Antimicrobial Activity of 9-*O*-Acyl- and 9-*O*-Alkylberberrubine Derivatives

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Abstract

For the structure-activity relationship study on berberrubine derivatives, a series of compounds bearing 9-*O*-acyl- and 9-*O*-alkyl-substituents were synthesized and tested for antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi. Octanoyl, decanoyl, lauroyl derivatives among the acyl analogs and hexyl, heptyl, octyl, nonyl, decyl, undecyl derivatives among the alkyl analogs showed strong antimicrobial activity against Gram-positive bacteria and fungi. As a whole, alkyl analogs were more active than acyl analogs for antimicrobial activity. Synthesized derivatives had no activity on Gram-negative bacteria. Too short or too long substituents decreased activity. These results suggest that the presence of lipophilic substituents with moderate sizes might be crucial for the optimal antimicrobial activity.

Berberine, isolated from a variety of plants [1], is widely used in Asia as a drug due to its antimicrobial activity [2–7]. Berberrubine is an isoquinoline alkaloid isolated from the plant *Berberis vulgaris* L. [8] and readily derived from berberine by pyrolysis [9].

In a previous paper, we reported that berberrubine showed a potent activity as a DNA topoisomerase II inhibitor [10], however, despite much resemblance in chemical structure, other protoberberine alkaloids such as berberine and palmatine [11] did not act on topoisomerase II. These results forced us to study the correlation between modified structure and biological activities. Recently, we reported that 9-*O*-lauroylberberrubine derivatives displayed active antibacterial potency against Gram-positive bacteria [12].

In the present study, the effects of variations in the length of the acyl side-chain at 9-*O*-position of berberrubine on the antimicrobial activity were examined. Also, antimicrobial activity of 9-*O*-alkylberberrubine derivatives with C_2-C_{18} alkyl chains were tested against representative strains of Gram-positive, Gram-negative bacteria and fungal microorganisms by 2-fold dilution methods. Acyl and alkyl derivatives were synthesized as present-

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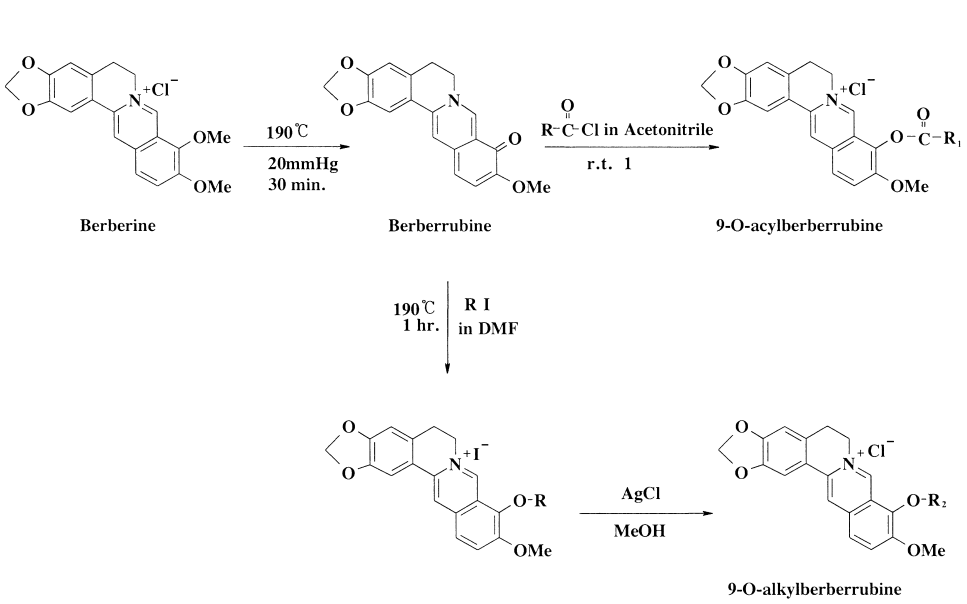


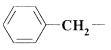
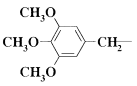
R_1	R_2
3. CH_3-	15. CH_3CH_2-
4. CH_3CH_2-	16. $CH_3\ CH_2\ CH_2$-
5. $CH_3\ CH_2\ CH_2$-	17. $CH_3\ (CH_2)_2CH_2$-
6. $CH_3\ (CH_2)_2CH_2$-	18. $CH_3\ (CH_2)_3CH_2$-
7. $CH_3\ (CH_2)_3CH_2$-	19. $CH_3\ (CH_2)_4CH_2$-
8. $CH_3\ (CH_2)_5CH_2$-	20. $CH_3\ (CH_2)_5CH_2$-
9. $CH_3\ (CH_2)_7CH_2$-	21. $CH_3\ (CH_2)_6CH_2$-
10. $CH_3\ (CH_2)_9CH_2$-	22. $CH_3\ (CH_2)_7CH_2$-
11. $CH_3\ (CH_2)_{11}CH_2$-	23. $CH_3\ (CH_2)_8CH_2$-
12. $CH_3\ (CH_2)_{13}CH_2$-	24. $CH_3\ (CH_2)_9CH_2$-
13. 	25. $CH_3\ (CH_2)_{10}CH_2$-
	26. $CH_3\ (CH_2)_{13}CH_2$-
	27. $CH_3\ (CH_2)_{16}CH_2$-
14. 	

Fig. **1** Synthesis of berberrubine (**2**), 9-*O*-acylberberrubines (**3 – 14**) and 9-*O*-alkylberberrubines (**15 – 27**).

Table **1** ^{1}H-NMR[a] data and mass spectra of 9-*O*-acyl-substituted derivatives of berberrubine (**8 – 10**)

	$CH_3(CH_2)_nCH_2$-CO- at C9-O													*ESIMS[b]*	
	CH_3-	*-CH_2-*	*-CH_2-CO-*	*H-5*	*10-OMe*	*H-6*	*OCH_2O*	*H-4*	*H-1*	*H-11*	*H-12*	*H-13*	*H-8*	*formula*	*m/z [M – Cl]$^+$*
8	0.91	1.25 ~ 1.42 (m, 8H)	2.85	3.23	4.03	4.94	6.18	7.10	7.81	8.21	8.28	9.05	9.93	$C_{27}H_{30}NO_5$	448
	(t, 3H)	1.76 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(2, H)	(d, H)	(d, 1H)	(s, 1H)	(s, 1H)		
9	0.89	1.23 ~ 1.42 (m, 12H)	2.85	3.22	4.02	4.93	6.18	7.10	7.81	8.19	8.29	9.03	9.87	$C_{29}H_{34}NO_5$	476
	(t, 3H)	1.74 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, H)	(d, H)	(d, 1H)	(s, 1H)	(s, 1H)		
10	0.88	1.22 ~ 1.42 (m, 16H)	2.86	3.23	4.03	4.94	6.18	7.10	7.82	8.22	8.29	9.03	9.92	$C_{31}H_{38}NO_5$	504
	(t, 3H)	1.74 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, H)	(d, H)	(d, 1H)	(s, 1H)	(s, 1H)		

[a] ^{1}H-NMR spectra were run in DMSO-d_6 on a Varian Mercury-300 MHz (δ from TMS).
[b] Mass spectra were determined on Fisons-VG platform in the positive ESI (electron spray ionization) mode.

Table **2** ^{1}H-NMR[a] data and mass spectra of 9-*O*-alkyl-substituted derivatives of berberrubine (**19 – 24**)

	$CH_3(CH_2)_nCH_2$- at C9-O													*ESIMS[b]*	
	CH_3-	*-CH_2-*	*-CH_2-CO-*	*H-5*	*10-OMe*	*H-6*	*OCH_2O*	*H-4*	*H-1*	*H-11*	*H-12*	*H-13*	*H-8*	*formula*	*m/z [M – Cl]$^+$*
19	0.99	1.29 ~ 1.45 (m, 6H)	4.28	3.23	4.05	4.94	6.18	7.09	7.80	8.01	8.18	8.93	9.74	$C_{25}H_{28}NO_4$	406
	(t, 3H)	1.85 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, 1H)	(d, 1H)	(d, 1H)	(s, 1H)	(s, 1H)		
20	0.96	1.24 ~ 1.37 (m, 8H)	4.28	3.21	4.05	4.94	6.17	7.10	7.80	7.98	8.19	8.92	9.76	$C_{26}H_{30}NO_4$	420
	(t, 3H)	1.74 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, 1H)	(d, 1H)	(d, 1H)	(s, 1H)	(s, 1H)		
21	0.96	1.28 ~ 1.41 (m, 10H)	4.28	3.21	4.07	4.96	6.17	7.10	7.80	7.98	8.19	8.92	9.74	$C_{27}H_{32}NO_4$	434
	(t, 3H)	1.84 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, 1H)	(d, 1H)	(d, 1H)	(s, 1H)	(s, 1H)		
22	0.96	1.27 ~ 1.47 (m, 12H)	4.28	3.21	4.05	4.96	6.17	7.09	7.80	7.98	8.19	8.92	9.74	$C_{28}N_{34}NO_4$	448
	(t, 3H)	1.85 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, 1H)	(d, 1H)	(d, 1H)	(s, 1H)	(s, 1H)		
23	0.89	1.24 ~ 1.47 (m, 14H)	4.28	3.21	4.05	4.96	6.17	7.09	7.80	7.98	8.19	8.92	9.74	$C_{29}N_{36}NO_4$	462
	(t, 3H)	1.86 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H)	(t, 2H)	(s, 2H)	(s, 1H)	(s, 1H)	(d, 1H)	(d, 1H)	(s, 1H)	(s, 1H)		
24	0.85	1.20 ~ 1.48 (m, 16H)	4.26	3.21	4.06	4.96	6.17	7.02	7.80	7.98	8.19	8.92	9.72	$CH_{38}NO_{430}$	476
	(t, 3H)	1.88 (m, 2H)	(t, 2H)	(t, 2H)	(s, 3H9	(t, 2H)	(s, 2H)	(s, 1H)	(s, 1H)	(d, 1H)	(d, 1H)	(s, 1H)	(s, 1H)		

[a] ^{1}H-NMR spectra were run in DMSO-d_6 on a Varian Mercury-300 MHz (δ from TMS).
[b] Mass spectra were determined on Fisons-VG platform in the positive ESI (electron spray ionization) mode.

Table **3** Antibacterial activities of berberine (**1**), berberrubine (**2**), 9-*O*-acyl (**3 – 14**), and 9-*O*-alkyl (**15 – 27**) berberrubine derivatives

Test organisms	*MIC (μg/ml)*													
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***
Enterococcus faecalis ATCC 29212	128	>128	>128	>128	>128	>128	128	32	32	32	>128	>128	>128	>128
Staphylococcus aureus ATCC 25923	128	>128	>128	>128	>128	>128	128	8	4	4	>128	>128	>128	>128
Micrococcus luteus ATCC 10240	32	64	64	64	64	64	64	16	4	1	2	4	16	64
Staphylococcus epidermidis ATCC 0155	>128	>128	>128	>128	>128	>128	>128	64	4	4	32	>128	>128	>128
Bacillus subtilis ATCC 6644	>128	>128	>128	>128	>128	>128	>128	>128	8	8	>128	>128	>128	>128
Escherichia coli ATCC 25922	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Escherichia coli ATCC 10536	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Proteus mirabilis ATCC 27853	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Pseudomonas aeurginosa ATCC 27853	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

	15	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***23***	***24***	***25***	***26***	***27***	***km***
Enterococcus faecalis ATCC 29212	128	128	64	64	8	8	2	2	2	8	64	128	>128	4
Staphylococcus aureus ATCC 25923	128	64	32	32	8	4	2	2	2	8	64	<128	<128	8
Micrococcus luteus ATCC 10240	8	4	4	1	0.5	0.125	0.125	0.125	0.125	0.5	0.5	4	32	2
Staphylococcus epidermidis ATCC 0155	64	64	8	8	2	2	1	0.5	0.5	1	1	64	128	0.5
Bacillus subtilis ATCC 6644	128	64	32	32	8	4	2	2	2	8	64	<128	<128	2
Escherichia coli ATCC 25922	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64
Escherichia coli ATCC 10536	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	8
Proteus mirabilis ATCC 27853	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128
Pseudomonas aeruginosa ATCC 27853	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

Determined after 24 hours of incubation at 37 °C for the bacteria.
All experiments were run in triplicates.
km = kanamycin sulfate.

ed in Fig. **1** for investigation of activity against Gram-positive (*Enterococcus faecalis, Staphylococcus aureus, Micrococcus luteus, Staphylococcus epidermidis and Bacillus subtilis*) and Gram-negative (*Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa*) bacteria as well as the fungi (*Candida krusei, Candida lusitaniae, Candida albicans and Cryptococcus neoformans*).

The structures of the new acyl and alkyl derivatives (**3 – 27**) were determined by NMR and MS experiments. Full signal assignment of ^{1}H and ^{13}C was carried out with various NMR techniques including DEPT, COSY, H,C-COSY, and long-range H,C-COSY. The complete assignment of ^{1}H-NMR chemical shifts and mass spectra for berberrubine derivative chlorides with antimicrobial activity are described in Table **1** and Table **2**.

The biological activities of the compounds against a panel of microorganisms are summarized in Tables **3** and **4**. The antibacterial activities of synthesized compounds (**3 – 27**) were compared with those of berberine (**1**), berberrubine (**2**) and kanamycin sulfate (**28**) as a positive control. The activities of the acyl- and alkylberberrubine derivatives against Gram-positive bacteria were increased as the length of the chain of the substituents increased. The change in lipophilicity of the protoberberinium salts caused by substituents influenced the antibacterial activity of derivatives.

Table **4** Antifungal activities of berberine (**1**), berberrubine (**2**), 9-*O*-acyl (**3 – 14**), and 9-*O*-alkyl (**15 – 27**) berberrubine derivatives.

Test organisms	*MIC (μg/ml)*													
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***
Candida krusei IFO 1664	32	>128	>128	>128	>128	>128	128	32	8	64	>128	>128	>128	>128
Candida lusitaniae ATCC 42720	>128	>128	>128	>128	>128	>128	128	16	2	>128	>128	>128	>128	>128
Candida albicans ATCC 10231	128	>128	>128	>128	>128	>128	128	32	2	4	>128	>128	>128	>128
Candida tropicalis IFO 10241	16	>128	>128	>128	>128	>128	128	32	4	4	64	>128	>128	>128
Cryptococcus neoformans ATCC 2344	8	>128	>128	>128	128	128	128	16	2	2	4	32	>128	>128

	15	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***23***	***24***	***25***	***26***	***27***	***AmpB***
Candida krusei IFO 1664	64	32	32	32	8	4	1	1	1	4	16	64	>128	0.5
Candida lusitaniae ATCC 42720	>128	32	16	16	4	2	1	1	1	4	16	64	>128	0.5
Candida albicans ATCC 10231	>128	64	64	64	8	4	2	1	1	4	16	64	>128	0.125
Candida tropicalis IFO 10241	128	32	16	16	4	2	1	1	2	8	16	32	>128	0.25
Cryptococcus neoformans ATCC 2344	16	32	16	16	1	1	0.5	0.5	1	4	8	32	64	0.125

Determined after 24 – 72 hours of incubation at 28 – 30 °C for the fungi.
All experiments were run in triplicates.
AmpB = Amphotericin B.

However, longer chains than lauroyl in acyl analogs and dodecyl in alkyl homologs decreased the antibacterial activity. Among the acyl derivatives, octanoyl (**8**), decanoyl (**9**) and lauroyl (**10**) berberrubine chlorides showed moderate activity. Among the alkyl derivatives, hexyl (**19**), heptyl (**20**), octyl (**21**), nonyl (**22**), decyl (**23**) and undecyl (**24**) exhibited strong antibacterial potency against the tested organisms. As a whole, 9-*O*-alkylberberrubine analogs were more active than 9-*O*-acylberberrubine analogs or the parent molecule, berberine and berberrubine, against Gram-positive bacteria. For Gram-negative bacteria, the synthesized compounds had little antibacterial activity. Additionally, antifungal activity of derivatives was compared with berberine (**1**) and berberrubine (**2**), starting material, as well as amphotericin B as a positive control. Similar results in antifungal activity were displayed as in antibacterial activity against Gram-positive bacteria.

As a result, 9-*O*-alkylberberrubine derivatives with C_6 – C_{11} alkyl chains (**19 – 24**) had potent antimicrobial activity and potential as new antimicrobial agents.

Materials and Methods

Berberine (**1**) is commercially available. Berberrubine (**2**) was synthesized from berberine [12]. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Mercury (300 MHz) using TMS as a internal standard and DMSO-d_6 as a solvent. The mass spectra were taken by a Fisons-VG platform in the positive ESI mode.

9-O-Acylberberrubine chlorides (**3 – 14**): Dried berberrubine (0.714 g) in acetonitrile (25 ml) was reacted with acetyl (0.17 ml), propionyl (0.18 m), butyryl (0.19 ml), valeryl (0.21 ml), hexanoyl (0.23 ml), octanoyl (0.31 ml), decanoyl (0.41 ml), lauroyl (0.51 ml), myristoyl (0.55 ml), palmitoyl (0.61 ml), benzoyl (0.31 ml) and 3,4,5-trimethoxybenzoyl (0.52 g) chlorides at room temperature for 1 h [13]. The mixture was diluted with diethyl ether (50 ml). The resulting crystals were filtered and washed with ether. Chromatography with $CHCl_3$: MeOH (4 : 1) on a silica gel column gave the corresponding 9-*O*-acylberberrubine chlorides.

9-O-Alkylberberrubine chlorides (**15 – 27**): Dried berberrubine (0.714 g) in DMF (100 ml) was reacted with ethyl (0.312 g), propyl (0.340 g), butyl (0.368 g), pentyl (0.396 g), hexyl (0.424 g), heptyl (0.452 g), octyl (0.480 g), nonyl (0.508 g), decyl (0.536 g), undecyl (0.564 g), dodecyl (0.592 g), hexadecyl (0.704 g), octadecyl (0.816 g) iodide at 120 °C for 5 h [9]. Evaporation and chromatography with $CHCl_3$: MeOH (4 : 1) on a silica gel column gave the corresponding 9-*O*-alkylberberrubine iodides. These iodides were converted into yellow-orange crystalline chlorides with AgCl in hot MeOH [4].

The test compound was dissolved in H_2O containing 2.5 % DMSO as a negative control and its antibacterial activity was measured by the broth dilution method in 96-well titer plates. After incubation for 24 h, the microbial growth was examined by measuring the optical density at 650 nm with a Model Emax Microplate

Reader (Molecular Devices) [9]. The concentration of compound were examined in the range of 0.125 – 128 μg/ml. The MIC of the test compound was defined as the lowest concentration at which there was no visible growth. Antifungal activities of the test compounds were examined by means of the broth dilution method in Sabouraud medium for fungi [14]. The concentration of compound were examined in the range of 0.125 – 128 μg/ml.

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