Antimicrobial Activity of 9-*O*-Acyl- and 9-*O*-Benzoyl-Substituted Berberrubines

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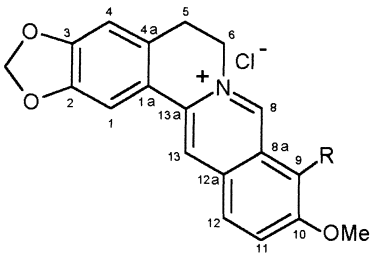
Abstract: **In the course of a structure-activity relationship study on berberrubine derivatives, a series of compounds bearing 9-*O*-acyl- (4–6) and 9-*O*-benzoyl- (7) substituents was synthesized with the expectation of increasing the antimicrobial activity. One of the berberrubine derivatives, 9-lauroylberberrubine chloride was the most active against Gram-positive bacteria *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus subtilis* as well as the Gram-negative bacterium *Klebsiella pneumoniae* in comparison to berberine, the currently used antibiotic in clinic. This result suggested that the presence of lipophilic substituents of certain structures and sizes might be crucial for the optimal antimicrobial activity.**

Berberine, an alkaloid derived from the plant *Berberis aristata* L. (Berberidaceae), has been shown to possess some antibacterial action, local anaesthetic action, antiheparin, anticoagulant action and antitrachoma action (1–4). Berberrubine is 9-demethylberberine, an alkaloid isolated from *Berberis vulgaris* L., and is readily derived from berberine (5). Berberine had no antitumor activity, but berberrubine and the ester derivatives of berberrubine were reported to have a strong antitumor activity (6). We reported previously that berberrubine represents a new class of antitumor agent which exhibits the topoisomerase II poison activity as well as catalytic inhibition activity and may have a potential clinical value in cancer treatment (7). Furthermore, as shown in Fig. **1**, several berberrubine derivatives and new acyl derivatives (**5** and **6**) were synthesized to study the correlation between their structures and biological activities. In this paper, we report the syntheses and the antimicrobial activities of 9-*O*-acyl- and 9-*O*-benzoyl-berberrubine derivatives.

The structures of the new acyl derivatives (**5** and **6**) 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 and ^{13}C chemical shifts for 9-valeryl- (**5**) and 9-lauroylberberrubine (**6**) chlorides are described in Table **1**.



	R
1	OCH_3
2	O^-
3	OH
4	$OCOCH_3$
5	$OCO(CH_2)_3CH_3$
6	$OCO(CH_2)_{10}CH_3$
7	$OCOC_6H_5$

Fig. 1 The structures of berberine, berberrubine, and berberrubine derivatives.

Table 1 NMR data for 9-valeryl- (**5**) and 9-lauroylberberrubine chlorides (**6**).

No.	**5** ^{1}H	**6** ^{1}H	**5** ^{13}C	**6** ^{13}C
1	7.81	7.81	105.39	105.41
1a	–	–	130.66	130.66
2	–	–	149.36	149.38
3	–	–	149.76	149.75
4	7.10	7.09	108.28	108.28
4a	–	–	120.19	120.20
5	3.22	3.22	26.32	26.19
6	4.95	4.93	55.27	55.20
8	9.93	9.98	145.11	144.24
8a	–	–	137.89	137.88
9	–	–	147.49	147.49
9-OCO	–	–	170.38	170.39
9-Valeryl	2.87	2.87	32.88	33.22
or	1.73	1.73	26.32	24.29
9-Lauroyl	1.48	1.44	21.54	24.42
	0.97	1.23–	13.69	28.36
	–	0.84	–	28.44
	–	–	–	28.74
	–	–	–	28.78
	–	–	–	28.87
	–	–	–	28.99
	–	–	–	29.03
	–	–	–	13.99
10	–	–	150.15	150.15
10-OMe	4.03	4.02	57.17	57.15
11	8.20	8.21	126.47	126.49
12	8.28	8.28	125.72	125.70
12a	–	–	133.38	133.40
13	9.04	9.06	120.19	120.42
13a	–	–	136.37	136.37
OCH_2O	6.18	6.17	102.00	102.00

The biological activities of the compounds tested against a panel of microorganisms are summarized in Tables **2** and **3**. The antibacterial activities of compounds **3–7** were compared

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with those of berberine (**1**) and berberrubine (**2**). The activities of the 9-*O*-acyl- and 9-*O*-benzoylberberrubine derivatives against Gram-positive bacteria were increased as the length of the chain at the C-9 position increased. However, only 9-lauroylberberrubine chloride showed inhibitory activity against Gram-negative bacteria. Compounds **2**, **3**, and **4** have no activity at 128 μg/ml against any strains tested. Compounds **5** and **7** exhibited weaker activity than kanamycin sulfate against Gram-positive bacteria and proved to be inactive against Gram-negative bacteria (> 128 μg/ml). In particular, compound **6**, 9-lauroylberberrubine chloride, showed less inhibitory activity against Gram-positive bacteria by 1/2 – 1/8 than kanamycin sulfate, while it exerted stronger activity against the Gram-negative bacterium *K. pneumoniae* (MIC 8) than kanamycin sulfate (Table **2**).

The MICs of nystatin for some fungi and yeasts were determined and served as positive control for comparison with the experimental compounds. Compounds **3** and **6** exhibited weaker activity than nystatin against some fungi and yeasts, while compounds **2** and **5** showed inhibitory activity against some fungi only at high concentration (250 μg/ml). Among the compounds tested, berberrubine hydrochloride (**3**) exhibited the highest activity against *T. mentagrophytes*, *C. immitis*, and *C. neoformans* (Table **3**).

Materials and Methods

Berberine is commercially available. ^{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 solvent. The mass spectra were taken on a JEOL JMS HX 100 – 110A in the positive FAB mode.

Berberrubine (**2**): Berberine (3 g) with urea (6 g) was stirred at 200 °C for 30 min. Boiling water was added to the mixture followed by extraction with chloroform for 8 ~ 10 times. The organic layer was evaporated and chromatographed with $CHCl_3$: MeOH (4 : 1) on a silica gel column to give berberrubine, yield: 2.3 g (76 %).

Berberrubine hydrochloride (**3**): Berberrubine (0.56 g) in boiling water (22.4 ml) was treated with 35 % hydrochloride (3 ml). After cooling of the mixture at 4 °C, the resulting crystals were filtered. Drying of the filtrate gave berberrubine hydrochloride, yield: 0.43 g (70 %).

Table 2 Antibacterial activities of berberine, berberrubine, and berberrubine derivatives.

Test organisms	MIC (μg/ml)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	KM
Staphylococcus aureus subsp. *aureus* ATCC 25923	128	>128	>128	>128	>128	4	>128	1
Staphylococcus epidermidis ATCC 0155	64	>128	>128	>128	32	4	128	1
Enterococcus faecalis ATCC 29212	>128	>128	>128	>128	>128	32	>128	16
Micrococcus luteus ATCC 10240	128	>128	>128	>128	32	8	128	4
Bacillus subtilis ATCC 6633	128	>128	>128	>128	64	8	128	1
Escherichia coli ATCC 25922	>128	>128	>128	>128	>128	>128	>128	64
Escherichia coli ATCC 10536	>128	>128	>128	>128	>128	>128	>128	8
Proteus mirabilis ATCC 21100	>128	>128	>128	>128	>128	>128	>128	128
Pseudomonas aeruginosa ATCC 27853	>128	>128	>128	>128	>128	>128	>128	>128
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 10031	>128	>128	>128	>128	>128	8	>128	<128

Determined after 24 hours of incubation at 37 °C for the bacteria.
All experiments were run in triplicate.
KM = Kanamycin sulfate.

Table 3 Antifungal activities of berberubine and berberrubine derivatives.

Test organisms	MIC (μg/ml)				
	2	**3**	**5**	**6**	NYS
Candida lusitaniae ATCC 42720	500	>500	500	250	1.56
Candida krusei ATCC 6258	>500	>500	>500	>500	6.25
Candida albicans ATCC 10231	>500	>500	>500	500	3.125
Candida tropicalis ATCC 13803	>500	>500	500	500	3.125
Cryptococcus neoformans ATCC 36556	>500	62.5	250	250	1.56
Coccidioides immitis ATCC 34020	250	62.5	500	62.5	3.125
Trichophyton mentagrophytes ATCC 9533	250	250	500	250	25

Determined after 24 ~ 48 hours of incubation at 28 ~ 30 °C for the yeast and fungi.
NYS = Nystatin.

9-O-Acyl- and 9-O-benzoylberberrubine chlorides (**4 – 7**): Dried berberrubine (0.714 g) in acetonitrile (25 ml) was reacted with acetyl (0.17 ml), valeryl (0.21 ml), lauroyl (0.51 ml) and benzoyl (0.31 ml) chlorides at room temperature for 1 h (8). The mixture was diluted with diethyl ether (50 ml). The resulting crystals were filtered and washed with diethyl ether to give 9-*O*-acetyl (0.575 g, 72 %), 9-*O*-valeryl (0.626 g, 71 %), 9-*O*-lauroyl (0.318 g, 73 %) and 9-*O*-benzyol (0.575 g, 72 %) berberrubine chlorides. **5**: FAB-MS 406, HRFAB-MS: $C_{24}H_{24}NO_5$ calc 406.1654, found 406.1659. **6**: FAB-MS 504, HRFAB-MS: $C_{31}H_{38}NO_5$ calc 504.2750, found 504.2748.

The test compound was dissolved in H_2O containing 2.5 % DMSO 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 concentrations 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 agar dilution method in Sabouraud medium for fungi and yeasts (10). The concentrations of compound were examined in the range of 0.975 – 500 μg/ml.

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