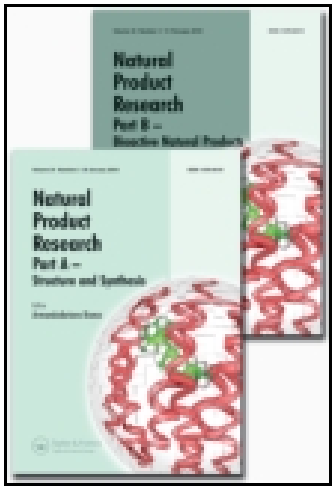

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Facile synthesis of tetrahydroprotoberberine and protoberberine alkaloids from protopines and study on their antibacterial activities

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Facile synthesis of tetrahydroprotoberberine and protoberberine alkaloids from protopines and study on their antibacterial activities

Pi Cheng[ab]*, Bin Wang[c], Xiubin Liu[a], Wei Liu[a], Weisong Kang[c], Jie Zhou[c] and Jianguo Zeng[abc]*

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A series of isoquinoline alkaloids including tetrahydroprotoberberines, *N*-methyl tetrahydroprotoberberines and protoberberines were facile synthesised with protopines as the starting material. All compounds were evaluated for their antibacterial activities against four pathogenic bacteria *Escherichia coli*, *Staphyloccocus aureus*, *Staphylococcus gallinarum* and *Salmonella choleraesuis*. Experimental results indicated that protoberberines were the most active compounds to the target bacteria among the tested alkaloids. It was suggested that planar molecule with high aromatisation level (e.g. coptisine **5** and berberine **6**) or a positive charge of the molecules (e.g. *N*-methyl tetrahydroprotoberberines **11** and **12**) had a positive influence on the antibacterial effects.

Keywords: protopines; isoquinoline alkaloids; synthesis; antibacterial activities

1. Introduction

The protopine alkaloids, for instance, protopine and allocryptopine, are a type of isoquinoline alkaloids mainly found in the plants of the Papaveraceae and Fumariaceae families and have been the focus of a number of biological studies such as anti-parasitic activity (Satou et al. 2002), anti-arrhythmic (Song et al. 2000), anti-thrombotic and hepatoprotective effects in animal models (Rathi et al. 2008).

Protopine alkaloids contain the unique structural feature of a nitrogen-containing 10-membered cyclic ketone. In biological systems, protopine alkaloids are considered to exist in equilibrium between their tricyclic bases and tetracyclic quaternary salts *N*-methyl-16-hydroxyl-tetrahydroprotoberberines (Vacek et al. 2010). In the metabolism pathway of protopines, (*S*)-reticuline that is derived from *L*-tyrosine underwent cyclisation, *N*-methylation and 14-hydroxylation to afford tetrahydroprotoberberine, *N*-methyl tetrahydroprotoberberine and *N*-methyl-16-hydroxyl-tetrahydroprotoberberine successively. *N*-methyl-16-hydroxyl-tetrahydroprotoberberines have been demonstrated to be the direct biosynthetic precursors of protopines (Zeng et al. 2013).

As the intermediates in the metabolism pathway of protopines, berbines (tetrahydroprotoberberine and *N*-methyl tetrahydroprotoberberine) were generally distributed in protopine-containing plants such as *Macleaya cordata,* a Papaveraceae berb medicine, with very low accumulation levels (Chen et al. 2009). In this study, the berbines and protoberberines were

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facile synthesised with protopine alkaloids as the starting material. To gain further insight into the pharmacological effects of alkaloids involved in the biosynthesis of protopines, all alkaloids were evaluated for their antibacterial activities against several animal pathogens.

2. Results and discussion

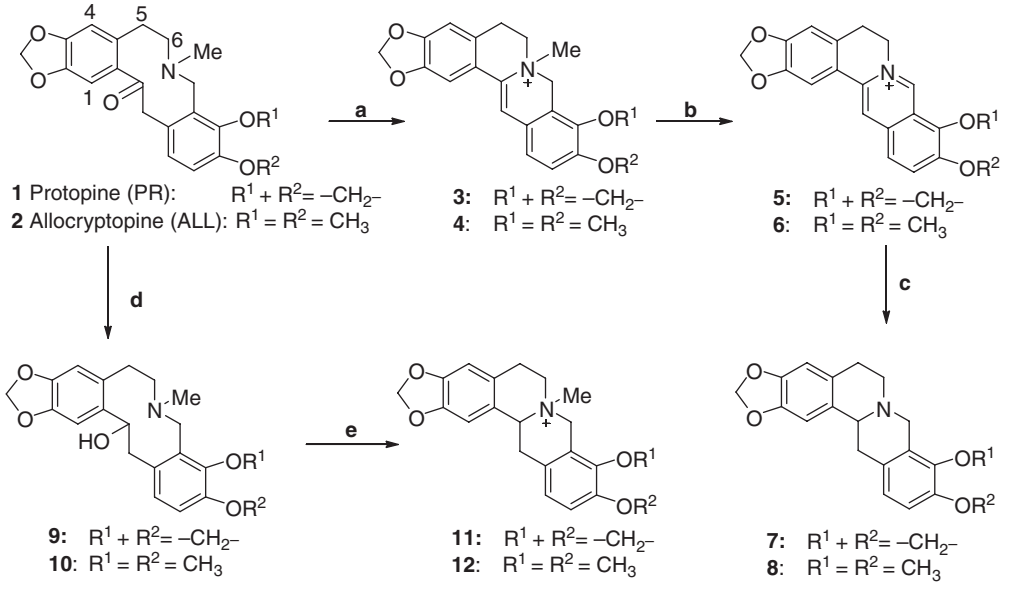
2.1. *Chemistry*

In protopines, the presence of nucleophilic amino group and electrophilic carbonyl group in the 10-membered ring generates a strong intramolecular interaction. As shown in Scheme 1, the reaction of protopine (**1**) or allocryptopine (**2**) with oxalyl chloride provided the *N*-methy-13,14-dehydroprotoberberine quaternary salts **3** and **4**, which contain four 6-membered ring system. A dimethyl sulfoxide (DMSO) solution of **3** or **4** was heated at 120°C to yield protoberberine salts coptisine (**5**) and berberine (**6**) as reported by Valpuesta et al. (2002). Coptisine and berberine were reduced by $NaBH_4$ to yield tetrahydroprotoberberine alkaloids stylopine (**7**) and canadine (**8**).

Dihydroprotopines (**9**, **10**) were obtained through an $NaBH_4$-mediated reduction of protopines, and then a reaction of compound **9** or **10** with CF_3COOH followed by treatment with HCl afforded *N*-methyl stylopine (**11**) and *N*-methyl canadine salts (**12**) successfully. With natural protopine-type alkaloids as starting materials, tetrahydroprotoberberine alkaloids (**7**, **8**) and their methylated salt (**11**, **12**) which were involved in the biosynthesis pathway of protopines were synthesised. Compounds **3** and **4** and hydroxyl derivatives **9** and **10** were unnatural and never existed in plants.

2.2. *Bioactivity and discussion*

Compounds **1–12** were evaluated for their antibacterial activities against four Gram-negative pathogenic bacteria: *Escherichia coli*, *Staphyloccocus aureus*, *Staphylococcus gallinarum* and



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, $CHCl_3$, reflux, 1 h; (b) DMSO, 120°C, 90 min; (c) $NaBH_4$ (10 equiv.), MeOH, room temperature, 24 h; (d) $NaBH_4$ (10 equiv.), MeOH, reflux, 48 h; (e) CF_3COOH, $CHCl_2$, room temperature 1.5 h, then HCl, MeOH.

Salmonella choleraesuis. Firstly, the Kirby-Bauer test (Kong & Jang 2008) was conducted to determine the sensitivity of the four selected bacteria to various alkaloids. The results are summarised in Table 1.

E. coli was resistant to the protopine-type alkaloids (**1, 2**) and their cyclisation salts (**3**, **4**) (inhibition zone < 10 mm). However, when the protopines were converted to the protoberberine-type alkaloids, berberine (**6**) exhibited increased inhibitory activity to *E. coli* (inhibition zone $= 15.8$ mm). For comparison, the tetrahydroprotoberberine-type (**7**, **8**), hydroxyl derivatives (**9**, **10**) and *N*-methyl tetrahydroprotoberberine salts (**11**, **12**) lost antibacterial activities. *S. aureus*, *S. gallinarum* and *S. choleraesuis* were moderately sensitive or resistant to naturally existed alkaloids **1**, **2** and their cyclisation salts **3**, **4**. *S. aureus*, *S. gallinarum* and *S. choleraesuis* showed higher sensitivity (inhibition zone = 30.6, 28.6 and 24.0 mm, respectively) to berberine **6** than that of *E. coli* and this is in accord with previous study (Cernakova & Kostalova 2002). Tetrahydroprotoberberine alkaloids **7** and **8** exhibited significantly decreased inhibitory activities to the bacteria compared with the aromatised protoberberine-type alkaloids **5** and **6**. But it was interesting to note that the *N*-methyl tetrahydroprotoberberine quaternary salts **11** and **12** showed moderate inhibitory activities to *S. aureus* and *S. gallinarum* compared to tetrahydroprotoberberines **7** and **8**. With respect to the positive control, all the four bacteria are moderately sensitive to Mequindox.

Minimum bactericidal concentration (MBC) values of all compounds were further determined for *E. coli*, *S. aureus*, *S. gallinarum* and *S. choleraesuis*. The results are listed in Table 2. For *E. coli*, all alkaloids showed a minimum inhibitory concentration (MIC) value above 100 $\mu g\,mL^{-1}$. The protoberberine-type salt berberine (**6**) displayed low MIC and MBC values against *S. aureus*, *S. gallinarum* and *S. choleraesuis*. This result is compatible with the data in Table 1. Coptisine (**5**) displayed better inhibitory activity against *S. choleraesuis* (MIC $= 52.5\ \mu g\,mL^{-1}$) compared to protopine derivative **1**. For *S. aureus* and *S. gallinarum*, the

Table 1. Kirby-Bauer antibacterial activities of **1** and **2** and their derivatives.

Compounds	Inhibition zone (mm)[a]			
	E. coli (CMCC44717)	*S. aureus* (CMCC56002)	*S. gallinarum* (NO2-2)	*S. choleraesuis* (CVCC504)
1	9.0	7.0	7.0	9.8
2	8.4	7.1	10.2	11.9
3	7.6	13.1	7.0	13.9
4	8.0	12.1	16.5	7.0
5	8.0	12.5	10.3	20.5
6	15.8	30.6	28.6	24.0
7	7.5	–[d]	–	7.0
8	8.0	7.0	–	7.0
9	7.5	–	–	7.0
10	7.4	7.1	7.0	7.0
11	8.0	15.0	15.3	6.4
12	7.9	18.9	19.3	7.0
Mequindox[b]	10.5	13.1	13.5	13.6
Control[c]	–	–	–	–

Note: All data are mean values of two separate experiments.
[a] Inhibition zone > 20 mm: sensitive; 20–10 mm: moderate sensitive; < 10 mm: resistant.
[b] Mequindox: an antibacterial agent used as positive control.
[c] Negative control: 20% DMSO.
[d] No inhibitory activity at the experimental conditions.

Table 2. MIC and MBC values ($\mu g\,mL^{-1}$) of **1** and **2** and synthetic compounds.

	E. coli (CMCC44717)		*S. aureus* (CMCC56002		*S. gallinarum* (NO2-2)		*S. choleraesuis* (CVCC504)	
Compounds	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	1000	>1000	500	1000	500	1000	500	500
2	500	1000	500	1000	250	1000	500	1000
3	1000	1000	>1000	>1000	>1000	>1000	1000	1000
4	500	1000	31.25	62.5	31.25	31.25	250	500
5	250	1000	250	500	500	500	62.5	62.5
6	250	500	15.63	31.25	15.63	15.63	62.5	125
7	>1000	>1000	>1000	>1000	>1000	>1000	500	1000
8	500	1000	>1000	>1000	>1000	>1000	250	500
9	500	1000	>1000	>1000	500	1000	250	500
10	500	1000	250	500	250	500	250	500
11	250	500	125	250	62.5	125	500	500
12	500	1000	31.25	62.5	31.25	62.5	250	250
Mequindox	62.5	125	31.25	62.5	31.25	62.5	7.8	15.63

Note: All data are mean values of two separated experiments.

antibacterial data of *N*-methyl tetrahydroprotoberberine quaternary salts **11** and **12** also exhibited good consonance with the Kirby-Bauer tests showed in Table 1, and this strongly suggests that *N*-methylation of tetrahydroprotoberberine will increase the antibacterial activities.

3. Experimental

3.1. *Chemistry*

Column chromatography silica gel (200–300 mesh) was used for compounds' purification; thin layer chromatography plate (Qingdao Meijin Chemical, Inc., Qingdao, China) was used for monitoring the chemical reactions; ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometers (Bruker Corporation, Germany) and chemical shifts were given in δ with tetramethylsilane as an internal reference. HRMS data were obtained on an Agilent UPLC-QTOF (6530) instrument (Agilent Technologies, USA). All the reagents are commercially available. Protopine and allocryptopine were isolated from *M. cordata*. All compounds were synthesised as reported in previous literatures with slight modifications (Valpuesta et al. 2002; Grycova et al. 2007), and the detailed procedure can be seen in the Supplementary material (available online).

Compound (**3**): obtained as pale yellow amorphous powder, isolated yield: 49.7%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.74 (s, 1H, H-1), 7.61 (s, 1H, H-13), 7.07 (d, 1H, $J = 8.6$ Hz, H-11), 7.05 (d, 1H, $J = 8.6$ Hz, H-12), 6.21, 6.11 (two brs, each 2H, 2 × OCH_2O), 5.08–5.00 (m, 2H, H-8), 4.23–4.18 (m, 1H, H-6), 4.02–3.89 (m, 1H, H-6′), 3.38–3.66 (m, 1H, overlapped, H-5), 3.13–3.09(m, 1H, overlapped, H-5′), 3.09 (s, 3H, NMe); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 149.5 (C-2), 149.2 (C-3), 148.3 (C-9), 144.9 (C-10), 135.2 (C-14), 126.6 (C-4a), 123.3 (C-12), 122.4 (C-12a), 118.9 (C-14a), 116.1 (C-13), 109.5 (C-1), 108.9 (C-11), 106.5 (C-8a), 103.7 (OCH_2O), 103.1 (C-4), 102.3 (OCH_2O), 60.7 (C-6), 59.6 (C-8), 45.9 (NMe), 23.9 (C-5); ESI-HRMS $[M]^+$ $C_{20}H_{18}NO_4$ 336.1230, found 336.1176.

Compound (**4**): obtained as yellow amorphous powder, isolated yield: 60.0%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.74 (s, 1H, H-1), 7.60 (s, 1H, H-13),7.26 (d, 1H, $J = 8.4$ Hz, H-11), 7.21 (d, 1H, $J = 8.4$ Hz, H-12), 6.94 (s, 1H, H-4), 6.12, 6.10 (two brs, each 1H, —OCH_2O—), 5.10 (d, 1H, $J = 14.8$ Hz, H-8), 4.94 (d, 1H, $J = 14.8$ Hz, H-8′), 4.12–4.02 (m, 1H, H-6), 3.99–3.89 (m, 1H, overlapped, H-6′), 3.89 (s, 3H, O-Me), 3.82 (s, 3H, OMe), 3.42–3.55 (m, 1H, overlapped, H-5), 3.11–3.06 (m, 1H, overlapped, H-5′), 3.06 (s, 3H, N-Me); ^{13}C NMR (DMSO-

d_6, 100 MHz) δ 154.2 (C-2), 149.2 (C-3), 148.2 (C-9), 145.8(C-10), 135.2 (C-14), 126.6 (C-4a), 124.3 (C-12), 122.1 (C-12a), 119.5 (C-14a), 118.8 (C-13), 115.9 (C-1), 113.9 (C-11), 108.9 (C-8a), 103.7 (C-4), 102.3 (OCH_2O), 61.5 (C-6), 60.7 (C-8), 60.4 (9-OMe), 56.5 (10-OMe), 45.6 (NMe), 23.9 (C-5); ESI-HRMS $[M]^+$ calcd for $C_{21}H_{22}NO_4$ 352.1543, found 352.1483.

Compound (**5**): obtained as yellow amorphous powder, isolated yield: 20.9%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.96 (s, 1H, H-8), 8.97 (s, 1H, H-13), 8.04 (d, 1H, $J = 8.8$ Hz, H-11), 7.82 (d, 1H, $J = 8.8$ Hz, H-12), 7.79 (s, 1H, H-1), 7.08 (s, 1H, H-4), 6.53, 6.17 (two s, each 2H, 2 × OCH_2O), 4.88 (t, 2H, $J = 6.0$ Hz, H-6), 3.19 (t, 2H, $J = 6.0$ Hz, H-5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.3 (C-2), 148.2 (C-3), 147.6 (C-8), 145.0 (C-9), 144.3 (C-10), 137.3 (C-14), 132.8 (C-4a), 131.0 (C-12a), 122.2 (C-12), 121.5 (C-13), 121.4 (C-11), 121.0 (C-14a), 112.1 (C-8a), 108.9 (C-1), 105.8 (C-4), 105.0, 102.6 (2 × OCH_2O), 55.7 (C-6), 26.8 (C-5); ESI-HRMS $[M]^+$ $C_{19}H_{14}NO_4$ 320.0917, found 320.0888.

Compound (**6**): obtained as yellow amorphous powder, isolated yield: 20.7%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.90 (s, 1H, H-8), 8.95 (s, 1H, H-13), 8.21 (d, 1H, $J = 9.2$ Hz, H-11), 8.00 (d, 1H, $J = 9.2$ Hz, H-12), 7.80 (s, 1H, H-1), 7.09 (s, 1H, H-4), 6.17 (s, 1H, OCH_2O), 4.93 (t, 2H, $J = 6.0$ Hz, H-6), 4.09 (s, 3H, 9-OMe), 4.07 (s, 3H, 10-OMe), 3.20 (t, 2H, $J = 6.0$ Hz, H-5); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.9 (C-2), 150.3 (C-3), 148.2 (C-9), 145.9 (C-10), 144.2(C-8), 138.0 (C-14), 133.5 (C-4a), 131.2 (C-12), 127.3 (C-12a), 124.0 (C-14a), 121.9 (C-13), 120.9 (C-1), 120.7 (C-11), 108.9 (C-8a), 105.9 (C-4), 102.6 (OCH_2O), 62.4 (C-6), 57.6 (9-OMe), 55.7 (10-OMe), 26.9 (C-5); ESI-HRMS $[M]^+$ calcd for $C_{20}H_{18}NO_4$ 336.1230, found 336.1185.

Compound (**7**) obtained as white amorphous powder, isolated yield: 68.0%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.92 (s, 1H, H-1), 6.75 (d, 1H, $J = 8.0$ Hz, H-11), 6.70 (s, 1H, H-4), 6.63 (d, 1H, $J = 8.0$ Hz, H-12), 6.00–5.94 (m, 4H, 2 × OCH_2O), 3.95 (d, 1H, $J = 16$ Hz, H-8), 3.46–3.32(m, 2H, overlapped, H-8′, H-14), 3.07 (1H, dd, $J = 12.0$, 4.0 Hz, H-6), 2.94–2.85 (m, 1H, H-13), 2.62–2.43 (m, 3H, H-13′, 8′, 6′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 146.2 (C-2), 145.9 (C-3), 145.0 (C-9), 143.2 (C-10), 131.2 (C-4a), 129.1 (C-12a), 128.0 (C-14a), 121.4 (C-12), 117.1 (C-8a), 108.6 (C-1), 107.0 (C-11), 106.2 (C-4), 101.3, 101.0 (2 × OCH_2O), 59.6 (C-14), 52.7 (C-8), 51.0 (C-6), 36.2 (C-13), 29.4 (C-5); ESI-HRMS $[M + H]^+$ $C_{19}H_{18}NO_4$ 324.1236, found 324.1182.

Compound (**8**): obtained as white amorphous powder, isolated yield: 58.4%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.91 (s, 1H, H-1), 6.88 (d, 1H, $J = 8.4$ Hz, H-11), 6.84 (d, 1H, $J = 8.4$ Hz, H-12), 6.66 (s, 1H, H-4), 5.94 (brs, 2H, OCH_2O), 4.05 (d, 1H, $J = 16$ Hz, H-8) 3.77, 3.72 (two s, 6H, 2 × OCH_3), 3.38–3.28 (m, 2H, overlapped, H-8′, H-14), 3.08 (1H, dd, $J = 11.2$, 4.0 Hz, H-6), 2.94–2.86 (m, 1H, H-13), 2.61–2.41 (m, 3H, H-13′, 8′, 6′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.3 (C-2), 146.2 (C-3), 145.9 (C-9), 144.9 (C-10), 131.4 (C-4), 128.7 (C-4a), 128.1 (C-12a), 128.0 (C-14a), 124.2 (C-12), 111.7 (C-1), 108.5 (C-8a), 106.2 (C-11), 101.0 (OCH_2O), 60.0 (9-OMe), 59.5 (C-14), 56.2 (10-OMe), 54.0 (C-8), 51.2 (C-6), 36.2 (C-13), 29.5 (C-5); ESI-HRMS $[M + H]^+$ $C_{20}H_{22}NO_4$ 340.1549, found 340.1539.

Compound (**9**): obtained as white amorphous powder, isolated yield: 65.8%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.00 (s, 1H, H-1), 6.84, 6.75 (two d, 1H each, $J = 8.0$ Hz, H-11, H-12), 6.70 (s, d, H-4), 6.00–5.95 (m, 4H, 2 × OCH_2O), 5.11–5.00 (m, 1H, H-14), 4.88–4.63 (m, 1H, H-8), 4.01 (d, 1H, $J = 14.0$ Hz, H-13), 3.48 (d, 1H, $J = 14.0$ Hz, H-13′), 3,40–3.35 (m, 1H, overlapped, H-8′), 2.96 (t, 1H, $J = 12.0$ Hz, H-5), 2.66 (d, 1H, $J = 12.0$ Hz, H-6), 2.55–2.43(m, 2H, H-5′, H-6′), 1.99 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.7 (C-2), 146.6 (C-3), 145.6 (C-9), 145.2 (C-10), 141.9 (C-12a), 132.1 (C-14a), 130.9 (C-4a), 130.3 (C-8a), 126.1 (C-12), 110.5 (C-1), 110.0 (C-11), 104.8 (C-4), 101.0, 100.8(2 × OCH_2O), 69.5 (C-14), 60.7 (C-6), 51.9 (C-8), 47.7 (C-13), 42.0 (N-CH_3), 32.0 (C-5); ESI-HRMS $[M + H]^+$ calcd for $C_{20}H_{22}NO_5$ 356.1498, found 356.1490.

Compound (**10**): obtained as white amorphous powder, isolated yield: 85.8%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.01 (s, 1H, H-1), 6.85, 6.80 (two d, 1H each, $J = 8.4$ Hz, H-11, H-12),

6.72 (s, d, H-4), 5.96, 5.92 (two brs, 1H each, OCH_2O), 5.07–5.04 (m, 1H, H-14), 4.86 (d, 1H, $J = 4.8$ Hz, H-8), 4.04 (d, 1H, $J = 14.8$ Hz, H-13), 3.76, 3,64 (two s, 3H each, $2 \times OCH_3$), 3.51 (d, 1H, $J = 14.8$ Hz, H-13′), 3,35–3.31 (m, 1H, overlapped, H-8′), 2.98 (t, 1H, $J = 12.8$ Hz, H-5), 2.71 (d, 1H, $J = 12.8$ Hz, H-6), 2.54–2.40 (m, 1H, H-5′, H-6′), 1.97 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 151.2 (C-2), 147.6 (C-3), 146.1 (C-9), 145.7 (C-10), 142.0 (C-12a), 133.1 (C-14a), 131.5 (C-4a), 131.3 (C-8a), 127.1 (C-12), 110.6 (C-1), 110.4 (C-11), 105.8 (C-4), 100.8 (OCH_2O), 69.2 (C-14), 60.5 (9-OCH_3), 60.2 (C-6), 56.0 (10-OCH_3), 52.0 (C-8), 47.5 (C-13), 42.9 (N-CH_3), 32.7 (C-5); ESI-HRMS $[M + H]^+$ calcd for $C_{21}H_{26}NO_5$ 372.1811, found 372.1807.

Compound (**11**): obtained as white amorphous powder, isolated yield 48.0%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.21 (d, 1H, $J = 8.4$ Hz, H-11), 7.17 (d, 1H, $J = 8.4$ Hz, H-12), 7.09 (s, 1H, H-1), 6.88 (s, 1H, H-4), 6.10–6.02 (m, 4H, $2 \times OCH_2O$), 5.00 (dd, 1H, $J = 12.0$, 6.0 Hz, H-14), 4.78 (d, 1H, $J = 15.2$ Hz, H-8), 4.70 (d, 1H, $J = 15.2$ Hz, H-8′), 4.10–4.00 (m, 2H, H-6), 3.33–3.15 (m, 1H, H-5), 3.10 (dd, 1H, $J = 18.0$, 4.0 Hz, H-5′), 2.90–2.81 (m, 2H, H-13), 2.88 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.3 (C-2), 147.0 (C-3), 146.3 (C-9), 142.5 (C-10), 141.1 (C-12a), 133.3 (C-14a), 131.9 (C-4a), 130.0 (C-8a), 119.1 (C-12), 110.8 (C-1), 110.0 (C-11), 106.6 (C-4), 101.9, 101.2 ($2 \times OCH_2O$), 70.1 (C-14), 60.2 (C-6), 51.5 (C-8), 40.8 (N-CH_3), 32.4 (C-13), 28.1 (C-5); ESI-HRMS $[M]^+$ calcd for $C_{20}H_{20}NO_4$ 338.1392, found 338.1377.

Compound (**12**): obtained as white amorphous powder, isolated yield 38.9%, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.15 (d, 1H, $J = 8.4$ Hz, H-11), 7.09 (d, 1H, $J = 8.4$ Hz, H-12), 7.10 (s, 1H, H-1), 6.90 (s, 1H, H-4), 6.07, 6.05 (s, 1H each, OCH_2O), 5.00 (dd, 1H, $J = 12.4$, 5.8 Hz, H-14), 4.84 (d, 1H, $J = 15.6$ Hz, H-8), 4.72 (d, 1H, $J = 15.6$ Hz, H-8′), 4.05–3.98 (m, 2H, H-6), 3.82 (brs, 3H, 10-OCH_3), 3.78 (brs, 3H, 9-OCH_3), 3.27–3.10 (m, 1H, H-5), 3.08 (dd, 1H, $J = 18.0$, 4.0 Hz, H-5′), 2.93 (dd, 2H, $J = 17.6$, 12.4 Hz, H-13), 2.81 (s, 3H, NCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 153.3 (C-2), 147.8 (C-3), 145.3 (C-9), 143.6 (C-10), 141.2 (C-12a), 133.4 (C-14a), 132.4 (C-4a), 130.9 (C-8a), 122.0 (C-12), 110.1 (C-1), 110.4 (C-11), 105.8 (C-4), 101.9 (OCH_2O), 69.5 (C-14), 61.2 (C-6), 60.9 (9-OCH_3), 56.8 (10-OCH_3), 52.0 (C-8), 39.6 (N-CH_3), 31.3 (C-13), 27.9 (C-5); ESI-HRMS $[M]^+$ calcd for $C_{21}H_{24}NO_4$ 354.1700, found 354.1640.

3.2. *Bioactivity assay*

Kirby-Bauer experiments and MIC and MBC determination were done as reported in the previous literature (Miao et al. 2011). The detailed procedure can be found in the Supplementary material.

4. Conclusions

In this study, several types of isoquinoline alkaloids involved in the biosynthesis of protopines were synthesised with protopines as starting materials and evaluated for their antibacterial activities. High aromatisation level of the alkaloids generates positive influence on the antibacterial activities. Planar cation molecules berberine and coptisine exhibited good antibacterial activities and act as bacterial DNA topoisomerase inhibitors as reported previously (Tse-Dinh 2007). *N*-methyl tetrahydroprotoberberines were found to display antibacterial activities for the first time.

Supplementary material

Further experimental details are available online alongside the original spectra of compounds **3**–**8**, **10** and **12**.

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