Two New Pyrrolidine Alkaloids, Radicamines A and B, as Inhibitors of α-Glucosidase from *Lobelia chinensis* Lour.

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Two new pyrrolidine alkaloids, radicamines A and B were isolated as inhibitors of α-glucosidase from *Lobelia chinensis* Lour. (Campanulaceae). Radicamines A and B were formulated as (2*S*,3*S*,4*S*,5*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(3-hydroxy-4-methoxyphenyl)-pyrrolidine (1) and (2*S*,3*S*,4*S*,5*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(4-hydroxyphenyl)-pyrrolidine (2) on the basis of spectroscopic analyses and chemical methods.

Key words pyrrolidine alkaloid; α-glucosidase inhibitor; *Lobelia chinensis*; Campanulaceae; radicamine A; radicamine B

Several structurally related monocyclic and bicyclic polyhydroxy pyrrolidines have been reported to show various biological activities, such as competitive inhibition of glycosidases,[1—4] as antiviral agents,[5] and as acaricides.[6] In the course of our survey for biologically active constituents extracted from crude drugs with hot water, we have reported many pyrrolidine alkaloids, broussonetines from *Broussonetia kazinoki* Sieb. (Moraceae).[7—14] Our continuing search led us to isolate two new pyrrolidine alkaloid, radicamines A and B, from *Lobelia chinensis* Lour. (Campanulaceae). This report deals with the structural elucidation and its inhibitory activities against α-glucosidase of these new natural products.

The plant, *Lobelia chinensis* Lour. (Japanese name "azemushiro"), is distributed throughout China, Taiwan, Korea, and Japan. The whole plants have been used as a diuretic, an antidote, hemostat, and as carcinostatic agents for stomach cancer in Chinese folk medicine. Various alkaloids, for example lobeline, lobelanine, lobelanidine and so on are known as constituents of this herb.

The herb was extracted with hot water and the alkaloidal constituents were concentrated as described in the Experimental section. Compounds **1** and **2** were isolated by preparative HPLC (column : Asahipak NH_2P [i.d. 4.6×250 mm]; solvent: CH_3CN–H_2O [80 : 20]) of the concentrated alkaloid mixture.

Compound **1** was obtained as a pale yellow oil, $[\alpha]_D$ +43.7° (c=0.13, H_2O), showing a brownish spot on TLC when sprayed with ninhydrin reagent followed by heating on a hot plate (ninhydrin reaction). The molecular formula was determined to be $C_{12}H_{17}NO_5$ on the basis of positive high-resolution secondary ion mass spectroscopy (pos. HR-SI-MS) (m/z: 256.1188, $[M+H]^+$, error, +0.4 mmu). The IR spectrum showed a strong OH and NH band at 3386 cm^{-1} and benzenoid bands at 1596 and 1516 cm^{-1}.

The ^{1}H-NMR spectrum of **1** showed the presence of an oxymethyl group (δ 3.84 [3H, s]), an oxymethylene group (δ 3.67 [1H, dd, J=6.4, 11.6 Hz], δ 3.73 [1H, dd, J=4.3, 11.6 Hz]), two oxymethine groups (δ 3.93 [1H, t, J=7.3 Hz], δ 4.06 [1H, dd, J=7.3, 8.9 Hz]), two methine groups attached to a nitrogen atom (δ 3.24 [1H, m], δ 3.86 [1H, d, J=8.9 Hz]), and a 1, 3, 4-trisubstituted benzene ring (δ 6.94 [1H, br s], δ 6.93 [1H, dd, J=2.0, 8.6 Hz], δ 7.02 [1H, d, J=8.6 Hz]).

Partial structures A—C were obtained by tracing ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY) cross-peaks and they were connected on the basis of the heteronuclear multiple bond correlation (HMBC) spectrum and characteristic mass fragment ion (m/z 131) to establish the planar structure (Fig. 2).

The ^{1}H- and ^{13}C-NMR signals were reasonably assigned by heteronuclear single quantum coherence (HSQC), and distortionless enhancement by polarization transfer (DEPT), as shown in Table 1.

The relative stereochemistry of the pyrrolidine moiety in **1** was determined by the vicinal coupling constants ($J_{2,3}$=$J_{3,4}$= 7.3 Hz, $J_{4,5}$=8.9 Hz) and nuclear Overhauser effects (NOEs) [between H-2 and H-4, H-3 and H-5, O-Me and H-5′] in the

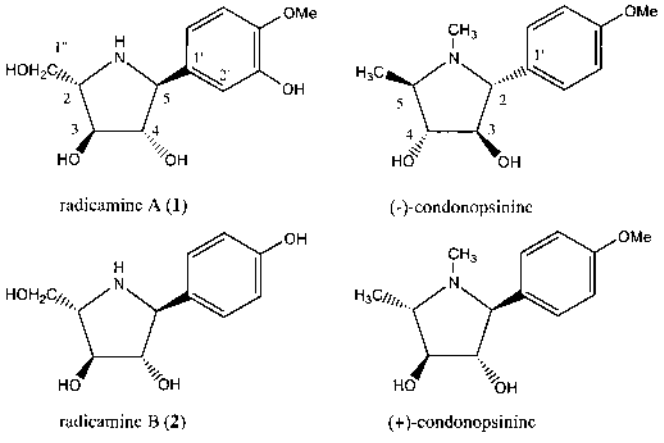

Fig. 1. Structures of **1**, **2**, (−)-Condonopsinine, and (+)-Condonopsinine

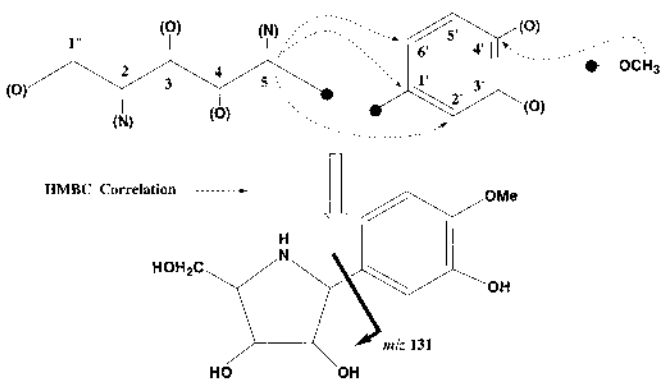

Fig. 2. The Planar Structure of **1**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** and **2**

	Radicamine A (**1**)		Radicamine B (**2**)	
	Proton	Carbon	Proton	Carbon
2	3.24 m	64.15	3.27 m	64.12
3	3.93[a)] t (7.3)	79.62	3.95[a)] t (7.6)	79.35
4	4.06 dd (7.3, 8.9)	84.16	4.12 dd (7.6, 9.0)	83.72
5	3.86[a)] d (8.9)	65.97	3.94[a)] d (9.0)	65.86
1′		134.90		131.78
2′	6.94 s	116.95	7.30 d (8.7)	131.68
3′		147.81	6.89 d (8.7)	118.34
4′		149.97		158.30
5′	7.02 d (8.6)	115.23	6.89 d (8.7)	118.34
6′	6.93 dd (2.0, 8.6)	122.35	7.30 d (8.7)	131.68
1″	3.67 dd (6.4, 11.6)	64.69	3.69 dd (6.4, 11.7)	64.38
	3.73 dd (4.3, 11.6)		3.75 dd (4.4, 11.7)	
O–Me	3.84 s	58.55		

a) Overlapped signal, ppm (Hz).

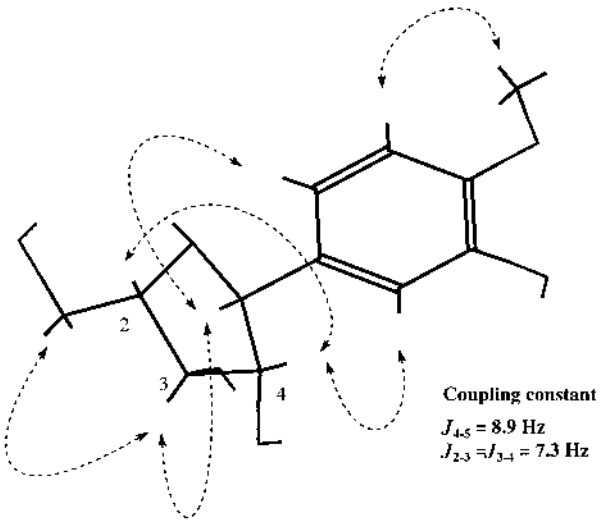

Fig. 3. NOEs of **1**

nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum to establish the structure as 2α-hydroxymethyl-3β,4α-dihydroxy-5β-(3-hydroxy-4-methoxyphenyl)-pyrrolidine (Fig. 3.).

Compound **2** was obtained as a pale yellow oil, $[\alpha]_D$ +72.0° (c=0.10, H_2O), showing a brownish spot on TLC by ninhydrin reaction. The molecular formula was determined to be $C_{11}H_{15}NO_4$ on the basis of pos. HR-SI-MS (*m/z*: 226.1073, $[M+H]^+$, error, −0.5 mmu). The IR spectrum showed a strong OH and NH band at 3402 cm^{-1} and benzenoid bands at 1615 and 1519 cm^{-1}.

The ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of **1**. The signals were assigned using ^{1}H–^{1}H COSY, HSQC, HMBC, and DEPT as shown in Table 1. These spectra suggested the presence of a 1,4-disubstituted benzene ring partial structure, instead of a 1,3,4-trisubstituted benzene ring.

The absolute configuration of the pyrrolidine moiety of **1** and **2** was derived from the $[\alpha]_D$ value by comparison with (+)-codonopsinine {$[\alpha]_D$ +12.5° (c=2.55, MeOH)} and (−)-codonopsinine {$[\alpha]_D$ −11.8° (c=0.69, MeOH)}.[15,16] *N*-Methyl-radicamines A (**1a**) and B (**2a**) were prepared from **1** and **2** by treatment with H_2, Pd–C, and HCHO in MeOH at room temperature. The $[\alpha]_D$ values of **1a** and **2a** were $[\alpha]_D$ +6.3° (c=0.80, MeOH) and $[\alpha]_D$ +8.3° (c=0.05, MeOH), respectively. Therefore, radicamines A and B were concluded to be (2*S*,3*S*,4*S*,5*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(3-hydroxy-4-methoxyphenyl)-pyrrolidine and (2*S*,3*S*,4*S*,5*S*)-2-hydroxymethyl-3,4-dihydroxy-5-(4-hydroxyphenyl)-pyrrolidine.

The absolute stereostructures were supported by the results of the benzoate chirality method, as follows.[17] A tribenzoate (**1d**) and two dibenzoate (**1b**, **1c**) were obtained by benzoylation of **1a** and purification of the products by preparative HPLC. The circular dichroism (CD) curve of **1b**, **1c**, and **1d**, are shown in Fig. 4. The difference CD curve between **1b**, **1c**, and **1d** showed a positive Cotton effect ($\Delta\varepsilon_{235\,nm}$ +7.9) and a negative Cotton effect ($\Delta\varepsilon_{218\,nm}$ −1.0) to establish the chiral arrangement in a clockwise manner.

The inhibitory activities of **1**, **2**, and (2*R*,3*R*,4*R*,5*R*) 2,5-dihydroxymethyl-3,4-dihydroxy-pyrrolidine (DMDP)[4] were assayed with respect to α-glucosidase. The IC_{50} values were found to be 6.7×10^{-6} M for **1**, 9.3×10^{-6} M for **2**, and 4.9×10^{-6} M for DMDP respectively. These two new compounds, polyhydroxy alkaloids having an aromatic ring, could be shown very interesting biological activities, similar to 1-deoxynojirimycin.[4] We will synthesize and survey related compounds.

Experimental

General The instruments used in this work were: a JASCO digital polarimeter (for specific rotation, measured at 25 °C), JASCO J-820 spectrometer (for CD measured at 25 °C), a Perkin-Elmer 1720X-FT-IR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); a Varian Mercury 300, unity Inova −500 (for NMR spectra measured in D_2O or CD_3OD, on the δ scale using tetramethylsilane as an internal standard) and a Shimadzu spectrophotometer UV 1200 (for enzyme assay).

Column chromatography was carried out on ion exchange resin (Amberlite CG-50, Amberlite IRA-67/Organo Company and Dowex 50W-X4/the Dow Chemical Company), and silica-gel (Chromatorex DM1020/Fuji Silysia Chemical Ltd.). HPLC was conducted with a Gilson 305 pump or a JASCO PU 980 equipped with a JASCO 830-RI or UV-970 as a detector. Silica gel 60 F254 (Merck) precoated TLC plates were used, developed with a $CHCl_3$–MeOH–AcOH–H_2O (20 : 10 : 7 : 5) solvent system, and detection was carried out by ninhydrin reagent followed by heating.

Isolation of 1 and 2 *Lobelia chinensis* Lour. (Campanulaceae) (0.6 kg, collected in Takatsuki City (Osaka) in 2000) were cut finely and then extracted with 50% MeOH (20 l) for 2 h. The extracted solution was chromatographed on an Amberlite CG-50 (H^+-form) column (i.d. 6.5×30 cm). After washing the column with water and 50% MeOH, the adsorbed material was eluted with 50% MeOH–28% ammonia solution (9 : 1). The eluted fraction was concentrated *in vacuo* to give a basic fraction (2.5 g). This fraction was chromatographed on a Dowex 50W-X4 column (200—400 mesh, i.d. 5.0×15 cm) pretreated with formic acid–ammonium formate buffer(0.2 M ammonia formate, adjusted to pH 5.7 with 1 N formic acid), with gradient elution (H_2O (1.0 l)→H_2O–28% ammonia solution (9 : 1, 1.0 l)). The fraction containing **1** and **2** was rechromatographed by HPLC (column : Shodex NH2-P (i.d. 4.6×250 mm); solvent: CH_3CN–H_2O (80 : 20), 1.0 ml/min; column temperature: ambient. **1** (15 mg) and **2** (1.2 mg) were finally obtained.

Radicamine A (**1**): Pale yellow oil, ninhydrin reaction: positive (a brownish spot on TLC), $[\alpha]_D$ +43.7° (c=0.13, H_2O), $C_{12}H_{17}NO_5$, pos. HR-SI-MS *m/z*: 256.1188 ($[M+H]^+$) error: 0.4 mmu, IR ν (KBr) cm^{-1}: 3386 (OH, NH), 1597, 1516 (benzen), UV λ_{max} nm (log ε): 228 (3.64), 281 (3.94), ^{1}H- and ^{13}C-NMR (D_2O): Table 1.

Radicamine B (**2**): Pale yellow oil, ninhydrin reaction: positive (a brownish spot on TLC), $[\alpha]_D$ +72.0° (c=0.10, H_2O), $C_{11}H_{15}NO_4$, pos. HR-SI-MS *m/z*: 226.1073 ($[M+H]^+$) error: −0.5 mmu, IR ν (KBr) cm^{-1}: 3402 (OH, NH), 1615, 1519 (benzen), UV λ_{max} nm (log ε): 227 (4.19), 277 (3.60), ^{1}H- and ^{13}C-NMR (D_2O): Table 1.

***N*-Methylation of 1 and 2** **1** (10 mg) and **2** (0.6 mg) were dissolved in MeOH (2 ml) and was treated with HCHO (1 ml), Pd–C, and H_2 at room temperature for 5 h, respectively and the reaction mixture was evaporated.

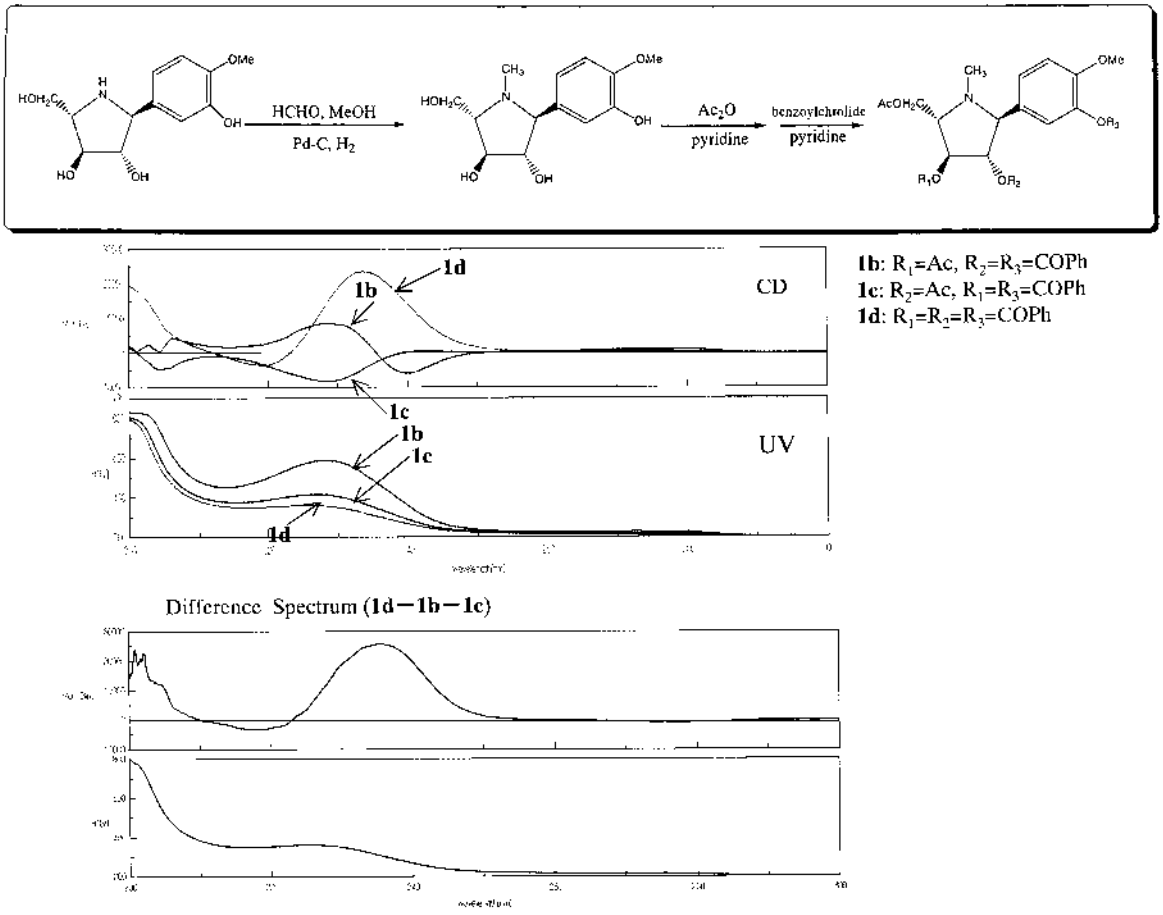

Fig. 4. CD Spectra of **1b**, **1c**, and **1d**

1a: pos. SI-MS *m/z* 270 $(M+H)^+$. ^{1}H-NMR (D_2O) δ: 2.60 (3H, s, N–$\underline{CH_3}$), 3.52 (1H, m, 2-H), 4.08 (3H, s, O–$\underline{CH_3}$), 4.18 (2H, d, J=5.9 Hz, 1″-H), 4.36* (2H, m, 3,5-H), 5.41 (1H, dd, J=5.5, 5.7 Hz, 4-H), 7.06—7.33 (3H, phenyl H). $[\alpha]_D$ +6.3° (c=0.80, MeOH).

2a: pos. SI-MS *m/z* 240 $(M+H)^+$. ^{1}H-NMR (D_2O) δ: 2.63 (3H, s, N–$\underline{CH_3}$), 3.55 (1H, m, 2-H), 4.22 (2H, d, J=5.9 Hz, 1″-H), 4.36* (2H, m, 3,5-H), 5.48 (1H, dd, J=5.5, 5.7 Hz, 4-H), 7.02 (2H, d, J=8.0 Hz, H-3′, 5′), 7.60 (2H, d, , J=8.0 Hz, H-2′, 6′). $[\alpha]_D$ +8.3° (c=0.05, MeOH).

Benzoate (1b—1d) **1a** (8 mg) was treated with acetic anhydride (20 mg) in pyridine at room temperature. After the usual work-up, the mixture was dissolved in pyridine (3 ml) and benzoylchloride (0.3 ml) was added, and the solution was stirred at room temperature for 12 h. The reaction products were subjected to HPLC [column, Crestpak C-18S (i.d. 4.6×250 mm); solvent, CH_3CN–H_2O (35 : 65→100 : 0 50 min); flow rate, 1.0 ml/min; detection, UV 254 nm; column temperature, 40 °C]. Dibenzoate (**1b**, **1c**) and tribenzoate (**1d**) were obtained as colorless oils (1.5 mg, 1.1 mg, 1.6 mg).

1b: pos. SI-MS *m/z* 562 $(M+H)^+$. ^{1}H-NMR (CD_3OD) δ: 2.13 (3H, s, CO$\underline{CH_3}$), 2.25 (3H, s, CO$\underline{CH_3}$), 2.34 (3H, s, N–$\underline{CH_3}$), 3.69 (1H, m, 2-H), 3.81 (3H, s, O–$\underline{CH_3}$), 4.17 (1H, d, J=5.5 Hz, 5-H), 4.55 (2H, m, 1″-H), 5.41 (1H, dd, J=3.0, 5.5 Hz, 4-H), 5.48 (1H, t, J=3.0 Hz, 3-H), 7.10—8.20 (13H, phenyl H). CD (c=1.95×10^{-4}, MeOH) $\Delta\varepsilon_{240\,nm}$ −1.8, $\Delta\varepsilon_{228\,nm}$ +2.6.

1c: pos. SI-MS *m/z* 562 $(M+H)^+$. ^{1}H-NMR (CD_3OD) δ: 1.98 (3H, s, CO$\underline{CH_3}$), 2.25 (3H, s, CO$\underline{CH_3}$), 2.34 (3H, s, N–$\underline{CH_3}$), 3.72 (1H, m, 2-H), 3.85 (3H, s, O–$\underline{CH_3}$), 4.20 (1H, d, J=5.5 Hz, 5-H), 4.60 (2H, m, 1″-H), 5.44 (1H, dd, J=3.0, 5.5 Hz, 4-H), 5.53 (1H, t, J=3.0 Hz, 3-H), 7.10—8.20 (13H, phenyl H). CD (c=1.60×10^{-4}, MeOH) $\Delta\varepsilon_{228\,nm}$ −2.5.

1d: pos. SI-MS *m/z* 624 $(M+H)^+$. ^{1}H-NMR (CD_3OD) δ: 2.25 (3H, s, CO$\underline{CH_3}$), 2.34 (3H, s, N–$\underline{CH_3}$), 3.80* (1H, 2-H), 3.84 (3H, s, O–$\underline{CH_3}$), 4.35 (1H, d, J=5.5 Hz, 5-H), 4.80* (2H, 1″-H), 5.50 (1H, dd, J=3.0, 5.5 Hz, 4-H), 5.65 (1H, t, J=3.0 Hz, 3-H), 7.10—8.20 (18H, phenyl H). CD (c=8.50×10^{-5}, MeOH) $\Delta\varepsilon_{233\,nm}$ +7.5, $\Delta\varepsilon_{219\,nm}$ −1.2. *: overlapped signals.

Assay of α-Glucosidase Inhibition The α-glucosidase activity was measured by the modified method of Dahlquist.[18] The reaction mixture consisted of the above basic extract solution (25 μl), 200 μl 50 mM phosphate buffer (pH 7.0), 175 μl 100 mM sucrose in 10 mM phosphate buffer (pH 7.0) and 100 μl α-glucosidase solution (A stock solution of 1.0 mg/ml in 10 mM phosphate buffer, pH 7.0 was diluted 40 times with the same buffer). The reaction mixture was incubated for 30 min at 37 °C. Then, to the incubated solution, 500 μl of an aqueous solution containing 1% 3,5-dinitrosalicylic acid, 5% sodium potassium tartate, 1% NaOH, 0.2% phenol and 0.05% sodium sulfite was added, and the mixture was heated at 100 °C for 10 min to stop the reaction. This solution was diluted with 2 ml water and the optical density at 540 nm was measured (ODtest). The control sample was prepared by adding water instead of the extract and by treating in the same way as test samples to give ODblank. The inhibition rates (%) were calculated from the formula 100−100×(ODtest−ODblank)/(control ODtest−control ODblank).

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