibited the most potent antifungal activities against *Cryptococcus neoformans*, *Candida* species (MIC = 0.25–1 μg/ml), and *Aspergillus* species (MIC = 2–4 μg/ml). The compound was found to be relatively safe up to 900 mg/kg in oral administration to mice.

The incidences of infections by opportunistic fungi are increasing especially in patients whose immune systems are compromised by AIDS, cancer, diabetes, aging, and other causes.[1,2] Although many antifungal compounds have been developed and some drugs are under clinical trials, safe and effective antifungal drugs have not yet been developed because of the similarity between fungi and mammalian cells.[3,4] Therefore, amphotericin B and the azole compounds remain the mainstream of fungal systemic infection therapy despite their serious side effects and the emergence of resistant strains.[5,6] This has consequently resulted in a strong demand for drugs that have low side effects and broad-spectrum activity.

Medicinal plants that have been used for a long time may be good sources of safe antifungal agents. Berberine **1**, which is an alkaloid isolated from Korean and Chinese medicinal plants, significantly inhibits growth of a wide range of *Candida* species.[7,8] Recently, berberine was combined with amphotericin B to treat disseminated candidiasis in mice and investigated for the synergistic interaction with fluconazole against fluconazole resistant clinical isolates of *Candida albicans*.[9–11] In addition, to develop the novel antifungal agents, various berberine derivatives with substituents at C-13 position were synthesized, which exhibited potent activity against *Candida* species.[12,13]

Previously, we reported that the introduction of various aromatic groups at C-13 position of berberine remarkably improved the antifungal activites.[13] Among them, 13-(4-isopropylbenzyl)berberine **2** including a lipophilic substituent at the *para* position of benzyl group exhibited the most potent antifungal activities (Fig. 1). The most significant structural feature leading to enhanced activity was the presence of a methyl group on 9-hydroxyl. Thus, 13-(4-isopropylbenzyl)berberine **2** showed over fourfold stronger activities than 13-(4-isopropylbenzyl)berberrubine **4** with a hydroxy group at 9-position instead against various the human pathogenic fungi.

The purpose of the present study was to investigate whether structural changes of the methyl group on 9-hydroxyl might enhance antifungal activities. A new series of 13-(4-isopropylbenzyl)berberine analogs **6a**–**6o** was synthesized by using other basic moieties such as alkyl chains, olefins and aromatic groups as the replacement of the methyl group in the 9-position, and examined for antifungal activities against seven human pathogenic fungi.

For the synthesis of 9-O-substituted-13-(isopropylbenzyl)berberines, 13-(4-isopropylbenzyl)berberrubine **4** was synthesized by the methods described in the previous study.[13] Acetonylberberine **3** used as a key intermediate was obtained by condensation of berberine with acetone. The treatment of **3** with 4-isopropylbenzyl bromide and sodium iodide (NaI) gave 13-(4-isopropylbenzyl)berberine **2** by displacement of the acetonyl group with the 4-isopropylbenzyl group. The pyrolysis of **2** was carried out in DMF with high temperature to give **4**. The treatment of **4** with various acyl chlorides, alkyl iodides and benzyl bromides gave a salt form of 9-O-substituted derivatives **5**. The iodide or bromide

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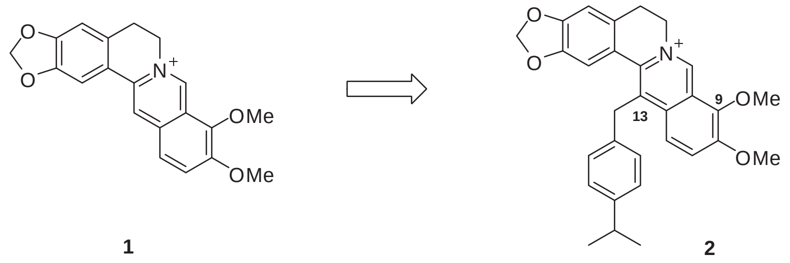
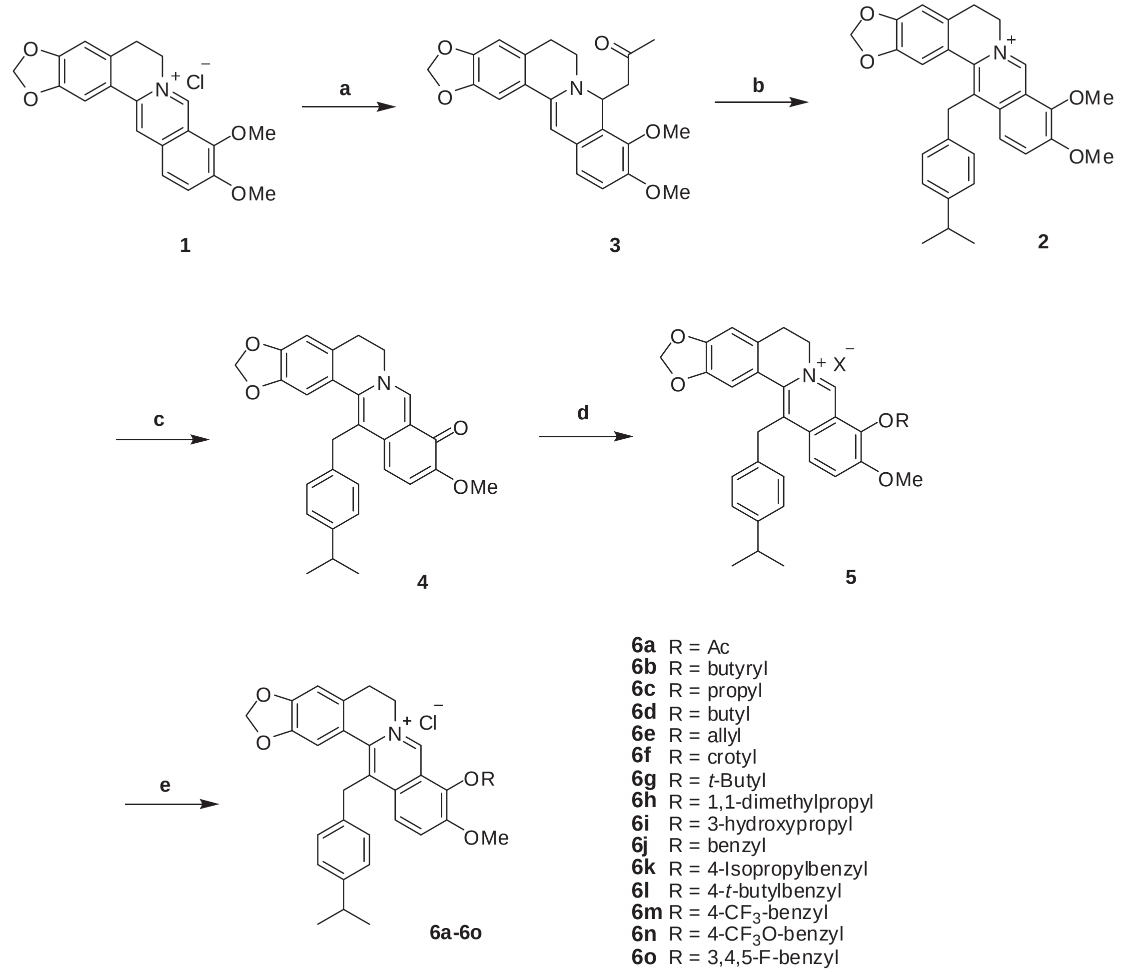


Figure 1. Structural optimization of berberine **1**.

salt forms of the target compounds were converted into yellow-orange crystalline chlorides **6** using AgCl in hot MeOH (Scheme 1).

All of the synthesized compounds were tested for in vitro antifungal activity against various *Candida* species, two *Aspergillus* species, and *Cryptococcus neoformans* using the procedure described by the broth microdilution method of the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards NCCLS).[14,15] The MIC was defined as the lowest concentration that produced an optically clear well. Determinations of MICs were performed in duplicate on at least three separate experiments.

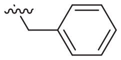
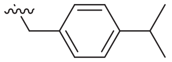
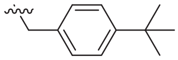
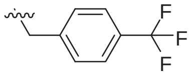
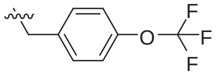
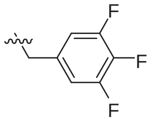
The antifungal activities of the compounds against human pathogenic fungi are summarized in Table 1. The results were compared with findings previously reported for **2**, **4**, and the proven antifungal agent amphotericin B. These results indicate that the replacement of the methyl group with various aliphatic groups at 9-O position remarkably improved antifungal activity. The most significant structural change leading to enhanced activity was the introduction of alkyl chains as an alkyloxy group. Among them, 9-*O*-butyl-13-(4-isopropylbenzyl)berberine **6d** exerted the most potent antifungal activities with MIC values of 0.25–1 μg/ml against several *Candida* species, and dramatically over eightfold stronger antifungal activities than the corresponding acyloxy substituted analog, 9-*O*-butyryl-13-(4-isopropylbenzyl)berberine **6b**. These results indicate that the acyl groups at 9-O position might be cleaved easily because the oxygen at C-9 position of berberrubine is present in the form of either ketone **4a** or hydroxyl **4b** and reversibly transformed due to oxidative rearrangement in isoquinolium salts (Fig. 2).[16,17] It can be seen that replacing the alkyl chains with olefin groups as either the allyl **6e** or crotyl **6f** does not



6a R = Ac
6b R = butyryl
6c R = propyl
6d R = butyl
6e R = allyl
6f R = crotyl
6g R = *t*-Butyl
6h R = 1,1-dimethylpropyl
6i R = 3-hydroxypropyl
6j R = benzyl
6k R = 4-Isopropylbenzyl
6l R = 4-*t*-butylbenzyl
6m R = 4-CF_3-benzyl
6n R = 4-CF_3O-benzyl
6o R = 3,4,5-F-benzyl

Scheme 1. Synthesis of 9-*O*-alkyl-13-(4-isopropylbenzyl)berberine derivatives. Reagents and conditions: (a) acetone, 5 N NaOH, rt, 1 h; (b) 4-isopropylbenzylbromide, NaI, CH_3CN, 80 °C, 4 h; (c) DMF, reflux, 190 °C, 2 h; (d) various acyl chlorides, alkyl iodides and benzyl bromides, CH_3CN, 120 °C, 4 h; (e) AgCl, MeOH, 60 °C, 2 h.

Table 1
Structures and in vitro antifungal activities for 9-substituted-13-(4-isopropylbenzyl)berberine derivatives **6**.

Compounds	R	MIC[a] (μg/ml)						
		Candida albicans[b]	*Candida tropicalis*	*Candida lusitaniae*	*Candida krusei*	*Aspergillus fumigatus*	*Aspergillus terreus*	*Cryptococcus neoformans*
2	CH_3	4	1	8	4	64	32	4
4	H	16	16	8	16	>128	>128	4
6a	$C(O)CH_3$	16	16	8	16	>128	>128	8
6b	$C(O)CH_2CH_2CH_3$	8	8	8	8	>128	>128	4
6c	$CH_2CH_2CH_3$	1	0.5	2	2	4	8	2
6d	$CH_2(CH_2)_2CH_3$	0.5	0.25	1	1	2	4	1
6e	$CH_2CH{=}CH_2$	1	1	2	2	8	8	2
6f	$CH_2CH{=}CHCH_3$	0.5	0.5	1	1	2	4	1
6g	$C(CH_3)_3$	4	1	8	4	32	32	4
6h	$C(CH_3)_2CH_2CH_3$	2	1	4	4	32	16	4
6i	$CH_2(CH_2)_2OH$	2	1	4	2	8	8	2
6j		4	4	8	4	64	32	4
6k		1	1	4	2	4	8	2
6l		1	1	4	4	4	8	2
6m		2	2	4	4	16	16	4
6n		2	2	4	4	32	16	4
6o		4	4	8	4	32	32	4
AmpB[c]		0.5	0.5	1	0.5	1	16	0.25

[a] The MIC value was determined by methods of CLSI. The final concentration of antifungal agents was between 0.125 and 128 μg/ml. The values are given as means of three experiments.

[b] Test organisms: *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, *Candida lusitaniae* ATCC 42720, *Candida krusei* ATCC 6258, *Aspergillus fumigatus* ATCC 16424, *Aspergillus terreus* ATCC 46941, and *Cryptococcus neoformans* ATCC 36556.

[c] AmpB: amphotericin B.

result in improved activity. Hence, the presence of unsaturation in the alkyl group did not enhance the activity as seen in compounds **6c**, **6d, 6e** and **6f**. Furthermore, comparison of **6c** and **6d** with **6g** and **6h** showed that the substitution of methyl groups on the C-1 position of the alkyl chains led to decreased activity. As the polarity is further increased in the cases of the hydroxy-propyl **6i**, there is a decrease in antifungal activity compared to the compound **6c**. In addition, the introduction of various benzyl groups at 9-O position showed diverse antifungal activities depending on the functional groups of the substituents on the aromatic ring. Compound **6k** and **6l** exhibited stronger activities than **6j** and **6o**, consistent with the previous requirement for potency, a lipophilic alkyl group at the benzyl moiety. The activity of these compounds with a benzyl group at 9-O position showed similar tendency to that of the previously synthesized compounds with a benzyl group at C-13 position.

To examine the toxicity of compound **6d,** the acute toxicity test of the compound was performed. Compound **6d** was orally administered to ICR mice with various doses (33–900 mg/kg), and the animals were closely monitored. No animal died in the 7-day treatment, indicating that LD_{50} of **6d** was over 900 mg/kg. No changes in clinical signs, body weights, and the gross necropsy of mice administrated with these dosages were observed. This result suggests that compound **6d** is considerably safe in vivo up to 900 mg/kg in oral administration.

On the basis of our previous[13] and current studies, our results suggest two structural requirements for enhancing the antifungal activity: 1) the introduction of a benzyl group including lipophilic substituents at C-13 position of berberine and 2) the modification of the methyl group at 9-O position with an alkyl group. Especially, the introduction of an alky group at 9-O position led to much better antifungal activity than that of an acyl group. Among novel ser-

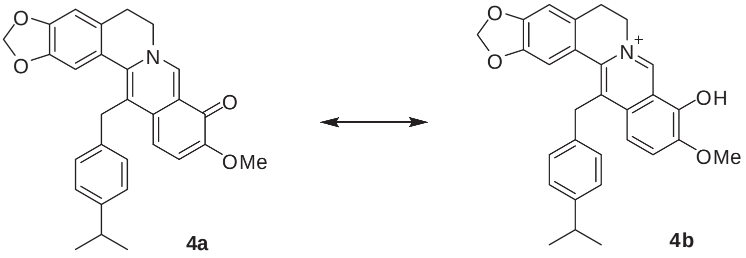


Figure 2. Rearrangement in compound **4**.

ies of 9-*O*-alkyl-13-(isopropylbenzyl)berberine derivatives, compound **6d** with a butyl group exhibited 4–32-fold stronger antifungal activities than compound **2** with methyl group, and showed similar antifungal activities to that of amphotericin B, a commercially available antifungal agent. The compound did not cause the toxicological change up to 900 mg/kg in mice for mortality, clinical signs, body weight, and necropsy in oral administration. Besides, no significant toxicity was observed from the necropsy performed on the last day of experiments, although additional toxicity data including administration over 900 mg/kg and long-term observation may be needed. Taken together, further analysis of structure–activity relationship for the series would make it possible to design novel antifungal agents.

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Supplementary data

Supplementary data (materials and methods, and ESI-MS, NMR, and elementary analysis data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.045.

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