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Research paper

The synthesis and antistaphylococcal activity of 9, 13-disubstituted berberine derivatives

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ABSTRACT

A series of novel 9, 13-disubstituted berberine derivatives have been synthesized and evaluated for the antibacterial activities against *Staphylococcus aureus*, including Newman strain and multidrug-resistant strains (NRS-1, NRS-70, NRS-100, NRS-108, and NRS-271). Compound **20** shows the most potent activity against the growth of Newman strain, with a MIC value of 0.78 μg/mL, which is comparable with the positive control vancomycin. In addition, compound **20**, **21**, and **33** are highly antistaphylococcal active against five strains of multidrug-resistant *S. aureus*, with MIC values of 0.78–1.56 μg/mL. Of note, theses antibacterial active compounds have no obvious toxicity to the viability of human fibroblast (HAF) cells at the MIC concentration.

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that causes many infections of ecological niches within the human body, leading to a wide range of diseases [1]. Methicillin-resistant *S. aureus* (MRSA) is a nosocomial and communal menace that is resistant to most antibacterial drugs and antiseptics [2]. MRSA accounts for 60–70% of *S. aureus* infections in hospitals and causes the highest number of invasive infections among all antibiotic-resistant bacteria [3–6]. It is estimated that by 2050, worldwide drug-resistant infections will cause an additional 10 million deaths annually [2]. Therefore the demand for the development of new antimicrobial agents with novel chemical scaffolds and biological mechanisms is necessary [7,8].

Natural products have been a rich resource for drug discovery [9]. Berberine (Scheme 1) is an isoquinoline alkaloid extracted from *Coptis chinensis* (Huang-Lian, a common herb in traditional Chinese medicine), and has been traditionally used as a nonprescription drug with a confirmed safety to treat diarrhea and gastrointestinal disorders for decades in China [10,11]. Recent studies show that berberine has various biological activities such as antimalarial [12], antileishmanial [13], anticancer [14–17], anti-alzheimer's disease [18,19], antiviral [20,21], cholesterol lowering effect [22], hypoglycemic effect [23], G-quadruplex binding ligands [24], and particularly antibacterial activity [25–28]. The lipophilic substituents at C-9 position of berberine could enhance the antimicrobial activities and broaden antimicrobial spectrum significantly. Zhou et al. showed the synthesis of 9-O-substituted berberine derivatives with antimicrobial activity by introducing various heterocycles such as triazole, metronidazole, and benzimidazole in C-9 position of berberine. Berberine-triazole **A**, berberine-metronidazole **B**, and berberine-benzimidazole **C** exhibited excellent activity against Gram-negative bacteria and fungus (Fig. 1) [29–31]. Su et al. revealed the antibacterial activity of 9-phenoxyalkyl berberine derivatives, for example, compound **D** (Fig. 1) strongly inhibited the proliferation of Gram-positive bacteria [32]. In addition, 9-*N*-

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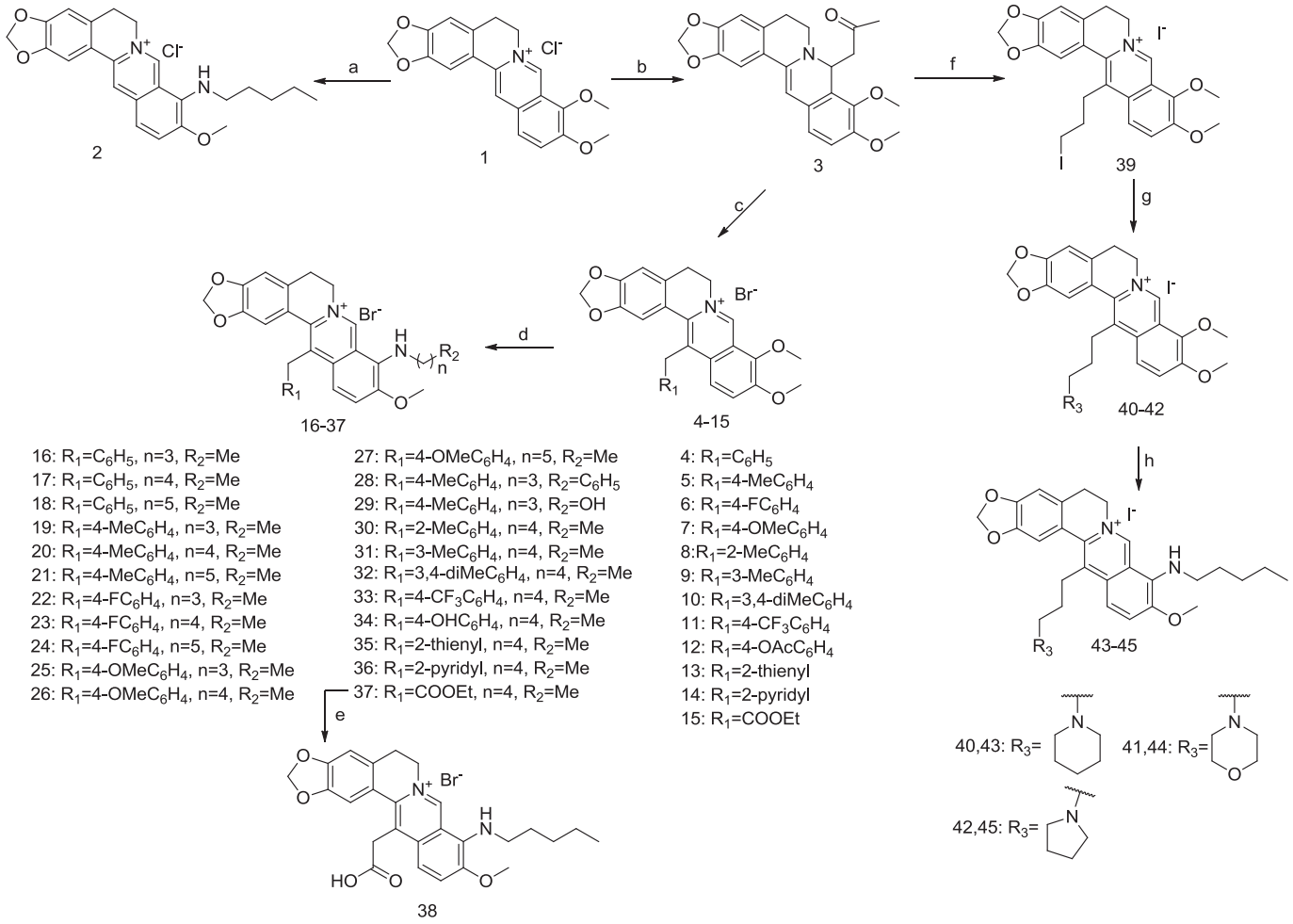

Scheme 1. Reagents and conditions (a) *n*-pentyl amine, EtOH, reflux, 8 h, 72%; (b) 5 N NaOH, acetone, rt, 1 h; (c) various bromides, NaI, CH_3CN, 80 °C, 4 h, 65%–77% (over two steps); (d) various amine, EtOH, reflux, 8–15 h, 60%–85%; (e) NaOH, MeOH, reflux, 1 h, 71%; (f) 1,3-diiodopropane, CH_3CN, 80 °C, 6 h, 50% (over two steps); (g) various amines, EtOH, reflux, 4 h; (h) *n*-pentyl amine, EtOH, reflux, 8 h, 33–42% (over two steps).

substituted berberine derivatives could serve as antioxidant, G-quadruplex binding ligands, inhibitors of acetylcholinesterase, and butyrylcholinesterase [33,34]. Several derivatives of 9-*N*-substituted berberine exhibited better antioxidant activity than 9-O-substituted berberine derivatives [33]. However, antibacterial activity of 9-*N*-substituted berberine has been poorly explored. Moreover, 13-substituted berberine derivatives have been shown a variety of bioactivities [35–38]. Kim et al. investigated the antifungal activities of 13-(substituted benzyl) berberine and showed that 13-(4-isopropyl benzyl) berberine **E** (Fig. 1) exerted the most potent antifungal activities against *Candida species* [35]. Berberine derivative **F** (Fig. 1) reported by Ball et al. showed even better antibacterial activity than berberine against *S. aureus* strain that overexpresses NorA protein [36]. To our knowledge, berberine derivatives bearing structural modification on both C-9 and C-13 positions have been rarely reported, however. In order to search for new chemical classes of potential antibacterial agents, we designed and synthesized a series of 9, 13-disubstituted berberine derivatives. The *in vitro* antibacterial activities against *S. aureus* Newman and five multidrug-resistant strains were evaluated.

2. Chemistry

All berberine derivatives were synthesized from commercially available berberine (**1**) as the starting material as described in Scheme 1. Compound **2** was obtained by treatment of berberine with *n*-pentylamine in refluxing EtOH. Compounds **4**–**15** were synthesized from a key intermediate **3** that was prepared by condensation of berberine with acetone. Treatment of **3** with various bromides and sodium iodide (NaI) in CH_3CN yielded 13-substituted berberine derivatives **4**–**15** respectively. The berberine derivatives **16**–**37** were made by reaction of compounds **4**–**15** with primary amines under refluxing EtOH. Compound **38** was synthesized by hydrolysis of **37** in the presence of 30% aqueous NaOH in MeOH at 80 °C. The intermediates **40**–**42** were obtained according to the published procedure [38]. Refluxing of compounds **40**–**42** with *n*-pentylamine in EtOH afforded berberine derivatives **43**–**45** respectively.

3. Result and discussion

3.1. Antibacterial activity

These novel berberine derivatives were first evaluated for the *in vitro* antibacterial activity (MIC; the minimum concentration of the compound that produced completely bacterial growth inhibition) against *S. aureus* Newman strain. The antibacterial activities of **16**, **17**, **20**, **21**, **23**, **26**, and **30**–**34** were shown in Table 1 and berberine, totarol, vancomycin were used as positive controls. The antibacterial activities of all synthesized compounds were shown in

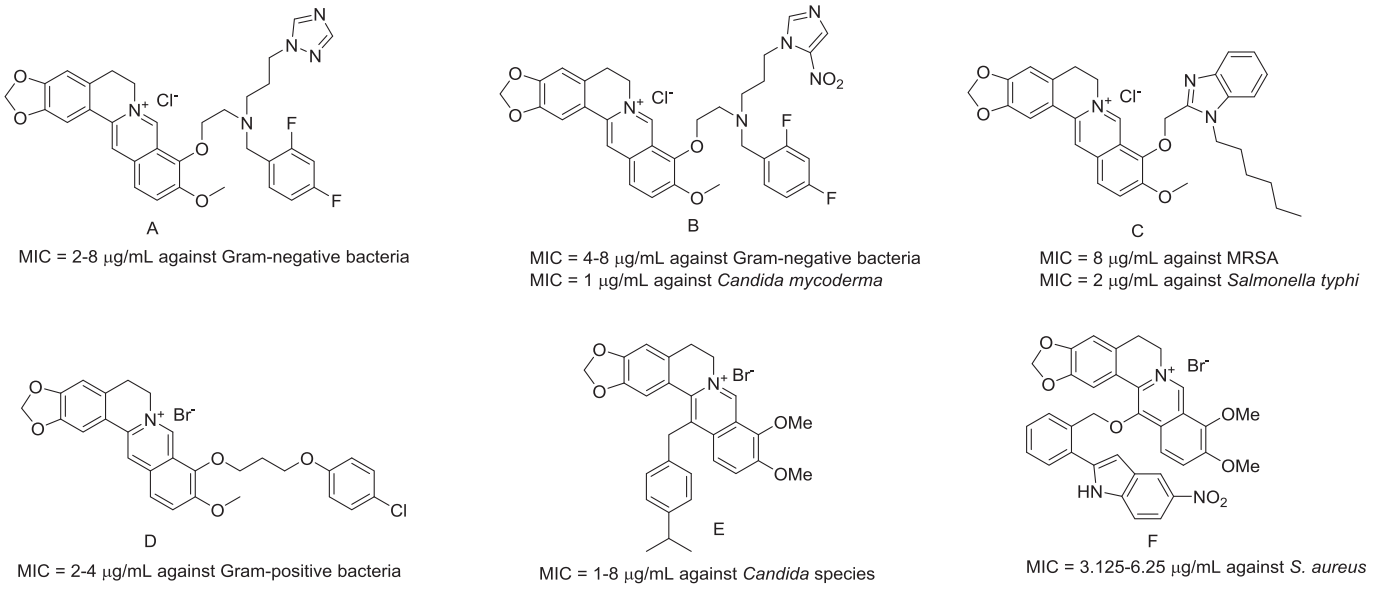

Fig. 1. Structures and antimicrobial activity of some reported compounds.

Supporting Information.

For *S. aureus* Newman strain, the MICs of most derivatives were between 0.78 and 25 μg/mL, except **43**–**45**. The 9-*N*- and 13-disubstituted berberine derivatives **16**–**18** and **19**–**21** exhibited remarkably improved antibacterial activity than 9-O- and 13-disubstituted berberine analogues **4** and **5**. For berberine analogues **16**–**18**, **19**–**21**, **22**–**24** and **25**–**27**, structure-activity relationship analysis showed that the antibacterial activity was relative to the length of 9-*N*-substituted alkyl chain, and the best alkyl length is n = 4. For instance, compounds **17**, **20**, **23**, and **26** (n = 4) exhibited more potent activity than **16**, **19**, **22**, and **25** (n = 3) and **18**, **21**, **24**, and **27** (n = 5). Replacement the methyl group (R_2) at 9-position of **19** with phenyl (**28**) and hydroxyl (**29**) dramatically decreased the antibacterial activities.

Compounds **17**, **20**, **23**, **26**, and **30**–**34** with various benzyl groups at 13-position showed better antibacterial activity than compound **2** without substituent at 13-position. Compounds **20**, **23**, **26**, and **30**–**33** with various hydrophobic groups substituted in phenyl at 13-position exhibited better activity than **17** which had no substituent on phenyl ring. Compound **34** with hydrophilic group (OH) substituted in phenyl ring slightly decreased the antibacterial potency compared to compound **17**. Compound **20** showed the highest antibacterial activity (0.78 μg/mL), which is better than totarol (1.56 μg/mL) and comparable to vancomycin. Replacement of phenyl ring at 13-position in compound **17** with heterocycles, such as thienyl (**35**) and pyridyl (**36**), decreased the antistaphylococcal activity. Similar, compounds with alkyl carboxylic acid (**38**) and its corresponding ethyl formate (**37**) at 13-postion displayed weakened inhibitory activities. In particular, compounds with (1-piperidino) propyl (**43**), (4-morpholino) propyl (**44**), and (1-pyrryl) propyl (**45**) substituents at 13-position lose inhibition on *Staphylococcal* growth (>25 μg/mL). Taken together, these 9, 13-disubstituted berberine derivatives (**16**–**35**) exhibited better antibacterial activity than berberine. Especially compounds **17**, **20**, **21**, **23**, **24**, **26** and **30**–**33** showed potent activity against *S. aureus* strain Newman with low MIC values of 0.78–1.56 μg/mL.

Table 1
MICs of berberine derivatives **16**, **17**, **20**, **21**, **23**, **26**, and **30**–**34** against *S. aureus* strain Newman (Newman), *S. aureus* NRS-1, NRS-70, NRS-100, NRS-108, NRS-271 and their IC_{50} values against the growth of HAF.

Compound	MIC (μg/mL)						IC_{50} (μg/mL)
	Newman	NRS-1	NRS-70	NRS-100	NRS-108	NRS-271	
16	3.13	n.t.[a]	n.t.	n.t.	n.t.	n.t.	>20
17	1.56	3.13	1.56	1.56	1.56	1.56	15.8 ± 3.48
20	0.78	1.56	1.56	0.78	1.56	1.56	12.512 ± 0.373
21	1.56	1.56	1.56	1.56	1.56	1.56	12.784 ± 0.452
23	1.25	3.13	1.56	3.13	1.56	1.56	12.702 ± 1.325
26	1.25	1.56	1.56	3.13	1.56	1.56	13.79 ± 1.602
30	1.25	3.13	1.56	3.13	1.56	3.13	13.013 ± 0.483
31	1.25	3.13	1.56	3.13	1.56	1.56	12.678 ± 1.039
32	1.25	3.13	1.56	1.56	1.56	1.56	12.498 ± 0.539
33	1.25	1.56	1.56	1.56	1.56	1.56	11.446 ± 1.291
34	2.5	6.25	1.56	3.13	3.13	3.13	>20
Berberine	>25	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Vancomycin	0.78	1.56	0.39	0.78	0.39	0.39	n.t.
Totarol[b]	1.56	3.13	1.56	1.56	3.13	3.13	8.46 ± 0.87

[a] n.t. = not tested.
[b] These data were reported previously in reference [8].

Since compounds **17**–**27** and **30**–**34** possessed excellent activity against *S. aureus* Newman strain with MIC values below 2.5 μg/mL, we further evaluated the antibacterial activities of these compounds against multidrug-resistant *S. aureus* strain NRS-1 (resistant to aminoglycosides and tetracycline), NRS-70 (resistant to erythromycin), NRS-100 (resistant to oxacillin and tetracycline), NRS-108 (resistant to gentamicin) and NRS-271 (linezolid-resistant, containing phage type E-MRSA 15). Compounds **20**, **21** and **33** displayed the most potent activity against drug-resistant strains with MIC values of 0.78–1.56 μg/mL, which was 1–4 folds more potent than totarol. For *S. aureus strain* NRS-1, compounds **18**–**21** and **26**–**33** showed equivalent antibacterial activity to vancomycin.

3.2. In vitro toxicity study

Berberine derivatives (**16**, **17**, **20**, **21**, **23**, **26**, and **30**–**34**) with low MIC values against Newman or multidrug-resistant strains were tested the cytotoxic activity on a human fibroblast (HAF) cell line using the MTS assay. The cytotoxicity results of these compounds on normal mammalian cells were summarized in Table 1. The tested compounds showed cytotoxicity with IC_{50} 11–20 μg/mL, which is lower than totarol. In addition, compounds **20**, **21**, **33**, and **34** were selected to investigate the cellular morphology of HAF under the concentration of MIC (1.56 μg/mL), and IC_{50} (12.5 μg/mL) respectively. As shown in Fig. 2, the compounds in a dose of 1.56 μg/mL exhibited minimal toxicity to HAF cells that showed normal morphology, however, the cells lost normal morphology when exposed to the tested compounds at 12.5 μg/mL. The above cytotoxicity assay demonstrated that the novel 9, 13-disubstituted berberine derivatives bear good selectivity for bacterial over mammalian cells.

4. Conclusion

A series of novel 9, 13-disubstituted berberine derivatives were synthesized and investigated for the antibacterial activity *in vitro* against *S. aureus* strain Newman and five multidrug-resistant S. *aureus* (NRS-1, NRS-70, NRS-100, NRS-108 and NRS-271). For these Gram-positive bacteria species, most synthetic derivatives showed satisfactory inhibitory activity with MIC values of 0.78–2.5 μg/mL. For Newman, compound **20** showed the highest activity (MIC, 0.78 μg/mL), which is comparable to the positive control vancomycin. For multidrug-resistant bacteria, compounds **20**, **21**, and **33** exhibited the most potent activity (MIC, 0.78–1.56 μg/mL) which are comparable or even superior to totarol. All the active antimicrobial compounds did not show obvious toxicity to human fibroblast (HAF) cells at MIC dose. In summary, we showed a series of 9, 13-disubstituted berberine derivatives for discovery of new chemical entities against multidrug-resistant *S. aureus*. The mechanism of these berberine derivatives for antistaphylococcal activity will be further explored.

5. Experimental section

5.1. General methods

Starting materials, reagents and chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. Flash column chromatography was performed using Qingdao Haiyang silica gel (200–300) with the indicated eluents. ^{1}H NMR spectra were recorded at a spectrometer frequency of 400 MHz, and ^{13}C NMR spectra at 100 MHz. Chemical shifts are reported in δ (ppm) using the δ 0 signal of tetramethylsilane (TMS) as internal standard. High resolution mass spectra were performed using a Bruker ESI-TOF high-resolution mass spectrometer. Melting points were uncorrected and were recorded on a Buchi B-54 melting point apparatus.

5.2. Synthesis of 9-N-n-pentyl-berberine **2**

Berberine chloride (0.37 g, 10 mmol) was dissolved in *n*-pentyl amine (40 mmol, 4.6 mL), the mixture was stirred and heated for 8 h at 100 °C. After the reaction completed (monitored by TLC), the mixture was concentrated in vacuo and the residual oil was purified on silica gel chromatography ($CHCl_3/CH_3OH$, 50/1–20/1 v/v) to give the proposed compound **2** as a red solid; yield: 72%; mp: 121.4–123.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.57 (s, 1H), 7.86 (s, 1H), 7.62 (br s, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.28 (s, 1H), 7.09 (d, J = 8.5 Hz, 1H), 6.78 (s, 1H), 6.06 (s, 2H), 5.09–5.06 (m, 2H), 3.91 (s, 3H), 3.82–3.76 (m, 2H), 3.16–3.13 (m, 2H), 1.82–1.75 (m, 2H),

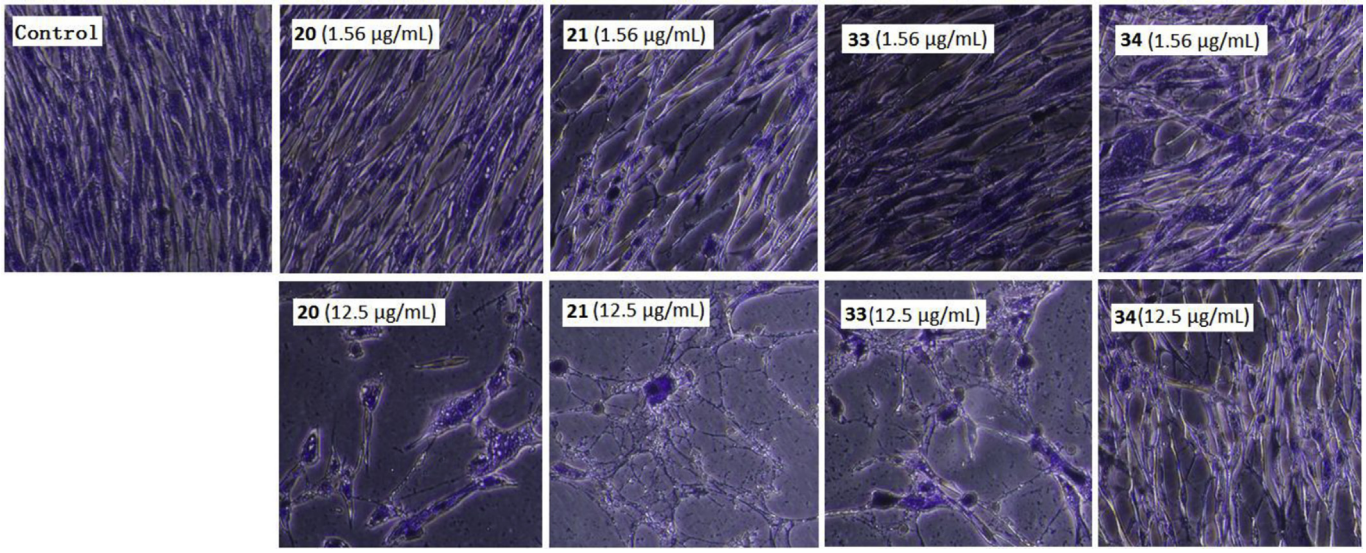

Fig. 2. Normal growth (control) of human fibroblast (HAF) cells and morphological changes in the cells exposure to compound **20**, **21**, **33**, and **34** at concentrations of 1.56 μg/mL and 12.5 μg/mL, respectively.

1.38–1.35 (m, 4H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.28, 148.45, 148.03, 146.17, 140.36, 135.51, 133.40, 129.91, 125.47, 120.73, 118.47, 117.16, 113.13, 108.78, 104.80, 102.18, 57.25, 54.17, 46.96, 31.23, 29.35, 28.14, 22.66, 14.28; HRMS (ESI): calcd for $C_{24}H_{27}N_2O_3$ $[M-Cl]^+$ 391.2016; found 391.2020.

5.3. Synthesis of 4-(bromomethyl) phenyl acetate

In a round bottomed flask were placed of 4-hydroxy benzaldehyde (2.00 g, 16.4 mmol), acetic anhydride (4.20 g, 41 mmol), pyridine (19 mL) and DMAP (0.10 g, 0.82 mmol). The reaction mixture was maintained under room temperature and the reaction was checked by TLC. After disappearance of the starting material, the mixture was hydrolyzed with a saturated solution $NaHCO_3$ (5 mL) and the medium was poured in a separating funnel containing 50 mL of diethyl ether. The aqueous layer was removed and the organic phase was washed with an aqueous solution of $CuSO_4$ (20%) until the aqueous layer remained light blue. The mixture was then washed several times with water (5 × 5 mL). The organic layer was dried and the solvents evaporated under vacuum. The crude obtained was purified over silica (petroleum ether/AcOEt, 5/1 v/v) to give the 4-formylphenyl acetate as clear oil, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 7.95 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 2.32 (s, 3H).

4-formylphenyl acetate (2.20 g, 13.4 mmol) which was dissolved in THF (40 mL) and the solution was cooled to 0 °C, then $NaBH_4$ (0.56 g, 14.7 mmol) was added. After the solution was stirred at 0 °C for 1.5 h, the reaction was complete according to TLC analysis. The solution was washed with NH_4Cl (2 × 50 mL) and the organic layer was dried with Na_2SO_4. The solvent evaporated under vacuum and the crude product 4-(hydroxymethyl) phenyl acetate was used for the next reaction without further purification. To the solution of 4-(hydroxymethyl) phenyl acetate (2.20 g, 13.2 mmol) and CBr_4 (5.80 g, 17.5 mmol) in CH_2Cl_2 (40 mL), then added PPh_3 (4.14 g, 15.8 mmol), the mixture was left at room under stirring and the reaction was checked by TLC. After disappearance of the starting material, the mixture was the residue was purified by silica gel chromatography (petroleum ether/AcOEt, 10/1 v/v) to give 4-(bromomethyl) phenyl acetate (2.40 g, 60% (over two steps)) as a white solid; mp: 61.7–63.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 8.0 Hz, 2H), 4.47 (s, 2H), 2.29 (s, 3H).

5.4. General procedure for the synthesis of compounds **4**–**15**

Berberine chloride (5.0 g, 13.5 mmol) 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 compound **3** without purified by silica gel chromatography. Compound **3** (4.0 g, 10.2 mmol) dissolved in acetonitrile was reacted with NaI (1.87 g, 12.5 mmol) and various bromide (15.3 mmol) at 80 °C for 4 h. The reaction mixture was concentrated and chromatographed on silica gel ($CHCl_3$/CH_3OH, 100/1–50/1 v/v) to give compounds **4**–**15**.

5.4.1. 13-Benzyl berberine ***4***

Yellow solid; yield: 77% (over two steps); mp: 186.2–188.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.44 (s, 1H), 7.69 (d, J = 9.4 Hz, 1H), 7.62 (d, J = 9.3 Hz, 1H), 7.39–7.35 (m, 2H), 7.34–7.28 (m, 1H), 7.12 (d, J = 7.3 Hz, 2H), 6.96 (s, 1H), 6.88 (s, 1H), 6.00 (s, 2H), 5.22–5.18 (m, 2H), 4.68 (s, 2H), 4.43 (s, 3H), 4.02 (s, 3H), 3.33–3.10 (m, 2H); HRMS (ESI): calcd for $C_{27}H_{24}NO_4$ $[M-Br]^+$ 426.1700; found 426.1714.

5.4.2. 13-(4-Methylbenzyl) berberine ***5***

Yellow solid; yield: 72% (over two steps); mp: 235.5–237.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.68 (d, J = 9.4 Hz, 1H), 7.62 (d, J = 9.3 Hz, 1H), 7.16 (d, J = 7.9 Hz, 2H), 7.00 (s, 1H), 6.98 (s, 2H), 6.86 (s, 1H), 5.99 (s, 2H), 5.21–5.17 (m, 2H), 4.62 (s, 2H), 4.41 (s, 3H), 4.01 (s, 3H), 3.31–3.27 (m, 2H), 2.34 (s, 3H); HRMS (ESI): calcd for $C_{28}H_{26}NO_4$ $[M-Br]^+$ 440.1856; found 440.1862.

5.4.3. 13-(4-Fluorobenzyl) berberine ***6***

Yellow solid; yield: 65% (over two steps); mp: 238.4–240.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 7.71 (d, J = 9.4 Hz, 1H), 7.58 (d, J = 9.3 Hz, 1H), 7.12–7.08 (m, 2H), 7.07–7.03 (m, 2H), 6.90 (s, 1H), 6.87 (s, 1H), 6.00 (s, 2H), 5.18–5.14 (m, 2H), 4.64 (s, 2H), 4.39 (s, 3H), 4.02 (s, 3H), 3.31–3.27 (m, 2H); HRMS (ESI): calcd for $C_{27}H_{23}FNO_4$ $[M-Br]^+$ 444.1606; found 444.1629.

5.4.4. 13-(4-Methoxybenzyl) berberine ***7***

Yellow solid; yield: 68% (over two steps); mp: 187.7–189.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.69 (d, J = 9.4 Hz, 1H), 7.63 (d, J = 9.4 Hz, 1H), 7.02 (d, J = 8.3 Hz, 2H), 6.99 (s, 1H), 6.90 (d, J = 8.6 Hz, 2H), 6.87 (s, 1H), 6.00 (s, 2H), 5.21–5.17 (m, 2H), 4.60 (s, 2H), 4.41 (s, 3H), 4.01 (s, 3H), 3.80 (s, 3H), 3.31–3.27 (m, 2H); HRMS (ESI): calcd for $C_{28}H_{26}NO_5$ $[M-Br]^+$ 456.1805; found 456.1824.

5.4.5. 13-(2-Methylbenzyl) berberine ***8***

Yellow solid; yield: 73% (over two steps); mp: 198.6–200.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.69 (d, J = 9.3 Hz, 1H), 7.52 (d, J = 9.3 Hz, 1H), 7.35 (d, J = 7.5 Hz, 1H), 7.25–7.21 (m, 1H), 7.08–7.04 (m, 1H), 6.86 (s, 1H), 6.82 (s, 1H), 6.64 (d, J = 7.6 Hz, 1H), 5.98 (s, 2H), 5.21–5.17 (m, 2H), 4.46 (s, 2H), 4.41 (s, 3H), 4.01 (s, 3H), 3.31–3.29 (s, 2H), 2.45 (s, 3H); HRMS (ESI): calcd for $C_{28}H_{26}NO_4$ $[M-Br]^+$ 440.1856; found 440.1833.

5.4.6. 13-(3-Methylbenzyl) berberine ***9***

Yellow solid; yield: 76% (over two steps); mp: 229.2–230.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H), 7.69 (d, J = 9.4 Hz, 1H), 7.61 (d, J = 9.3 Hz, 1H), 7.23 (d, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 1H), 6.98 (s, 1H), 6.95–6.82 (m, 3H), 5.99 (s, 2H), 5.21–5.17 (m, 2H), 4.63 (s, 2H), 4.41 (s, 3H), 4.01 (s, 3H), 3.30–3.28 (m, 2H), 2.31 (s, 3H); HRMS (ESI): calcd for $C_{28}H_{26}NO_4$ $[M-Br]^+$ 440.1856; found 440.1842.

5.4.7. 13-(3, 4-Dimethylbenzyl) berberine ***10***

Yellow solid; yield: 71% (over two steps); mp: 227.4–229.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H), 7.65 (d, J = 9.3 Hz, 1H), 7.61 (d, J = 9.3 Hz, 1H), 7.16 (s, 1H), 7.00 (s, 1H), 6.98 (s, 2H), 6.86 (s, 1H), 6.00 (s, 2H), 5.21–5.17 (m, 2H), 4.62 (s, 2H), 4.41 (s, 3H), 4.01 (s, 3H), 3.31–3.27 (m, 2H), 2.21 (s, 3H), 2.16 (s, 3H); HRMS (ESI): calcd for $C_{29}H_{28}NO_4$ $[M-Br]^+$ 454.2013; found 454.2041.

5.4.8. 13-(4-Trifluoromethylbenzyl) berberine ***11***

Yellow solid; yield: 66% (over two steps); mp: 221.6–223.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.85 (s, 1H), 7.61 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.9 Hz, 2H), 7.25 (s, 1H), 6.92 (d, J = 8.8 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 6.00 (s, 2H), 5.18–5.14 (m, 2H), 4.64 (s, 2H), 4.39 (s, 3H), 4.02 (s, 3H), 3.31–3.27 (m, 2H); HRMS (ESI): calcd for $C_{28}H_{23}F_3NO_4$ $[M-Br]^+$ 494.1574; found 494.1561.

5.4.9. 13-(4-Acetoxybenzyl) berberine ***12***

Yellow solid; yield 65% (over two steps); mp: 202.8–204.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 7.71 (d, J = 9.4 Hz, 1H), 7.58 (d, J = 9.3 Hz, 1H), 7.12–7.08 (m, 2H), 7.07–7.03 (m, 2H), 6.90 (s, 1H), 6.87 (s, 1H), 6.00 (s, 2H), 5.18–5.14 (m, 2H), 4.64 (s, 2H), 4.39 (s, 3H), 4.02 (s, 3H), 3.31–3.27 (m, 2H), 2.29 (s, 3H); HRMS (ESI): calcd for $C_{29}H_{26}NO_6$ $[M-Br]^+$ 484.1755; found 484.1742.

5.4.10. 13-(2-Thienylmethyl) berberine ***13***

Yellow solid; yield: 65% (over two steps); mp: 217.4–219.0 °C;

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 7.79 (s, 2H), 7.30 (d, J = 8.2 Hz, 1H), 7.11 (s, 1H), 6.96 (s, 1H), 6.88 (s, 1H), 6.67 (s, 1H), 6.03 (s, 2H), 5.17–5.13 (m, 2H), 4.73 (s, 2H), 4.39 (s, 3H), 4.03 (s, 3H), 3.29–3.25 (m, 2H); HRMS (ESI): calcd for $C_{25}H_{22}NO_4S$ $[M-Br]^+$ 432.1264; found 432.1249.

5.4.11. 13-(2-Pyridylmethyl) berberine ***14***

Yellow solid; yield: 69% (over two steps); mp: 221.3–222.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25 (s, 1H), 8.45 (d, J = 4.2 Hz, 1H), 7.78–7.74 (m, 1H), 7.68 (d, J = 9.4 Hz, 1H), 7.56 (d, J = 9.3 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.33 (s, 1H), 7.21 (d, J = 7.0 Hz, 1H), 6.85 (s, 1H), 5.97 (s, 2H), 5.14–5.10 (m, 2H), 4.82 (s, 2H), 4.29 (s, 3H), 3.96 (s, 3H), 3.28–3.24 (m, 2H); HRMS (ESI): calcd for $C_{26}H_{23}N_2O_4$ $[M-Br]^+$ 427.1652; found 427.1649.

5.4.12. 13-(Ethyl acetate) berberine ***15***

Yellow solid; yield: 65% (over two steps); mp: 216.2–218.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.81 (s, 1H), 7.56 (d, J = 8.7 Hz, 1H), 7.28 (s, 1H), 7.15 (d, J = 8.7 Hz, 1H), 6.88 (s, 1H), 6.09 (s, 2H), 5.07–5.03 (m, 2H), 4.33 (q, J = 7.0 Hz, 2H), 4.17 (s, 2H), 3.97 (s, 3H), 3.80 (s, 3H), 3.33–3.29 (m, 2H), 1.22 (t, J = 7.0 Hz, 3H); HRMS (ESI): calcd for $C_{24}H_{24}NO_6$ $[M-Br]^+$ 422.1598; found 422.1563.

5.5. General procedure for the synthesis of compounds ***16–37***

To a solution of intermediates **4**–**15** (1 mmol) in anhydrous ethanol (2 mL), the substituted amine (40 mmol) were added. The mixture was stirred and heated for 8–15 h at 78 °C. After the reaction completed (monitored by TLC), the mixture was concentrated in vacuo and the residual oil was purified on silica gel chromatography ($CHCl_3$/CH_3OH, 100/1–50/1, v/v) to give the proposed compound.

5.5.1. 9-N-n-butyl-13-benzyl berberine ***16***

Red solid, yield: 82%; mp: 169.5–171.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.92 (s, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.38–7.34 (m, 2H), 7.30 (d, J = 7.2 Hz, 1H), 7.12 (d, J = 7.5 Hz, 2H), 7.06 (d, J = 8.8 Hz, 1H), 6.93 (s, 1H), 6.85 (s, 1H), 6.63 (br s, 1H), 5.99 (s, 2H), 5.11 (br s, 2H), 4.58 (s, 2H), 3.91 (s, 3H), 3.89–3.83 (m, 2H), 3.14–3.11 (m, 2H), 1.82–1.75 (m, 2H), 1.52–1.42 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.26, 149.39, 148.54, 147.03, 140.28, 140.20, 137.38, 134.78, 134.42, 131.56, 130.28 (2C), 129.22 (2C), 127.97, 123.58, 121.88, 118.60, 116.82, 109.82, 109.32, 103.53, 58.48, 57.34, 50.89, 37.16, 34.47, 29.41, 21.14, 14.30; HRMS (ESI): calcd for $C_{30}H_{31}N_2O_3$ $[M-Br]^+$ 467.2329; found 467.2342.

5.5.2. 9-N-n-pentyl-13-benzyl berberine ***17***

Red solid, yield: 76%; mp: 208.5–210.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (s, 1H), 7.41–7.29 (m, 4H), 7.11 (d, J = 7.4 Hz, 2H), 7.05 (d, J = 8.8 Hz, 1H), 6.92 (s, 1H), 6.84 (s, 1H), 6.60 (br s, 1H), 5.98 (s, 2H), 5.11 (br s, 2H), 4.57 (s, 2H), 3.90 (s, 3H), 3.88–3.83 (m, 2H), 3.13–3.10 (m, 2H), 1.83–1.76 (m, 2H), 1.43–1.33 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.20, 149.30, 148.50, 147.05, 140.27, 140.17, 137.33, 134.76, 134.37, 131.49, 130.26 (2C), 129.23 (2C), 127.95, 123.51, 121.87, 118.51, 116.74, 109.79, 109.31, 103.51, 58.46, 57.32, 51.10, 37.16, 32.04, 30.21, 29.39, 23.59, 14.46; HRMS (ESI): calcd for $C_{31}H_{33}N_2O_3$ $[M-Br]^+$ 481.2486; found 481.2500.

5.5.3. 9-N-n-hexyl-13-benzyl berberine ***18***

Red solid, yield: 72%; mp: 175.2–177.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.87 (s, 1H), 7.44–7.26 (m, 4H), 7.11 (d, J = 7.5 Hz, 2H), 7.05 (d, J = 8.8 Hz, 1H), 6.92 (s, 1H), 6.84 (s, 1H), 6.57 (br s, 1H), 5.98 (s, 2H), 5.10 (br s, 2H), 4.57 (s, 2H), 3.90 (s, 3H), 3.87–3.82 (m, 2H), 3.13–3.10 (t, J = 5.8 Hz, 2H), 1.82–1.73 (m, 2H), 1.45–1.38 (m, 2H), 1.35–1.28 (m, 4H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.20, 149.29, 148.49, 147.06, 140.27, 140.19, 137.33, 134.75, 134.36, 131.48, 130.26 (2C), 129.23 (2C), 127.94, 123.48, 121.86, 118.49, 116.73, 109.78, 109.31, 103.51, 58.46, 57.31, 51.11, 37.16, 32.79, 32.31, 29.39, 27.65, 23.71, 14.42; HRMS (ESI): calcd for $C_{32}H_{35}N_2O_3$ $[M-Br]^+$ 495.2642; found 495.2640.

5.5.4. 9-N-n-butyl-13-(4-methylbenzyl) berberine ***19***

Red solid, yield: 70%; mp: 179.1–181.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 7.8 Hz, 2H), 7.06 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 7.7 Hz, 2H), 6.95 (s, 1H), 6.84 (s, 1H), 6.58 (br s, 1H), 5.98 (s, 2H), 5.10 (br s, 2H), 4.52 (s, 2H), 3.90 (s, 3H), 3.88–3.83 (m, 2H), 3.13–3.10 (m, 2H), 2.34 (s, 3H), 1.81–1.73 (m, 2H), 1.50–1.41 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.64, 147.20, 146.59, 145.76, 139.97, 136.80, 135.44, 135.23, 133.52, 132.98, 130.13 (2C), 129.42, 127.86 (2C), 123.54, 120.60, 116.36, 112.86, 109.05, 108.52, 102.00, 77.16, 56.83, 47.53, 36.21, 33.73, 28.99, 21.18, 20.34, 14.16; HRMS (ESI): calcd for $C_{31}H_{33}N_2O_3$ $[M-Br]^+$ 481.2483; found 481.2483.

5.5.5. 9-N-n-pentyl-13-(4-methylbenzyl) berberine ***20***

Red solid, yield: 83%; mp: 170.2–171.9 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 9.77 (s, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 9.0 Hz, 1H), 7.16 (d, J = 7.8 Hz, 2H), 7.07–7.01 (m, 3H), 7.00 (s, 1H), 5.99 (s, 2H), 4.82–4.79 (m, 2H), 4.64 (s, 2H), 4.00 (s, 3H), 3.67–3.63 (m, 2H), 3.17–3.14 (m, 2H), 2.32 (s, 3H),1.76–1.69 (m, 2H) 1.46–1.33 (m, 4H), 0.93 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.23, 149.38, 148.52, 146.96, 140.12, 137.76, 137.30, 137.14, 134.72, 134.45, 131.81, 130.88 (2C), 129.11 (2C), 123.53, 121.89, 118.61, 116.92, 109.84, 109.29, 103.52, 58.47, 57.32, 51.17, 36.79, 32.04, 30.22, 29.39, 23.59, 21.08, 14.45; HRMS (ESI): calcd for $C_{32}H_{35}N_2O_3$ $[M-Br]^+$ 495.2642; found 495.2674.

5.5.6. 9-N-n-hexyl-13-(4-methylbenzyl) berberine ***21***

Red solid, yield: 75%; mp: 190.6–192.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.88 (s, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.15 (d, J = 7.7 Hz, 2H), 7.06 (d, J = 8.9 Hz, 1H), 6.99 (d, J = 7.7 Hz, 2H), 6.95 (s, 1H), 6.83 (s, 1H), 6.57 (br s, 1H), 5.98 (s, 2H), 5.10 (br s, 2H), 4.52 (s, 2H), 3.90 (s, 3H), 3.87–3.82 (m, 2H), 3.13–3.10 (m, 2H), 2.34 (s, 3H), 1.82–1.74 (m, 2H), 1.45–1.38 (m, 2H), 1.35–1.28 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.23, 149.38, 148.53, 146.97, 140.15, 137.76, 137.29, 137.13, 134.72, 134.43, 131.81, 130.88 (2C), 129.10 (2C), 123.47, 121.89, 118.59, 116.92, 109.83, 109.28, 103.52, 58.45, 57.30, 51.23, 36.78, 32.80, 32.31, 29.38, 27.66, 23.72, 21.08, 14.42; HRMS (ESI): calcd for $C_{33}H_{37}N_2O_3$ $[M-Br]^+$ 509.2799; found 509.2862.

5.5.7. 9-N-n-butyl-13-(4-fluorobenzyl) berberine ***22***

Red solid, yield: 70%; mp: 188.3–190.1 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 9.79 (s, 1H), 7.73 (d, J = 9.0 Hz, 1H), 7.40 (d, J = 9.0 Hz, 1H), 7.24–7.16 (m, 2H), 7.09–7.05 (m, 2H), 7.00 (s, 1H), 6.98 (s, 1H), 6.00 (s, 2H), 4.80 (br s, 2H), 4.69 (s, 2H), 4.00 (s, 3H), 3.70–3.66 (m, 2H), 3.18–3.15 (m, 2H), 1.75–1.68 (m, 2H), 1.52–1.43 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.10 (d, $J_{C\text{-}F}$ = 245.4 Hz), 151.21, 149.28, 148.49, 147.10, 140.24, 137.35, 136.14 (d, $J_{C\text{-}F}$ = 3.2 Hz), 134.78, 134.21, 131.27, 131.06, 130.98, 123.55, 121.78, 118.49, 116.97, 116.75, 116.55, 109.68, 109.32, 103.52, 58.44, 57.32, 50.78, 36.36, 34.43, 29.36, 21.09, 14.28; HRMS (ESI): calcd for $C_{30}H_{30}FN_2O_3$ $[M-Br]^+$ 485.2235; found 485.2243.

5.5.8. 9-N-n-pentyl-13-(4-fluorobenzyl) berberine ***23***

Red solid, yield: 76%; mp: 204.2–206.1 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 9.77 (s, 1H), 7.74 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 9.0 Hz, 1H), 7.24–7.16 (m, 2H), 7.10–7.06 (m, 2H), 7.01 (s, 1H), 6.98 (s, 1H), 6.01 (s, 2H), 4.82–4.79 (m, 2H), 4.70 (s, 2H), 4.01 (s, 3H), 3.68–3.65 (m,

2H), 3.18–3.15 (m, 2H), 1.77–1.70 (m, 2H), 1.46–1.36 (m, 4H), 0.93 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.72 (d, $J_{C\text{-}F}$ = 247.4 Hz), 148.63, 146.13, 145.51, 144.86, 139.01, 134.40, 132.87 (d, $J_{C\text{-}F}$ = 4.0 Hz), 132.08, 131.93, 128.45, 128.37, 127.64, 122.30, 119.30, 115.37, 115.16, 115.08, 111.44, 107.64, 107.48, 100.95, 55.72, 46.72, 34.72, 30.21, 29.93, 28.13, 27.83, 21.50, 13.11; HRMS (ESI): calcd for $C_{31}H_{32}FN_2O_3$ $[M-Br]^+$ 499.2391; found 499.2364.

5.5.9. 9-N-n-hexyl-13-(4-fluorobenzyl) berberine ***24***

Red solid, yield: 66%; mp: 188.6–190.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.82 (s, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.14–7.01 (m, 5H), 6.88 (s, 1H), 6.86 (s, 1H), 6.54 (br s, 1H), 6.01 (s, 2H), 5.09 (br s, 2H), 4.56 (s, 2H), 3.92 (s, 3H), 3.88–3.83 (m, 2H), 3.15–3.12 (t, J = 5.9 Hz, 2H), 1.82–1.75 (m, 2H), 1.44–1.39 (m, 2H), 1.34–1.29 (m, 4H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.77 (d, $J_{C\text{-}F}$ = 247.4 Hz), 149.69, 147.19, 146.63, 145.88, 139.99, 135.49, 133.94 (d, $J_{C\text{-}F}$ = 3.0 Hz), 133.12, 132.98, 129.53, 129.45, 128.80, 123.32, 120.36, 116.42, 116.21, 116.19, 112.68, 108.70, 108.54, 102.03, 56.82, 56.79, 47.93, 35.77, 31.67, 31.53, 28.88, 26.69, 22.67, 14.12; HRMS (ESI): calcd for $C_{32}H_{34}FN_2O_3$ $[M-Br]^+$ 513.2548; found 513.2563.

5.5.10. 9-N-n-butyl-13-(4-methoxybenzyl) berberine ***25***

Red solid, yield: 66%; mp: 212.4–214.3 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 9.76 (s, 1H), 7.90 (s, 1H), 7.73 (d, J = 9.1 Hz, 1H), 7.44 (d, J = 9.1 Hz, 1H), 7.12–7.02 (m, 3H), 7.00 (s, 1H), 6.91 (s, 1H), 6.89 (s, 1H), 6.00 (s, 2H), 4.82–4.79 (m, 2H), 4.63 (s, 2H), 4.01 (s, 3H), 3.78 (s, 3H), 3.68–3.64 (m, 2H), 3.17–3.14 (m, 2H), 1.75–1.68 (m, 2H), 1.52–1.43 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.51, 149.54, 147.10, 146.50, 145.62, 139.81, 135.32, 133.36, 132.84, 130.06, 129.42, 128.91 (2C), 123.38, 120.46, 116.26, 114.72 (2C), 112.82, 108.90, 108.40, 101.90, 56.74, 56.72, 55.31, 47.47, 35.64, 33.61, 28.85, 20.22, 14.04; HRMS (ESI): calcd for $C_{31}H_{33}N_2O_4$ $[M-Br]^+$ 497.2435; found 497.2400.

5.5.11. 9-N-n-pentyl-13-(4-methoxybenzyl) berberine ***26***

Red solid, yield: 72%; mp: 205.6–207.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.81 (s, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.11 (d, J = 8.9 Hz, 1H), 7.03 (d, J = 8.2 Hz, 2H), 6.98 (s, 1H), 6.88 (d, J = 8.6 Hz, 2H), 6.85 (s, 1H), 6.49 (br s, 1H), 6.00 (s, 2H), 5.10 (br s, 2H), 4.52 (s, 2H), 3.92 (s, 3H), 3.87–3.81 (m, 2H), 3.80 (s, 3H), 3.14–3.11 (m, 2H), 1.83–1.75 (m, 2H), 1.45–1.32 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.53, 149.57, 147.14, 146.58, 145.61, 139.74, 135.37, 133.39, 132.84, 130.10, 129.56, 128.94 (2C), 123.41, 120.48, 116.34, 114.75 (2C), 113.04, 108.93, 108.41, 101.95, 56.84, 56.80, 55.35, 47.86, 35.67, 31.22, 29.17, 28.88, 22.53, 14.15; HRMS (ESI): calcd for $C_{32}H_{35}N_2O_4$ $[M-Br]^+$ 511.2591; found 511.2583.

5.5.12. 9-N-n-hexyl-13-(4-methoxybenzyl) berberine ***27***

Red solid, yield: 69%; mp: 202.3–204.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.86 (s, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.07 (d, J = 8.9 Hz, 1H), 7.01 (d, J = 8.2 Hz, 2H), 6.96 (s, 1H), 6.87 (d, J = 8.3 Hz, 2H), 6.83 (s, 1H), 6.56 (br s, 1H), 5.99 (s, 2H), 5.09 (br s, 2H), 4.49 (s, 2H), 3.90 (s, 3H), 3.87–3.81 (m, 2H), 3.79 (s, 3H), 3.12–3.09 (m, 2H), 1.81–1.74 (m, 2H), 1.45–1.38 (m, 2H), 1.33–1.28 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.00, 151.14, 149.24, 148.45, 146.89, 140.05, 137.20, 134.65, 134.41, 131.93, 131.88, 130.22 (2C), 123.52, 121.85, 118.55, 116.82, 115.59 (2C), 109.81, 109.25, 103.47, 58.46, 57.32, 55.77, 49.43, 36.33, 32.76, 32.28, 29.36, 27.62, 23.67, 14.39; HRMS (ESI): calcd for $C_{33}H_{37}N_2O_4$ $[M-Br]^+$ 525.2748; found 525.2769.

5.5.13. 9-N-(3-phenylpropyl)-13-(4-methylbenzyl) berberine ***28***

Red solid, yield: 66%; mp: 185.9–187.8 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.63 (s, 1H), 7.90 (s, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.43 (d, J = 9.1 Hz, 1H), 7.25–7.07 (m, 6H), 7.04–7.00 (m, 4H), 6.00 (s, 2H), 4.72–4.69 (m, 2H), 4.65 (s, 2H), 3.98 (s, 3H), 3.66–3.63 (m, 2H), 3.15–3.12 (m, 2H), 2.75–2.72 (m, 2H), 2.33 (s, 3H), 2.06–1.99 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.52, 147.10, 146.45, 145.75, 142.21, 139.56, 136.67, 135.28, 135.11, 133.43, 132.80, 130.00 (2C), 129.26, 128.58 (2C), 128.17 (2C), 127.73 (2C), 125.51, 123.76, 120.45, 116.50, 112.90, 108.92, 108.39, 101.90, 56.56, 46.87, 41.02, 36.05, 33.38, 32.94, 28.88, 21.05; HRMS (ESI): calcd for $C_{36}H_{35}N_2O_3$ $[M-Br]^+$ 543.2642; found 543.2660.

5.5.14. 9-N-(3-hydroxypropyl)-13-(4-methylbenzyl) berberine ***29***

Red solid, yield: 66%; mp: 223.1–225.0 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.89 (s, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.16 (d, J = 7.7 Hz, 2H), 7.08–6.95 (m, 4H), 6.00 (s, 2H), 4.81–4.79 (m, 2H), 4.65 (s, 2H), 4.01 (s, 3H), 3.82–3.79 (m, 2H), 3.77–3.73 (m, 2H), 3.18–3.15 (m, 2H), 2.33 (s, 3H), 1.99–1.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.77, 147.25, 147.08, 145.84, 139.65, 136.87, 135.80, 135.20, 133.54, 133.11, 130.17 (2C), 129.91, 127.88 (2C), 123.19, 120.50, 116.93, 114.03, 109.06, 108.57, 102.03, 59.23, 57.39, 56.79, 45.66, 36.24, 33.26, 29.03, 21.18; HRMS (ESI): calcd for $C_{30}H_{31}N_2O_4$ $[M-Br]^+$ 483.2278; found 483.2280.

5.5.15. 9-N-n-pentyl-13-(2-methylbenzyl) berberine ***30***

Red solid, yield: 82%; mp: 216.3–218.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.91 (s, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.24–7.21 (m, 1H), 7.09–7.05 (m, 1H), 6.95 (d, J = 8.8 Hz, 1H), 6.84 (s, 1H), 6.80 (s, 1H), 6.68 (d, J = 7.6 Hz, 1H), 6.61 (br s, 1H), 5.97 (s, 2H), 5.12 (br s, 2H), 4.36 (s, 2H), 3.90 (s, 3H), 3.89–3.84 (m, 2H), 3.14–3.11 (m, 2H), 2.43 (s, 3H), 1.84–1.77 (m, 2H), 1.47–1.31 (m, 4H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.21, 149.44, 148.56, 147.00, 140.15, 138.18, 137.55, 137.33, 134.78, 134.61, 131.92, 131.75, 128.76, 128.22, 127.66, 123.70, 121.91, 118.56, 116.77, 109.40, 109.31, 103.53, 58.49, 57.35, 51.17, 35.57, 32.05, 30.23, 29.39, 23.59, 19.90, 14.45; HRMS (ESI): calcd for $C_{32}H_{35}N_2O_3$ $[M-Br]^+$ 495.2642; found 495.2658.

5.5.16. 9-N-n-pentyl-13-(3-methylbenzyl) berberine ***31***

Red solid, yield: 85%; mp: 225.4–227.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.88 (s, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.25–7.21 (m, 1H), 7.09 (d, J = 7.6 Hz, 1H), 7.05 (d, J = 8.9 Hz, 1H), 6.95 (s, 1H), 6.91–6.88 (m, 2H), 6.83 (s, 1H), 6.59 (br s, 1H), 5.98 (s, 2H), 5.10 (br s, 2H), 4.52 (s, 2H), 3.90 (s, 3H), 3.87–3.82 (m, 2H), 3.11–3.10 (m, 2H), 2.31 (s, 3H), 1.83–1.76 (m, 2H), 1.45–1.30 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.53, 147.10, 146.46, 145.66, 139.83, 139.09, 138.20, 135.34, 133.36, 132.86, 129.19, 129.16, 128.51, 127.83, 124.94, 123.40, 120.47, 116.22, 112.77, 108.93, 108.40, 101.89, 56.75, 56.71, 47.70, 36.41, 31.24, 29.16, 28.88, 22.52, 21.52, 14.14; HRMS (ESI): calcd for $C_{32}H_{35}N_2O_3$ $[M-Br]^+$ 495.2642; found 495.2660.

5.5.17. 9-N-n-pentyl-13-(3,4-dimethylbenzyl) berberine ***32***

Red solid, yield: 81%; mp: 199.3–201.3 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.87 (s, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 7.09 (d, J = 7.7 Hz, 1H), 7.05 (s, 1H), 6.96–6.94 (m, 2H), 6.84 (d, J = 7.9 Hz, 1H), 6.00 (s, 2H), 4.88–4.85 (m, 2H), 4.61 (s, 2H), 4.00 (s, 3H), 3.70–3.67 (m, 2H), 3.19–3.16 (m, 2H), 2.25 (s, 3H), 2.22 (s, 3H), 1.79–1.72 (m, 2H), 1.47–1.36 (m, 4H), 0.93 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CD_3OD/CDCl_3$) δ 150.76, 148.67, 148.13, 146.21, 139.61, 138.25, 136.75, 136.72, 135.97, 134.07, 133.90, 131.54, 131.09, 129.86, 126.13, 123.25, 121.30, 117.98, 116.38, 109.62, 108.99, 103.00, 58.17, 57.12, 50.61, 36.64, 31.70, 29.80, 29.22, 23.18, 19.98, 19.43, 14.31; HRMS (ESI): calcd for $C_{33}H_{37}N_2O_3$ $[M-Br]^+$ 509.2799; found 509.2780.

5.5.18. 9-N-n-pentyl-13-(4-trifluoromethylbenzyl) berberine ***33***

Red solid, yield: 79%; mp: 204.2–206.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.85 (s, 1H), 7.62 (s, 1H), 7.60 (s, 1H), 7.40 (d, J = 8.9 Hz,

1H), 7.27 (s, 1H), 7.25 (s, 1H), 6.92 (d, J = 8.8 Hz, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 6.61 (br s, 1H), 5.99 (s, 2H), 5.08 (br s, 2H), 4.63 (s, 2H), 3.90 (s, 3H), 3.88–3.83 (m, 2H), 3.14–3.11 (m, 2H), 1.82–1.75 (m, 4H), 1.42–1.33 (m, 2H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.74, 147.20, 146.52, 146.33, 142.53, 140.31, 135.55, 133.09, 132.85, 129.43 (q, $J_{C\text{-}F}$ = 32.4 Hz), 128.35 (2C), 127.73, 126.29 (q, $J_{C\text{-}F}$ = 3.7 Hz, 2C), 124.00 (q, $J_{C\text{-}F}$ = 270.2 Hz), 123.42, 120.23, 116.07, 112.18, 108.62, 108.50, 102.03, 56.74, 56.59, 47.68, 36.39, 31.22, 29.15, 28.91, 22.51, 14.12; HRMS (ESI): calcd for $C_{32}H_{32}N_2O_3F_3$ $[M-Br]^+$ 529.2360; found 529.2365.

5.5.19. 9-N-n-pentyl-13-(4-hydroxylbenzyl) berberine ***34***

Red solid, yield: 68%; mp: 218.7–220.6 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.81 (s, 1H), 7.70 (d, J = 9.0 Hz, 1H), 7.42 (d, J = 9.0 Hz, 1H), 7.06 (s, 1H), 7.00–6.91 (m, 3H), 6.75 (s, 1H), 6.73 (s, 1H), 5.98 (s, 2H), 4.80 (br s, 2H), 4.56 (s, 2H), 3.98 (s, 3H), 3.67–3.63 (m, 2H), 3.16–3.13 (m, 2H), 1.76–1.69 (m, 2H), 1.43–1.34 (m, 4H), 0.92 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.43, 151.19, 149.38, 148.51, 146.81, 140.02, 137.21, 134.66, 134.53, 132.15, 130.68, 130.16 (2C), 123.65, 121.89, 118.69, 117.02, 116.97 (2C), 109.88, 109.23, 103.48, 58.42, 57.30, 51.08, 36.31, 31.98, 30.19, 29.37, 23.54, 14.39; HRMS (ESI): calcd for $C_{31}H_{33}N_2O_4$ $[M-Br]^+$ 497.2435; found 497.2460.

5.5.20. 9-N-n-pentyl-13-(2-thienylmethyl) berberine ***35***

Red solid, yield: 80%; mp: 176.1–178.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.85 (s, 1H), 7.49 (d, J = 8.9 Hz, 1H), 7.29 (s, 1H), 7.24 (d, J = 8.9 Hz, 1H), 7.08 (s, 1H), 6.96 (s, 1H), 6.86 (s, 1H), 6.67 (s, 1H), 6.55 (br s, 1H), 6.04 (s, 2H), 5.10 (br s, 2H), 4.64 (s, 2H), 3.94 (s, 3H), 3.89–3.84 (m, 2H), 3.14–3.11 (m, 2H), 1.83–1.76 (m, 2H), 1.43–1.34 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.65, 147.24, 146.47, 146.20, 141.16, 140.03, 134.99, 133.07, 132.97, 128.75, 127.45, 126.03, 124.98, 123.59, 120.25, 116.36, 112.33, 108.91, 108.43, 102.01, 56.78, 56.50, 47.61, 31.68, 31.17, 29.13, 28.88, 22.49, 14.12; HRMS (ESI): calcd for $C_{29}H_{31}N_2O_3S$ $[M-Br]^+$ 487.2050; found 487.2033.

5.5.21. 9-N-n-pentyl-13-(2-pyridylmethyl) berberine ***36***

Red solid, yield: 78%; mp: 143.6–145.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.77 (s, 1H), 8.54 (s, 1H), 7.74–7.70 (m, 1H), 7.37 (d, J = 8.9 Hz, 1H), 7.29 (s, 1H), 7.27 (s, 1H), 7.24–7.20 (m, 1H), 6.98 (d, J = 8.9 Hz, 1H), 6.84 (s, 1H), 6.39 (br s, 1H), 5.98 (s, 2H), 5.08 (br s, 2H), 4.71 (s, 2H), 3.88 (s, 3H), 3.84–3.79 (m, 2H), 3.13–3.10 (m, 2H), 1.78–1.72 (m, 2H), 1.39–1.30 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.80, 150.02, 149.59, 147.14, 146.37, 146.01, 140.04, 137.45, 135.59, 133.20, 132.97, 128.76, 123.43, 123.20, 122.19, 120.70, 116.50, 112.45, 109.17, 108.39, 101.90, 56.77, 56.42, 47.79, 39.23, 31.16, 29.13, 28.97, 22.50, 14.13; HRMS (ESI): calcd for $C_{30}H_{32}N_3O_3$ $[M-Br]^+$ 482.2438; found 482.2439.

5.5.22. 9-N-n-pentyl-13-(2-ethoxy-2-oxoethyl) berberine ***37***

Red solid, yield: 60%; mp: 146.2–148.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.81 (s, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.28 (s, 1H), 7.15 (d, J = 8.8 Hz, 1H), 6.88 (s, 1H), 6.51 (br s, 1H), 6.09 (s, 2H), 5.05 (br s, 2H), 4.36–4.30 (m, 2H), 4.17 (s, 2H), 3.97 (s, 3H), 3.88–3.83 (m, 2H), 3.11–3.08 (m, 2H), 1.78–1.76 (m, 2H), 1.39–1.33 (m, 7H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.83, 149.84, 147.46, 146.67, 145.97, 140.35, 135.66, 133.30, 133.07, 124.80, 123.21, 120.22, 115.83, 110.71, 109.22, 108.58, 102.11, 62.15, 56.83, 56.39, 47.93, 37.12, 31.18, 29.09, 28.82, 22.49, 14.25, 14.10; HRMS (ESI): calcd for $C_{28}H_{33}N_2O_5$ $[M-Br]^+$ 477.2384; found 477.2376.

5.6. Synthesis of 9-N-n-pentyl-13-(carboxymethyl) berberine ***38***

Compound **37** (0.28 g, 0.5 mmol) was dissolved in warm MeOH (12 mL) and 30% aqueous NaOH (7.5 mL) and the mixture was heated to reflux for 1 h. The reaction mixture was concentrated under reduced pressure and treated with 20 mL of water. The solution was extracted three times with CH_2Cl_2, and the combined extracts were dried over Na_2SO_4. The organic phase was evaporated under reduced pressure and crude product was recrystallized from EtOAc to obtain compound **38** (0.185 g, 71%) as a red solid; mp: 138.6–139.9 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.62 (s, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.68–7.65 (m, 2H), 6.96 (s, 1H), 6.04 (s, 2H), 4.71–4.68 (m, 2H), 4.09 (s, 2H), 4.02 (s, 3H), 3.55–3.51 (m, 2H), 3.09–3.07 (m, 2H), 1.69–1.62 (m, 2H), 1.39–1.33 (m, 4H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 177.26, 151.01, 149.31, 148.58, 145.78, 139.60, 136.73, 134.99, 134.58, 131.03, 123.62, 122.18, 118.75, 116.11, 111.00, 109.06, 103.42, 58.17, 57.30, 50.92, 41.21, 31.87, 30.18, 29.31, 23.52, 14.39; HRMS (ESI): calcd for $C_{26}H_{29}N_2O_5$ $[M-Br]^+$ 449.2071; found 449.2096.

5.7. General procedure for the synthesis of compounds ***43–45***

Compound **3** (2.65 g, 6.7 mmol) dissolved in CH_3CN (75 mL) and 1, 3-diiodopropane (18.92 g, 64 mmol) was added, then stirred at 80 °C for 6 h. After cooling, the reaction mixture was concentrated under reduced pressure and crude product was purified by column chromatography on silica gel ($CHCl_3$/CH_3OH, 100/1–50/1 v/v) to give 13-(3-iodopropyl) berberine **39** 2.11 g (yield 50%, over two steps); mp: 207.3–209.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.79 (s, 1H), 7.88 (d, J = 9.1 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.23 (s, 1H), 7.03 (s, 1H), 6.01 (s, 2H), 5.10 (br s, 2H), 4.38 (s, 3H), 4.08 (s, 3H), 3.63–3.58 (m, 2H), 3.13–3.11 (m, 2H), 2.93–2.88 (m, 2H), 2.30–2.14 (m, 2H). Compound **39** (370 mg, 0.59 mmol) dissolved in EtOH (36 mL) and various amines (8.8 mmol) was added, then stirred at 78 °C for 4 h. After cooling, the reaction mixture was dried under vacuum and the crude product **40–42** was used for the next reaction without further purification. To a solution of compound **40** (0.5 mmol) in anhydrous ethanol (1 mL), *n*-pentylamine (2.3 mL, 20 mol) were added. The mixture was stirred and heated for 8 h at 78 °C. After the reaction completed, the mixture was concentrated in vacuo and the residual oil was purified on silica gel chromatography ($CHCl_3$/CH_3OH, 100/1–50/1 v/v) to give compound **43**.

5.7.1. 9-N-n-pentyl-13-(3-(piperidin-1-yl) propyl) berberine ***43***

Red solid; yield: 35% (over two steps); mp: 163.2–165.3 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.64 (s, 1H), 7.88 (d, J = 9.1 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.23 (s, 1H), 7.03 (s, 1H), 6.11 (s, 2H), 4.71–4.68 (m, 2H), 4.08 (s, 3H), 3.62–3.59 (m, 2H), 3.51 (d, J = 12.3 Hz, 2H), 3.46–3.42 (m, 2H), 3.22–3.18 (m, 2H), 3.11–3.08 (m, 2H), 2.91 (t, J = 12.3 Hz, 2H), 2.30–2.14 (m, 2H), 1.93–1.90 (m, 2H), 1.85–1.65 (m, 5H), 1.56–1.32 (m, 5H), 0.96–0.84 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.99, 147.65 (2C), 145.41, 138.88, 134.49, 132.90, 131.96, 131.76, 122.09, 120.13, 116.72, 115.34, 109.59, 108.43, 102.31, 57.27, 56.62, 55.75, 52.87 (2C), 49.98, 31.10, 28.99, 28.72, 26.79, 24.46, 22.72 (2C), 22.46, 21.53, 14.05; HRMS (ESI): calcd for $C_{32}H_{42}N_3O_3$ $[M-I]^+$ 516.3221; found 516.3252.

5.7.2. 9-N-n-pentyl-13-(3-morpholinopropyl) berberine ***44***

Compound **41** was treated with *n*-pentylamine according to general procedure to give the desired product **44** as a red solid, yield 33% (over two steps), mp: 152.5–154.3 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.74 (s, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.21 (s, 1H), 7.00 (s, 1H), 6.10 (s, 2H), 4.76–4.74 (m, 2H), 4.04 (s, 3H), 3.69–3.67 (m, 4H), 3.63–3.60 (m, 2H), 3.39–3.35 (m, 2H), 3.12–3.09 (m, 2H), 2.60–2.50 (m, 6H), 2.06–1.98 (m, 2H), 1.72–1.65 (m, 2H), 1.40–1.32 (m, 4H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 150.90, 149.17, 148.48, 146.01, 139.97, 135.88, 134.83, 134.75, 133.76, 123.65, 122.03, 118.54, 116.03, 110.43, 109.30,

103.57, 67.35 (2C), 58.79, 58.38, 57.43, 54.58 (2C), 50.77, 31.94, 30.12, 29.42, 28.30, 27.89, 23.49, 14.43; HRMS (ESI): calcd for $C_{31}H_{40}N_3O_4$ $[M-I]^+$ 518.3013; found 518.3028.

5.7.3. 9-N-n-pentyl-13-(3-(pyrrolidin-1-yl) propyl) berberine ***45***

Compound **42** was treated with *n*-pentylamine according to general procedure to give the desired product **45** as a red solid, yield 42% (over two steps), mp: 149.3–151.3 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.74 (s, 1H), 7.90 (d, $J = 9.0$ Hz, 1H), 7.85 (d, $J = 9.0$ Hz, 1H), 7.21 (s, 1H), 7.01 (s, 1H), 6.10 (s, 2H), 4.76–4.72 (m, 2H), 4.04 (s, 3H), 3.65–3.62 (m, 2H), 3.49–3.45 (m, 2H), 3.43–3.32 (m, 6H), 3.16–3.13 (m, 2H), 2.26–2.20 (m, 2H), 2.10–2.01 (m, 4H), 1.73–1.66 (m, 2H), 1.40–1.32 (m, 4H), 0.91 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 150.92, 149.26, 148.47, 145.98, 140.01, 135.91, 134.99, 134.86, 133.85, 123.67, 122.14, 118.65, 116.10, 110.50, 109.31, 103.57, 58.38, 58.16, 57.44, 55.40, 53.05, 50.84, 49.00, 45.40, 31.95, 30.13, 29.44, 28.46, 28.36, 23.50, 14.41; HRMS (ESI): calcd for $C_{31}H_{40}N_3O_3$ $[M-I]^+$ 502.3064; found 502.3087.

5.8. Biological assay

5.8.1. In vitro antibacterial assay

The strains used in this study were *S. aureus* Newman and five multidrug-resistant *S. aureus* (NRS-1, NRS-70, NRS-100, NRS-108 and NRS-271). All strains were grown at 37 °C overnight in TSB without the antibiotic. Overnight cultures diluted 1000 fold were grown at 37 °C for 2–3 h until A_{600} 0.6. Then bacteria were diluted 1:400 into fresh TSB medium, compounds were prepared in DMSO and diluted serially by two-fold to final concentrations in the range of 0.39–50 μg/mL. Equal volume of bacteria and compound were added to 96 well plates and mixed well by shaking. After 18 h incubation, the A_{600} of each well was visualized by Bioteksynergy2. Experiments were performed three times for each condition.

5.8.2. Cytotoxicity assay

The cytotoxic activity against human fibroblast (HAF) cells *in vitro* was measured using the MTS assay. Stock solutions (25 mg/mL) of the test compounds were dissolved in DMSO. Cells were cultured in a 96-well plate at a density of 1×10^5 cells and different concentrations of compounds were respectively added to each well. The incubation was permitted at 37 °C, 5% CO_2 atmosphere. After incubation for 24 h, the cells were treated with various concentrations of the compound for 24 h. 20 μL MTS reagent was added into each well of the 96-well assay plate containing the compounds in 100 μL of culture medium. The samples were incubated at 37 °C, 5% CO_2 atmosphere for 1–2 h. Response of HAF cells to the test compounds was determined spectrophotometrically at a single wavelength of 490 nm. The assay was measured three times, after which the average of IC_{50} was calculated. The cytotoxicity of each compound was expressed as the concentration of compound that reduced cell viability to 50% (IC_{50}).

5.8.3. Morphological test

The morphology of human fibroblast (HAF) cells was observed using cell HE staining assay. In brief, cells were cultured in a 12-well plate at the appropriate cell densities. After incubation for 24 h, cells were exposed to 1.56 μg/mL and 12.5 μg/mL concentrations of the compounds. After treatment for 24 h, the supernatant medium was removed and the cells were fixed by 95% alcohol. About 30 min later, the plate was washed by water for three times. After dying, the cell nucleuses were stained with 500 μL hematoxylin solution each well for 10 min. After staining, the hematoxylin was removed and the plate was washed by water for three times. After dying, the cells were differentiated with 500 μL 0.3% acid alcohol each well for several seconds until the background was almost colorless. After differentiation, the cells were immersed in 500 μL 0.5% dilute ammonia water for 10 min to make the cell nucleuses turn to blue. Then the plate was washed by water for three times and each well was added 500 μL eosin solutions for 5 min to stain the cytoplasm. After staining, the plate was washed by water for five times until the background was almost colorless. At last, the morphology was observed under a contrast microscope.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.01.012.

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