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Synthesis of 13-(substituted benzyl) berberine and berberrubine derivatives as antifungal agents

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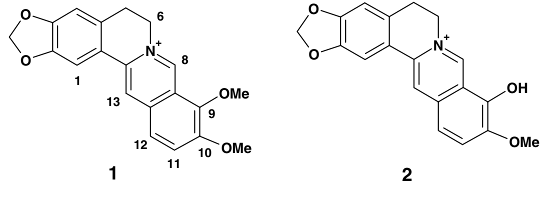
Abstract—By introducing various aromatic groups in 13-C of berberine and berberrubine, a series of 13-(substituted benzyl) berberine and berberrubine derivatives were synthesized and examined for antifungal activities against various human pathogenic fungi. The synthesized compounds exhibited more potent antifungal activities than berberine and berberrubine. Among them, 13-(4-isopropyl benzyl) berberine (**6e**) exerted the most potent antifungal activities against *Candida* species (MIC = 1–8 μg/ml) and a 4-fold stronger activity than 13-(4-isopropyl benzyl) berberrubine (**7e**) synthesized by pyrolysis of compound **6e**.

During the past two decades, life-threatening fungal infections are frequently emerging through opportunistic infections with the advent of cancer chemotherapy, organ transplantation or HIV.[1,2] Most of systemic fungal infections are mainly caused by opportunistic pathogens such as *Candida* and *Aspergillus* species.[3,4] Although many researchers have carried out intensive studies in attempts to develop new antifungal agents and some drugs are under clinical trials, amphotericin B and the azole compounds remain the mainstay of fungal systemic infection therapy.[5] However, the adverse effects of polyenes and the emergence of *Candida* strains resistant to commercial azole compounds make the treatment of patients with deeply invasive mycoses difficult.[6] Therefore, demand for the development of new antifungal agents which have low side effects and broad-spectrum activity against various fungi is greater than ever before.

In these aspects, berberine (**1**), a representative protoberberine of isoquinoline alkaloid, is attractive as a lead compound for the development of antifungal agents, because berberine, isolated from Korean and Chinese medicinal plants, significantly inhibits the growth of various *Candida* species.[7,8] In addition, berberrubine (**2**), isolated from the plant *Berberis vulgaris* L.[9] and readily derived from berberine by pyrolysis,[10,11] exhibits a broad range of pharmacological activities (Fig. 1).[12] Recently, to develop the compounds with an enhanced antifungal activity from berberine, 13-(4-*tert*-butyl benzyl) berberine was synthesized and showed potent activity.[13]

In this study, berberine derivatives were synthesized by means of the introduction of various aromatic groups in the 13-C to improve the antifungal activity and all the synthesized compounds were demethylated at 9-O position to give novel berberrubine derivatives.

For the synthesis of 13-(substituted benzyl) berberines and berberrubines, acetonyl-berberine (**3**) used as a key intermediate was obtained by condensation of



1 **2**

Figure 1. Structure of berberine (**1**) and berberrubine (**2**).

Keywords: Antifungal activity; 13-(Substituted benzyl) berberine; 13-(Substituted benzyl) berberrubine.

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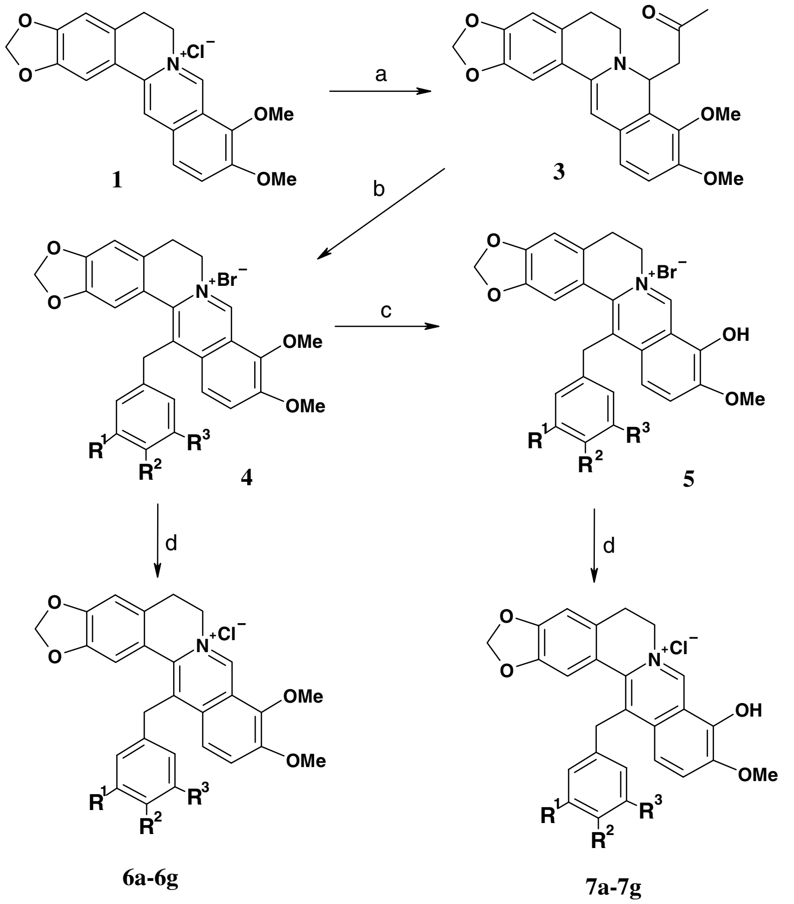
berberine with acetone. The treatment of **3** with various benzyl bromides and sodium iodide (NaI) gave 13-(substituted benzyl) berberine bromides (**4**) by displacement of the acetonyl group with various benzyl groups. The pyrolysis of 13-(substituted benzyl) berberine bromides (**4**) was carried out in DMF with high temperature to give 13-(substituted benzyl) berberrubine bromides (**5**).[14] These bromides were converted into yellow-orange crystalline chlorides (**6** and **7**) using AgCl in hot MeOH (Scheme 1).

All of the above compounds were investigated for in vitro antifungal activity against various *Candida* species, *Aspergillus* species, and *Cryptococcus neoformans* according to the broth microdilution method of the NCCLS.[15,16] The MIC values were determined as the lowest concentration of the agent that inhibited growth up to 90% after incubation for 48 and 72 h.

The antifungal activities of the compounds against human pathogenic fungi are summarized in Table 1. These results indicate that the chemical modifications of the natural product, berberine and berberrubine, such as introduction of various aromatic groups at 13-position, can remarkably improve the antifungal activity. This modification is likely to make the compounds more lipophilic, which may increase the permeability of the cell membrane. As a result, 13-(substituted benzyl) berberine bromides (**4**) showed same activity as 13-(substituted benzyl) berberine chlorides (**6**) (data not shown) and berberine derivatives (**6a–6g**) exhibited more potent activity than berberrubine derivatives (**7a–7g**) against *Candida* and *Aspergillus*. Since compounds **6a–6g** only differ structurally from compounds **7a–7g** by the presence of a methyl group on 9-hydroxyl, the methyl group is perceived to be important for the enhanced antifungal activity. However, it remains to be further investigated whether structural change of the methyl group is effective to generate more potent antifungal agents. On the other hand, berberrubine derivatives showed similar activity to berberine derivatives against *C. neoformans*. The most significant structural change leading to enhanced activity was the introduction of benzyl group including lipophilic substituents at the *para*-position. The high potency shown by derivatives **6d–6e** supported the importance of lipophilic substituents of the benzyl group on 13-C of berberine. Among them, 13-(4-*tert*-butyl benzyl) berberine (**6d**)[17] and 13-(4-isopropyl benzyl) berberine (**6e**)[17] exerted the most potent antifungal activities with MIC values of 1–8 μg/ml against various *Candida* species, and 4-fold stronger antifungal activities than 13-(4-trifluoromethyl benzyl) berberine (**6f**) and 13-(4-trifluoromethoxy benzyl) berberine (**6g**). These results indicated that the derivatives (**6f** and **6g**) bearing electron-withdrawing group, such as tri-



Scheme 1. Reagents and conditions: (a) acetone, 5 N NaOH, rt, 1 h; (b) various benzyl bromide, NaI, CH_3CN, 80 °C, 4 h; (c) DMF, reflux, 190 °C, 2 h; (d) AgCl (1 equiv), MeOH, 60 °C, 2 h.

Table 1. Structures and in vitro antifungal activity for 13-(substituted benzyl) berberine derivatives (**6**) and berberrubine derivatives (**7**)

Compound	R^1	R^2	R^3	MIC[a] (μg/ml)						
				C. albicans[b]	*C. tropicalis*	*C. lusitaniae*	*C. krusei*	*A. fumigatus*	*A. terreus*	*C. neoformans*
1				128	16	>128	32	>128	>128	64
2				>128	128	>128	>128	>128	>128	>128
6a	OMe	OMe	OMe	128	64	128	64	>128	>128	32
6b	F	F	F	128	64	128	64	>128	>128	64
6c	F	H	F	128	32	64	64	>128	>128	32
6d	H	$C(CH_3)_3$	H	4	2	8	8	64	64	4
6e	H	$CH(CH_3)_2$	H	4	1	8	4	64	32	4
6f	H	CF_3	H	32	16	32	32	>128	>128	16
6g	H	OCF_3	H	16	8	32	16	>128	>128	16
7a	OMe	OMe	OMe	>128	128	128	>128	>128	>128	32
7b	F	F	F	>128	128	128	128	>128	>128	64
7c	F	H	F	>128	128	128	128	>128	>128	32
7d	H	$C(CH_3)_3$	H	16	16	8	32	>128	>128	8
7e	H	$CH(CH_3)_2$	H	16	16	8	16	>128	>128	4
7f	H	CF_3	H	128	64	64	64	>128	>128	16
7g	H	OCF_3	H	64	32	32	32	>128	>128	16
AmpB				0.5	0.5	1	0.5	1	16	0.25

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

[b] Tested 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.

fluoromethyl and trifluoromethoxy, at the *para*-position were less active than the derivatives (**6d** and **6e**) bearing bulky hydrocarbon group, such as *tert*-butyl and isopropyl. In addition, the berberrubine derivatives (**7d**–**7e**) showed similar tendencies as berberine derivatives (**6d**–**6e**).

In summary, a series of 13-(substituted benzyl) berberine and berberrubine derivatives has been established as a new class of antifungal agents. Structural features as mentioned above can provide useful information for synthesizing berberine and berberrubine derivatives with an enhanced activity in the future.

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14. Berberine chloride was purchased from Sigma–Aldrich and used without further purification. Berberine chloride (5 g) was dissolved in 5 N NaOH (23 ml). While stirring, acetone (5 ml) was added dropwise. After stirring for 1 h at room temperature, the reaction mixture was filtered and washed with 80% MeOH to give 4.15 g of **3** (83% yield). Compound **3** (4 g) dissolved in acetonitrile was reacted with NaI (1.87 g) and various benzyl bromide (1.5 equiv) at 80 °C for 4 h. The reaction mixture was concentrated and chromatographed on silica gel ($CHCl_3$/MeOH = 12:1) to give compound **4** (44–52% yields). The pyrolysis of compound **4** was carried out with high temperature (190 °C) in DMF. The reaction mixture was evaporated and purified by column chromatography on silica gel ($CHCl_3$/MeOH = 9:1) to give compound **5** (72–76% yields).
15. NCCLS, reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-second edition, M27-A2, National Committee for Clinical Laboratory Standards, Villanova, PA, 2002.
16. NCCLS, reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, M38-A, National Committee for Clinical Laboratory Standards, Villanova, PA, 2002.

17. Spectral data for compound **6d**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.6 (s, 1H), 7.34 (s, 1H), 7.31 (s, 1H), 7.18 (d, 1H), 7.08 (s, 1H), 7.06 (s, 1H), 6.98 (s, 1H), 6.78 (s, 1H), 6.43 (d, 1H), 5.97 (s, 2H), 4.42 (t, 2H), 4.40 (s, 2H) 3.91 (s, 3H) 3.88 (s, 3H) 3.03 (t, 2H) 1.31 (s, 9H). MS (EI) *m*/*z*: 336 (M$^+$). Compound **6e**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.5 (s, 1H), 7.31 (s, 1H), 7.29 (s, 1H), 7.17 (d, 1H), 7.04 (s, 1H), 7.02 (s, 1H), 6.93 (s, 1H), 6.73 (s, 1H), 6.39 (d, 1H), 5.94 (s, 2H), 4.41 (t, 2H), 4.38 (s, 2H) 3.89 (s, 3H) 3.85 (s, 3H) 3.01 (t, 2H) 2.80 (m, 1H) 1.29 (s, 6H). MS (EI) *m*/*z*: 322 (M$^+$).