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Original article

Synthesis, structure–activity relationship and in vitro anti-mycobacterial evaluation of 13-n-octylberberine derivatives

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ABSTRACT

Twenty-eight new 13-n-octylberberine derivatives were synthesized and evaluated for their activities against drug-susceptible *Mycobacterium tuberculosis* (*M. tuberculosis*) strain $H_{37}Rv$. Among these compounds, compound **16e** was the most effective anti-tubercular agent with a MIC value of 0.125 μg/mL. Importantly, compound **16e** exhibited more potent effect against rifampicin (RIF)- and isoniazid (INH)-resistant *M. tuberculosis* strains than both RIF and INH, suggesting a new mechanism of action. Therefore, it has been selected as a drug candidate for further investigation, or as a chemical probe for identifying protein target and studying tuberculosis biology. We consider 13-n-octylberberine analogs to be a promising novel class of antituberculars against multi-drug-resistant (MDR) strains of *M. tuberculosis*.

1. Introduction

Tuberculosis (TB) is more prevalent in the world today than at any other time in human history. It is anticipated that there will be about 9.8 million new TB cases this year, more than in any other year in history [1,2]. Five percent of all TB cases are now estimated to be multi-drug-resistant TB (MDR-TB), which is resistant to at least rifampin (RIF) and isoniazid (INH), the two most important first-line drugs used currently in clinic [3a,3b]. The overall incidence of TB in HIV positive patients is 50 times that of the rate for HIV negative individuals [4–6]. In recent years, the emergence of MDR-TB and extensively-drug-resistant TB (XDR-TB) becomes one of the biggest challenges in the treatment of this disease. Although some TB drug candidates are being currently evaluated in clinical trials, there have been no approved new chemical entities for treatment of TB in the past 40 years [7a,7b]. Therefore, there is an urgent need to discover the novel scaffold of anti-TB candiates with new mechanism of action, or without cross-resistance with current anti-mycobacterial drugs [7b].

In search for new chemical classes of anti-mycobacterial agents, the compound libraries constructed in our laboratories were screened against drug-susceptible *Mycobacterium tuberculosis* (*M. tuberculosis*) strain $H_{37}Rv$ with RIF and INH as reference drugs [8]. 13-n-Octylberberine (**1**, Fig. 1), identified from the library of berberines, appeared to show a moderate anti-tubercular activity with a MIC of 2.0 μg/mL (Table 1). In particular, compound **1** demonstrated a mild antimycobaterial activity (Table 2) against RIF- and INH-resistant strains isolated from patients in China (MIC = 4.0 μg/mL), suggesting a new mechanism of action [9]. Therefore, the new chemical entity and ideal actitity with no cross resistance of hit **1** provoked our strong interest to explore the structure–activity relationship (SAR) so as to discover candidates with novel mode of action for treatment of TB, especially MDR-TB.

Among the 13-substituted berberine analogs, compounds **2**–**9** (Fig. 1) showed no anti-mycobacterial activities against *M. tuberculosis* $H_{37}Rv$ in comparison with **1**. The primary SAR analysis revealed that n-octyl side-chain at the 13-position of **1** might be essential for the bactericidal activity. Therefore, in the present study we retained the n-octyl group at position 13, and focused our SAR study on the variation of substituents at positions 2-, 3- and/or 9-position(s) with **1** as the lead. On the basis of this

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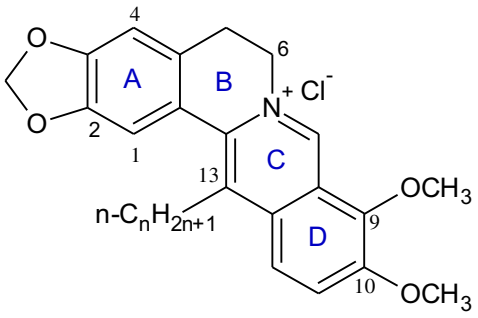
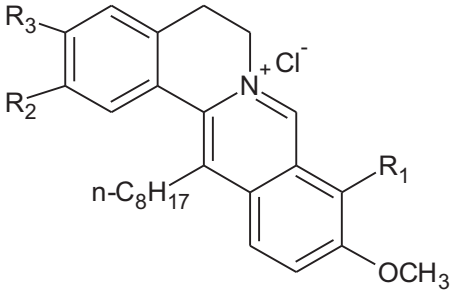


compound **1**: n=8
compounds **2–9**: n=0-7

Fig. 1. Chemical structures of compounds **1**–**9**.

strategy, 28 new 13-n-octylberberine analogs were designed, synthesized and evaluated for their in vitro anti-tubercular activities against drug-susceptible and drug-resistant stains of *M. tuberculosis*.

Table 1
Structures and anti-mycobacterial activities of aimed compounds against *M. tuberculosis* strain H37Rv.



Compd	R_1	R_2	R_3	MIC[a] (μg/mL)
1	OCH_3		OCH_2O	2.0
11	OH		OCH_2O	4.0
12a	OCH_2CH_3		OCH_2O	4.0
12b	n-OC_3H_7		OCH_2O	2.0
12c	n-OC_4H_9		OCH_2O	8.0
12d	OCH_2Ph		OCH_2O	4.0
12e	*p*–CH_3–PhCOO		OCH_2O	4.0
12f	*p*–CH_3O–PhCOO		OCH_2O	4.0
12g	*p*–CF_3–$PhSO_3$		OCH_2O	16.0
12h	*p*–CN–$PhSO_3$		OCH_2O	8.0
13	OCH_3	OH	OH	16.0
14a	OCH_3	OH	OCH_3	2.0
14b	OCH_3	OH	OCH_2CH_3	2.0
14c	OCH_3	OH	n-OC_3H_7	4.0
14d	OCH_3	OH	n-OC_4H_9	8.0
14e	OCH_3	OH	OCH_2Ph	2.0
14f	OCH_3	OCH_3	OCH_3	2.0
14g	OCH_3	OCH_2CH_3	OCH_2CH_3	0.5
14h	OCH_3	n-OC_3H_7	n-OC_3H_7	2.0
14i	OCH_3	n-OC_4H_9	n-OC_4H_9	2.0
14j	OCH_3	OCH_2Ph	OCH_2Ph	8.0
14k	OCH_3	OCH_2CH_2O		2.0
15	OH	OH	OH	32.0
16a	OCH_2CH_3	OH	OCH_2CH_3	4.0
16b	n-OC_3H_7	OH	n-OC_3H_7	4.0
16c	n-OC_4H_9	OH	n-OC_4H_9	8.0
16d	OCH_2Ph	OH	OCH_2Ph	0.5
16e	OCH_2CH_3	OCH_2CH_3	OCH_2CH_3	0.125
16f	OCH_2Ph	OCH_2Ph	OCH_2Ph	8.0
INH				0.0625
RIF				0.0625

[a] MIC: minimum inhibitory concentration.

Table 2
In vitro anti-tubercular activities of the key compounds against MDR strains of *M. tuberculosis*[a] (MIC: μg/mL[b])

Compd	44	83	164	431	926	$H_{37}Rv$
1	4	4	4	4	ND	2.0
14g	4	4	4	ND	4	0.5
16d	0.5	1.0	1.0	0.25	0.5	0.5
16e	0.25	0.5	0.125	0.5	0.125	0.125
RIF	64	> 64	> 64	> 64	> 32	0.0625
INH	4	4	4	64	32	0.0625

[a] MDR strains were isolated from patients with tuberculosis in China.
[b] MIC: minimum inhibitory concentration.

2. Chemistry

Twenty-eight 13-n-octylberberine analogs were synthesized with commercially available berberine (**2**) as the starting material as described in Scheme 1, which includes three synthetic methods (Route A, B and C). The intermediate **10** was obtained via a selective reduction reaction, in which $NaBH_4$ was used as a reducing agent and methanol as the solvent [10]. Then, the intermediate **10** reacted with n-octyl aldehyde in the solvent mixture of EtOH (80%) and HOAc, and then acidified with 2 N HCl to yield the desired compound **1** in a good yield [10,11].

Compound **1** was heated at 195–210 °C for 10–15 min under vacuum (30–40 mmHg) to afford the black oil [12], which was recrystallized with ethanol/concentrated HCl (95:5) to afford **11**. The desired products **12a**–**d** (Route A) were obtained through nucleophilic substitution of **11** with different RX (X = Cl, Br, I) respectively in yields of 36–40%, in which K_2CO_3/KOH was used as the base and DMF as the solvent [13–15]. The products **12e**–**h** were prepared by esterification of **11** with the different acyl chloride, using pyridine as the base and acetonitrile as the solvent with yields of 21%–47% [16–17]. The key intermidiate **13** (Route B) was synthesized by demethyl reaction of compound **1** with phloroglucinol in H_2SO_4 (60%) at 90–95 °C [18]. In the third synthetic route (Route C), another key intermediate **15** was gotten through demethyl reaction of **1** with $AlCl_3$ (4.4 eq) in CH_2Cl_2 [19–21]. The final compounds in **12**, **14** and **16** series were purified by flash chromatography using CH_2Cl_2/CH_3OH (95: 5) as the gradient eluent.

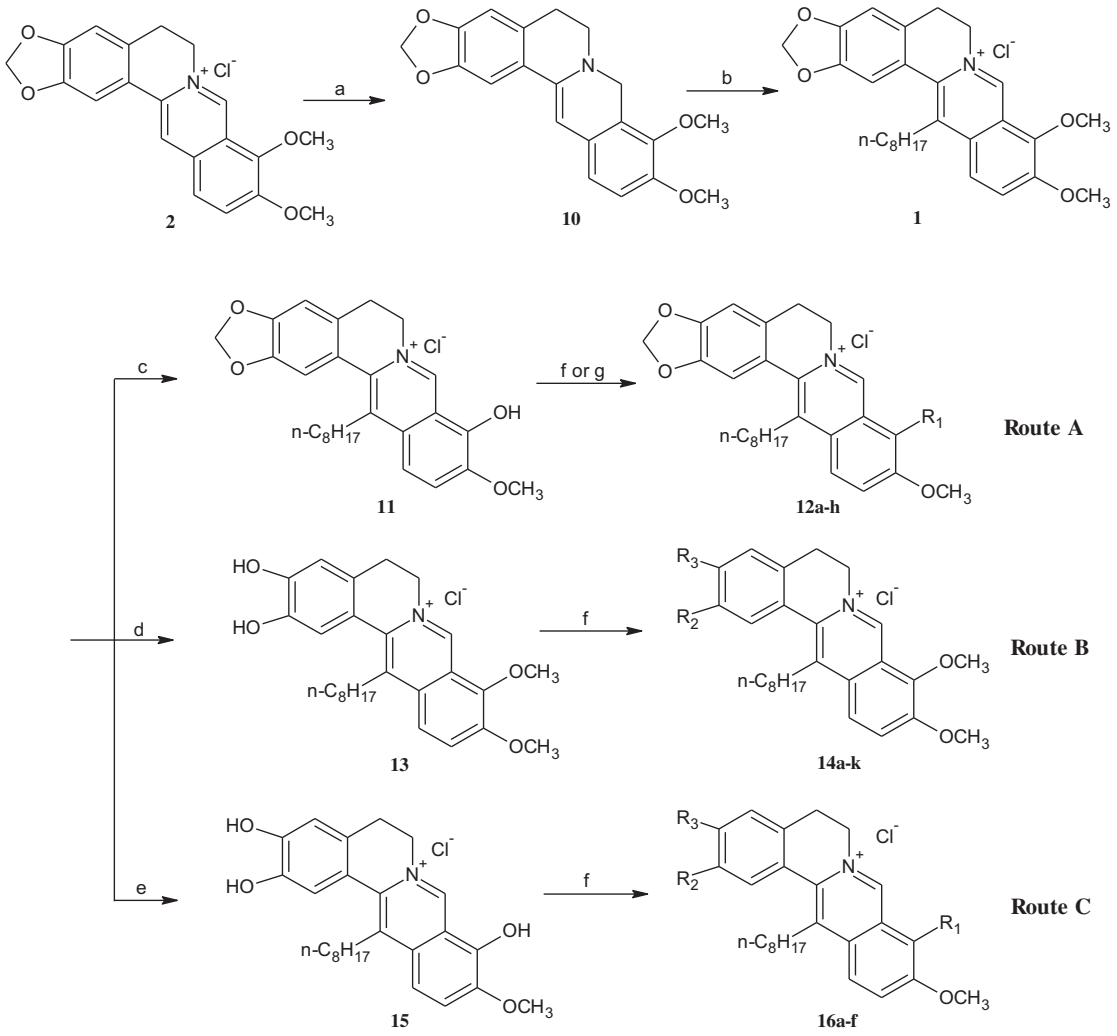
3. Results and discussion

3.1. SAR analysis for anti-mycobacterial activity

Twenty-eight 13-substituted berberine derivatives were first evaluated for their anti-tubercular activities against the multiplication of drug-susceptible *M. tuberculosis* strain $H_{37}Rv$ with the microplate alamar blue assay (MABA). RIF and INH were used as reference drugs. Structures of 28 analogs and their anti-mycobacterial activities are shown in Table 1.

SAR study was first focused on the substituents at the 9-position on the aromatic ring D of **1**. Replacement methoxyl at the 9-position with hydroxyl, ethoxyl, n-propoxy, n-butoxy, benzyloxy, benzoyloxy or benzenesulfonyl respectively, generated nine new analogs (**11**, **12a**–**h**). The result showed that compound **12b** bearing an ethoxyl afforded a mediated anti-tubercular activity at a MIC value of 2.0 μg/mL, similar to that of the lead. The other compounds (**11**, **12a**, **12c**–**h**) showed decreased activities with MIC ranges between 4.0 and 16.0 μg/mL, regardless of the size of the side-chains.

Next, SAR analysis was moved to the modifications at the 2- and 3-positions on the ring A. The oxacyclopentene was opened, the resulting compound **13** bearing 2,3-dihydroxyl almost lost the activity. A methoxyl, ethoxyl, n-propoxy, n-butoxy or benzyloxy



Scheme 1. Reagents and conditions for the chemical synthesis: (a) $NaBH_4$, NaOH; (b) octyl aldehyde, acetic acid, 80% ethanol; (c) 195 °C, 30–40 mmHg, 10–15 min; (d) phloroglucinol, 60% H_2SO_4, 90–95 °C; (e) $AlCl_3$ (4.4 eq), rt, CH_2Cl_2; (f) RX, K_2CO_3/KOH, 70 °C; (g) RCOX, pyridine, CH_3CN, reflux.

was attached at the 3-position of **13**, respectively, with which five new analogs (**14a**–**e**) were prepared and tested. Compounds **14a**–**b** and **14e** afforded a similar anti-mycobacterial activity to the lead with a MIC of 2.0 μg/mL. Then, introduction of a methoxyl, ethoxyl, n-propoxy, n-butoxy, benzyloxy or ethylenedioxy at the positions 2 and 3 of **13** simultaneously, produced six compounds **14f**–**k**. The results showed that compounds **14f**, **14h**–**i** and **14k** demonstrated a moderate activity at a MIC of 2.0 μg/mL. Compound **14g** possessing 2, 3-diethoxyl exhibited an increased activity with a MIC value of 0.5 μg/mL, greater than that of the lead. It seemed that introducting a substituent at the 2- and/or 3-position(s) might be benefitable for the anti-mycobacterial activity.

In another variation, SAR study was carried out to explore the substituents at positions 2, 3 and 9. The substituents including a hydroxyl, ethoxyl, n-propoxy, n-butoxy or benzyloxy were added at the 2-, 3- and 9-positions, respectively, by which seven new analogs (**15**, **16a**–**f**) were made and tested. Compounds **16d**–**e** showed a potent activity at a MIC of 0.5 and 0.125 μg/mL, respectively, in comparison with the lead. Especially, compound **16e** possessing tri-ethoxyl exhibited the strongest anti-tubercular activity among the synthezied compounds, with 16-fold increase over that of the lead. Thus, attachment of the suitable substituents at the 2- 3- and 9-positions, especially an ethoxyl, might significantly improve the activity against *M. tuberculosis* strain.

3.2. Anti-resistance TB effect of the key compounds

As compounds **14g**, **16d** and **16e** possessed excellent activity against drug-susceptible *M. tuberculosis* strain $H_{37}Rv$ with MIC values below 0.5 μg/mL, they were further examined the effect on the MDR strains of *M. tuberculosis*. In this experiment, *M. tuberculosis* strains 44, 83, 164, 431 and 926 isolated from patients with tuberculosis in China, were resistant to both RIF and INH. As described in Table 2, RIF and INH showed a decreased activity against the drug-resistant stains partially or completely with a MIC range between 4 and >64 μg/mL. However, compounds **16d** and **16e** inhibited the drug-resistant strains with a potency (MIC = 0.125–1.0 μg/mL) similar to its effect on drug-susceptible strain (MIC = 0.125–0.5 μg/mL). The results indicated that compounds **16d** and **16e** were active for either drug-susceptible *M. tuberculosis* or those resistant to RIF and INH, suggesting a novel mechanism of action against TB.

4. Conclusion

In conclusion, 28 new 13-n-octylberberines with different substituents on the ring A and D were synthesized and evaluated for their anti-mycobacterial activities against *M. tuberculosis* $H_{37}Rv$ with **1** as the lead. SAR analysis revealed that (i) n-octyl group at

position 13 might be essential for the activity; (ii) introduction of substituents at the 2-, 3- and/or 9-positions, especially an ethoxyl, might significantly enhance the activity. Among the test compounds, compound **16e** exhibited a potential activity against both drug-susceptible and MDR strains of *M. tuberculosis*, suggesting a novel mode of action against TB. Thus, it has been selected as a drug candidate for further investigation. In particular, compound **16e** might be used as a chemical probe for identifying new protein targets, unraveling mechanism of action as well as studing TB biology. We consider 13-n-octylberberines to be a promising new class of anti-tubercular agents with an advantage of inhibiting MDR strains of *M. tuberculosis*.

5. Experimental section

5.1. Chemistry

Melting point (mp) was obtained with CXM-300 melting point apparatus and uncorrected. The ^{1}H NMR spectra was performed on a Varian Inova 400 MHz spectrometer (Varian, San Francisco, CA) and ^{13}C NMR on a Bruker Avance III 400 spectrometer in CD_3OD or DMSO-d_6, with Me_4Si as the internal standard. ESI high-resolution mass spectra (HRMS) were recorded on an Autospec Ultima-TOF mass spectrometer (Micromass UK Ltd, Manchester, UK). Flash chromatography was performed on CombiflashRf 200 (Teledyne, Nebraska, USA), particle size 0.038 mm.

5.2. 2,3-Methylenedioxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**1**)

To a stirred solution of BBR (7.43 g, 20 mmol) and K_2CO_3 (8.30 g, 60 mmol) in methanol (250 mL), 5% NaOH (10 mL) solution containing $NaBH_4$ (0.80 g, 21 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 h and the precipitated product was filtered, washed with water (20 mL) and 30% ethanol (20 mL), and then recrystallized from absolute ethanol to provide the intermediate **10** as a dark green solid.

To a stirred solution of the intermediate **10** (5.0 g, 15 mmol) in 80% ethanol (200 mL), n-octyl aldehyde (10 mL), HOAc (50 mL) was added. The reaction mixture was heated to 85–95 °C for 5 h. The solvent was removed by evaporation to get the red oil. Diethyl ether (200 mL) was added to this system and stirred at room temperature for 2 h. After filtration, the diethyl ether layer was collected and concentrated, and the residue was acidified by 2% HCl, stirred at room temperature for 0.5 h. The solid was obtained by filtration to afford the title compound **1** (4.6 g, 48%) as a yellow solid. mp: 111–113 °C ^{1}H NMR (CD_3OD) δ: 0.84 (t, 3H, J = 6.8 Hz), 1.24–1.28 (m, 8H), 1.39–1.41 (m, 2H), 1.79–1.81 (m, 2H), 3.06 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.4 Hz), 4.07 (s, 3H), 4.15 (s, 3H), 4.73 (t, 2H, J = 5.6 Hz), 6.07 (s, 2H), 6.98 (s, 1H), 7.24 (s, 1H), 8.10 (d, 2H, J = 9.6 Hz), 9.72 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.2, 149.0, 146.5, 144.3, 144.2, 135.7, 134.2, 134.0, 132.2, 125.9, 121.4, 121.2, 120.3, 109.1, 108.3, 102.1, 62.0, 57.0 (2), 31.2 (2), 30.4, 28.8, 28.5, 28.3, 27.3, 22.0, 13.9. HRMS: calcd for $C_{28}H_{34}NO_4Cl$ $[M-Cl]^+$ 448.2484, found 448.2506.

5.3. 2,3-Methylenedioxy-9-hydroxy-10-methoxy-13-n-octylprotoberberine chloride (**11**)

Compound **1** (1.2 g, 2.48 mmol) was heated at 195–210 °C for 10–15 min under vacuum (30–40 mmHg) to afford the black oil, which was acidified with ethanol/concentrated HCl (95:5). The solvent was removed by evaporation, the residue was collected and then purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH as the gradient eluent, affording the title compound **11** (1.14 g, 98%) as a dark red soild. mp: 122–124 °C ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 7.2 Hz), 1.23–1.41 (m, 10H), 1.82 (s, 2H), 3.04 (t, 2H, J = 6.0 Hz), 3.33 (t, 2H, J = 8.4 Hz), 4.03 (s, 3H), 4.67 (t, 2H, J = 6.0 Hz), 6.06 (s, 2H), 6.96 (s, 1H), 7.23 (s, 1H), 7.79 (d, 1H, J = 9.2 Hz), 7.96 (d, 1H, J = 9.2 Hz), 9.72 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 148.8, 146.5, 145.2, 144.6, 144.4, 134.8, 133.9, 133.6, 131.6, 124.6, 120.5, 117.2, 115.9, 109.1, 108.3, 102.0, 56.9, 56.7, 31.2, 30.2, 28.9, 28.6, 28.5, 28.4, 27.5, 22.0, 13.9. HRMS: calcd for $C_{27}H_{32}NO_4Cl$ $[M-Cl]^+$ 434.2331, found 434.2340.

5.4. General procedure for the synthesis of compounds **12a–d** (Route A)

To a stirred solution of **11** (200 mg, 0.43 mmol) and KOH (78.4 mg, 1.4 mmol) in DMF (10 mL), RX (1.0–1.5 eq) was added. The reaction mixture was stirred at 70 °C for 5–6 h. The solvent was removed by evaporation, the residue was acidified by 2 N HCl, the soild was collected by filtration and then purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH (95:5) as the gradient eluent, affording the title compounds **12a–d**. Compounds **12a–d** were gained following the same procedure using purchased alkyl halide as material.

5.4.1. 2,3-Methylenedioxy-9-ethoxy-10-methoxy-13-n-octylprotoberberine chloride (**12a**)

Compound **11** (200 mg, 0.43 mmol) was treated with ethyl bromide (37.3 μl , 0.5 mmol) according to the general procedure to give the desired product **12a** [8a,8c] as a brown solid, yield: 36%; mp: 86–88 °C; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.84 (t, 3H, J = 7.2 Hz), 1.24–1.48 (m, 13H), 1.81 (t, 2H, J = 7.6 Hz), 3.06 (t, 2H, J = 6.0 Hz), 3.37 (t, 2H, J = 8.0 Hz), 4.06 (s, 3H), 4.43 (q, 2H, J = 6.8, 14.2 Hz), 4.74 (t, 2H, J = 6.0 Hz), 6.07 (s, 2H), 6.98 (s, 1H), 7.24 (s, 1H), 8.08, 8.12 (dd, 2H, J = 9.6 Hz), 9.67 (s, 1H); ^{13}C NMR (CD_3OD) δ: 151.8, 151.3, 148.7, 145.3, 145.1, 137.7, 136.3, 135.1, 134.4, 127.0, 123.5, 122.1, 121.8, 110.4, 109.3, 103.7, 71.5, 58.9, 57.5, 32.9, 31.9, 30.5, 30.3 (2), 30.1, 29.3, 23.6, 15.9, 14.4. HRMS: calcd for $C_{29}H_{36}NO_4Cl$ $[M-Cl]^+$ 462.2644, found 462.2635.

5.4.2. 2,3-Methylenedioxy-9-n-propoxy-10-methoxy-13-n-octylprotoberberine chloride (**12b**)

Compound **11** (150 mg, 0.32 mmol) was treated with propyl iodide (47 μl, 0.48 mmol) according to the general procedure to give the desired product **12b** as a brown solid, yield: 39%; mp: 95–97 °C; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.84 (t, 3H, J = 6.8 Hz), 1.06 (t, 3H, J = 7.6 Hz), 1.24–1.26 (m, 10H), 1.41 (t, 2H, J = 7.2 Hz), 1.79–1.92 (m, 2H), 3.06 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 7.6 Hz), 4.06 (s, 3H), 4.31 (t, 2H, J = 6.8 Hz), 4.74 (t, 2H, J = 5.6 Hz), 6.07 (s, 2H), 6.98 (s, 1H), 7.24 (s, 1H), 8.08, 8.12 (dd, 2H, J = 9.2 Hz), 9.63 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.2, 149.0, 146.5, 144.1, 143.4, 135.7, 134.2, 134.0, 132.2, 125.8, 121.4, 121.2, 120.3, 109.1, 108.3, 102.1, 75.9, 57.1, 56.9, 31.2, 30.4, 28.9, 28.6, 28.5, 28.3, 27.4, 22.8, 22.0, 13.9, 10.2. HRMS: calcd for $C_{30}H_{38}NO_4Cl$ $[M-Cl]^+$ 476.2801, found 476.2806.

5.4.3. 2,3-Methylenedioxy-9-n-butoxy-10-methoxy-13-n-octylprotoberberine chloride (**12c**)

Compound **11** (150 mg, 0.32 mmol) was treated with butyl iodide (55 μl, 0.48 mmol) according to the general procedure to give the desired product **12c** as a brown solid, yield: 39%; mp: 114–116 °C; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.84 (t, 3H, J = 6.8 Hz), 0.98 (t, 3H, J = 7.6 Hz), 1.23–1.88 (m, 16H), 3.06 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.0 Hz), 4.06 (s, 3H), 4.31 (t, 2H, J = 6.4 Hz), 4.74 (t, 2H, J = 5.6 Hz), 6.07 (s, 2H), 6.98 (s, 1H), 7.24 (s, 1H), 8.08, 8.11 (dd, 2H, J = 9.2 Hz), 9.60 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.2, 149.0, 146.5, 144.1, 143.4, 135.7, 134.2, 134.0, 132.2, 125.8, 121.4, 121.2, 120.3, 109.1, 108.3, 102.1, 74.0, 57.1, 56.9, 31.5, 31.2, 30.3, 28.9, 28.6,

28.5, 28.3, 27.4, 22.0, 18.5, 13.9, 13.7. HRMS: calcd for $C_{31}H_{40}NO_4Cl$ $[M-Cl]^+$ 490.2957, found 490.2969.

5.4.4. 2,3-Methylenedioxy-9-benzyloxy-10-methoxy-13-n-octylprotoberberine chloride (**12d**)

Compound **11** (150 mg, 0.32 mmol) was treated with benzyl bromide (42 μl, 0.35 mmol) according to the general procedure to give the desired product **12d** as a brown solid, yield: 40%; mp: 102–103 °C; ^{1}H NMR (400 MHz, CD_3OD) δ: 0.84 (t, 3H, J = 6.8 Hz), 1.12–1.40 (m, 10H), 1.78–1.80 (m, 2H), 3.00 (t, 2H, J = 5.6 Hz), 3.36 (s, 2H), 4.11 (s, 3H), 4.61 (t, 2H, J = 5.6 Hz), 5.40 (s, 2H), 6.06 (s, 2H), 6.96 (s, 2H), 7.21–7.29 (m, 4H), 7.42 (d, 1H, J = 6.4 Hz), 8.11 (s, 2H), 9.42 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.5, 149.0, 146.5, 144.2, 142.6, 136.4, 135.7, 134.2, 134.0, 132.1, 128.8 (2), 128.4, 128.3 (2), 125.7, 121.7, 121.6, 120.2, 109.1, 108.3, 102.1, 75.5, 57.1, 57.0, 31.2, 30.4, 28.8, 28.6 (2), 28.3, 27.4, 22.0, 13.9. HRMS: calcd for $C_{34}H_{38}NO_4Cl$ $[M-Cl]^+$ 524.2801, found 524.2811.

5.5. General procedure for the synthesis of compounds **12e–h** (Route A)

To a solution of CH_3CN (5 mL) was added **11** (105 mg, 0.22 mmol) at room temperature, then heated to form a dark-red solution, to which was then added anhydrous pyridine (20 μl) and acyl chloride or sulfuryl chloride. The resulting mixture was refluxed at 75–80 °C for 5–6 h and cooled to precipitate completely. The crude product was filtered and purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH (95:5) as the gradient eluent. Compounds **12e–h** were gained following the same procedure using purchased acyl chloride or sulfuryl chloride as material.

5.5.1. 2,3-Methylenedioxy-9-O-(4-methylbenzoyl)-10-methoxy-13-n–octylprotoberberine chloride (**12e**)

Compound **11** (108 mg, 0.23 mmol) was treated with anhydrous pyridine (20 μl, 0.25 mmol) and 4-methylbenzoyl chloride (134 ul, 1.0 mmol), according to the general procedure to give the desired product **12e** as a brown solid, yield: 47%; mp: 118–121 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.25–1.31 (m, 8H), 1.44 (t, 2H, J = 6.8 Hz), 1.86 (m, 2H), 2.45 (s, 3H), 3.03 (t, 2H, J = 5.6 Hz), 3.44 (t, 2H, J = 8.0 Hz), 4.03 (s, 3H), 4.71 (t, 2H, J = 5.6 Hz), 6.08 (s, 2H), 6.97 (s, 1H), 7.27 (s, 1H), 7.40 (d, 2H, J = 8.0 Hz), 8.14 (d, 2H, J = 8.0 Hz), 8.21, 8.41 (dd, 2H, J = 9.6 Hz), 9.65 (s, 1H); ^{13}C NMR (CD_3OD) δ: 165.4, 152.3, 151.5, 148.8, 147.0, 144.0, 138.5, 137.1, 136.6, 135.2, 134.4, 131.8, 131.3, 130.7, 130.2, 126.7, 126.2, 125.9, 123.3, 121.7, 110.5, 109.3, 103.8, 59.0, 57.7, 33.0, 32.2, 30.6, 30.3 (2), 30.1, 29.1, 23.7, 21.8, 14.4. HRMS: calcd for $C_{35}H_{38}NO_5Cl$ $[M-Cl]^+$ 552.2750, found 552.2743.

5.5.2. 2,3-Methylenedioxy-9-O-(4-methoxylbenzoyl)-10-methoxy-13-n-octylprotoberberine chloride (**12f**)

Compound **11** (129 mg, 0.27 mmol) was treated with anhydrous pyridine (24 μl, 0.30 mmol) and 4-methoxylbenzoyl chloride (0.21 g , 1.23 mmol), according to the general procedure to give the desired product **12f** as a brown solid, yield: 43%; mp: 118–120 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.25–1.29 (m, 8H), 1.44 (t, 2H, J = 6.8 Hz), 1.86 (m, 2H), 3.03 (t, 2H, J = 5.6 Hz), 3.44 (t, 2H, J = 8.0 Hz), 3.89 (s, 3H), 4.03 (s, 3H), 4.72 (t, 2H, J = 5.6 Hz), 6.07 (s, 2H), 6.97 (s, 1H), 7.09 (d, 2H, J = 8.8 Hz), 7.27 (s, 1H), 8.20 (m, 3H), 8.40 (d, 1H, J = 9.6 Hz), 9.64 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 164.2, 163.0, 150.2, 149.1, 146.6, 143.4, 136.4, 134.7, 134.4, 134.2, 132.7, 132.1, 131.3, 125.0, 124.7, 121.2, 120.2, 120.0, 114.4, 113.8, 109.2, 108.3, 102.1, 57.2, 57.0, 55.8, 31.2 (2), 30.5, 28.9, 28.6, 28.4, 27.2, 22.0, 13.9. HRMS: calcd for $C_{35}H_{38}NO_6Cl$ $[M-Cl]^+$ 568.2699, found 568.2685.

5.5.3. 2,3-Methylenedioxy-9-O-(4-trifluoromethylbenzenesulfonyl)-10-methoxy-13-n- octylprotoberberine chloride (**12g**)

Compound **11** (110 mg, 0.23 mmol) was treated with anhydrous pyridine (20 μl, 0.25 mmol) and 4-trifluoromethyl-benzenesulfonyl chloride (0.24 g, 1.0 mmol), according to the general procedure to give the desired product **12g** as a brown solid, yield: 21%; mp: 103–104 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.24–1.27 (m, 8H), 1.42 (t, 2H, J = 6.8 Hz), 1.82 (t, 2H, J = 6.8 Hz), 3.09 (t, 2H, J = 5.6 Hz), 3.42 (t, 2H, J = 8.0 Hz), 3.67 (s, 3H), 4.83 (s, 2H), 6.09 (s, 2H), 7.02 (s, 1H), 7.29 (s, 1H), 7.67 (d, 2H, J = 8.4 Hz), 7.92 (d, 2H, J = 8.0 Hz), 8.01, 8.17 (dd, 2H, J = 8.4 Hz), 9.62 (s, 1H); ^{13}C NMR (CD_3OD) δ: 153.0, 151.8, 150.3, 148.9, 143.9, 140.8, 139.2, 137.4, 135.3, 134.9 (3), 127.7 (3), 126.4 (2), 126.1, 124.0, 121.5, 110.5, 109.4, 103.9, 59.4, 57.3, 33.0, 32.2, 30.7, 30.3 (2), 30.1, 29.0, 23.7, 14.4. HRMS: calcd for $C_{34}H_{35}F_3NO_6SCl$ $[M-Cl]^+$ 642.2137, found 642.2171.

5.5.4. 2,3-Methylenedioxy-9-O-(4-cyanobenzenesulfonyl)-10-methoxy-13-n-octylprotoberberine chloride (**12h**)

Compound **11** (105 mg, 0.22 mmol) was treated with anhydrous pyridine (20 μl, 0.25 mmol) and 4-cyano-benzenesulfonyl chloride (0.20 g, 1.0 mmol), according to the general procedure to give the desired product **12h** as a brown solid, yield: 40%; mp: 99–100 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.24–1.28 (m, 8H), 1.41 (m, 2H), 1.81 (m, 2H), 3.09 (t, 2H, J = 5.6 Hz), 3.42 (t, 2H, J = 8.0 Hz), 3.70 (s, 3H), 4.83 (s, 2H), 6.09 (s, 2H), 7.01 (s, 1H), 7.27 (s, 1H), 7.73 (d, 2H, J = 8.0 Hz), 7.89 (d, 2H, J = 8.4 Hz), 8.04, 8.14 (dd, 2H, J = 8.4 Hz), 9.62 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 152.6, 151.0, 149.4, 146.6, 142.5, 138.7, 137.2, 135.2, 134.2, 133.8, 132.0, 131.3, 129.3, 126.4, 125.2, 121.6, 120.0, 118.6, 117.6, 111.0, 109.2, 108.3, 102.2, 57.3, 56.8, 31.2, 30.6, 29.0, 28.5 (2), 28.3, 27.1, 22.0, 13.9. HRMS: calcd for $C_{34}H_{35}N_2O_6ClS$ $[M-Cl]^+$ 599.2216, found 599.2237.

5.6. 2,3-Dihydroxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**13**)

To a stirred solution of 60% H_2SO_4 (100 mL), phloroglucin (1.16 g, 7.14 mmol) was added portionwise to form a colorless solution, then compound **1** (1.00 g, 2.07 mmol) was added portionwise and the mixture was stirred at 90–95 °C for 10–15 min. Then the mixture was poured into saturated brine (20 mL) immediately with violent stirring, and the resulting mixture was stirred at room temperature for 2 h and cooled down to precipitate completely, filtration, water washing, filter cake layer was adjusted to neutral with NaOH (1 N), then adjusted to acidity with HCl (2 N), stirred, filtration, collecting solid precipitation, recrystallization with ethanol/concentrated HCl (95:5) to afford the title compound **13** (0.86 g, 88%) as a dark red soild. mp: 114.4–116.0 °C; ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.24–1.29 (m, 8H), 1.42–1.44 (m, 2H), 1.78 (m, 2H), 2.97 (t, 2H, J = 5.6 Hz), 3.30 (t, 2H, J = 8.0 Hz), 4.07 (s, 6H), 4.75 (t, 2H, J = 5.6 Hz), 6.85 (s, 1H), 7.18 (s, 1H), 8.13, 8.17 (dd, 2H, J = 9.6 Hz), 9.83 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 149.8, 148.0, 144.2, 144.1 (2), 136.5, 133.2, 132.4, 130.3, 125.9, 121.1, 121.0, 117.8, 116.5, 114.7, 62.0, 57.4, 57.0, 31.2, 30.6, 28.9, 28.8, 28.7, 28.5, 26.7, 22.0, 13.9. HRMS: calcd for $C_{27}H_{34}NO_4Cl$ $[M-Cl]^+$ 436.2488, found 436.2480.

5.7. General procedure for the synthesis of compounds **14a–k** (Route B)

To a stirred solution of **13** (300 mg , 0.62 mmol) and KOH (139 mg , 2.48 mmol) in DMF (10 mL), RX (1.0 or > 2.0 eq) was added. The reaction mixture was stirred at 70 °C for 5–6 h. The solvent was removed by evaporation, the residue was acidified by 2 N HCl, the soild was collected by filtration and then purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH (95:5) as

the gradient eluent, affording the title compounds **14a**–**k**. Compounds **14a**–**k** were gained following the same procedure using purchased alkyl halide as material.

*5.7.1. 2-Hydroxy-3,9,10-trimethoxy-13-n-octylprotoberberine chloride (**14a**)*

Compound **13** (150 mg , 0.32 mmol) was treated with methyl iodide (20 μl , 0.32 mmol), according to the general procedure to give the desired product **14a** as a brown solid, yield: 29%; mp: 88–90 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.24 (m, 8H), 1.43–1.45 (m, 2H), 1.83 (m, 2H), 3.06 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.0 Hz), 3.93 (s, 3H), 4.07 (s, 3H), 4.15 (s, 3H), 4.74 (s, 2H), 7.04 (s, 1H), 7.25 (s, 1H), 8.09 (s, 2H), 9.70 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.0, 149.7, 145.4, 144.3, 144.2, 136.1, 133.6, 132.3, 130.0, 125.9, 121.2, 121.1, 119.1, 116.2, 111.3, 62.0, 57.3, 57.0, 55.8, 31.2 (2), 30.5, 28.9, 28.7, 28.4, 26.9, 22.0, 13.9. HRMS: calcd for $C_{28}H_{36}NO_4Cl$ $[M-Cl]^+$ 450.26443, found 450.26432.

*5.7.2. 2-Hydroxy-3-ethoxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14b**)*

Compound **13** (150 mg , 0.32 mmol) was treated with ethyl bromide (23.8 μl , 0.32 mmol), according to the general procedure to give the desired product **14b** as a brown solid, yield: 23%; mp: 92–93 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 7.2 Hz), 1.24–1.27 (m, 8H), 1.38–1.46 (m, 5H), 1.83 (m, 2H), 3.05 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.0 Hz), 4.06 (s, 3H), 4.15 (s, 3H), 4.16–4.21 (m, 2H), 4.73 (t, 2H, J = 5.6 Hz), 7.02 (s, 1H), 7.25 (s, 1H), 8.09 (s, 2H), 9.69 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.0, 148.8, 145.3, 144.2 (2), 136.1, 133.6, 132.3, 130.2, 125.9, 121.2, 121.1, 119.0, 116.1, 112.0, 64.0, 62.0, 57.3, 57.0, 31.2 (2), 30.5, 28.8, 28.7, 28.4, 26.9, 22.0, 14.6, 13.9. HRMS: calcd for $C_{29}H_{38}NO_4Cl$ $[M-Cl]^+$ 464.2801, found 464.2798.

*5.7.3. 2-Hydroxy-3-n-propoxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14c**)*

Compound **13** (150 mg , 0.32 mmol) was treated with propyl iodide (31.2 μl , 0.32 mmol), according to the general procedure to give the desired product **14c** as a brown solid, yield: 29%; mp: 93–94 °C; ^{1}H NMR (DMSO-d_6) δ: 0.84 (t, 3H, J = 6.8 Hz), 1.01 (t, 3H, J = 7.2 Hz), 1.22–1.27 (m, 10H), 1.40 (m, 2H), 1.74–1.81 (m, 4H), 3.02 (s, 2H), 4.03 (t, 2H, J = 6.4 Hz), 4.07 (s, 6H), 4.78 (s, 2H), 7.06 (s, 1H), 7.26 (s, 1H), 8.14, 8.18 (dd, 2H, J = 9.6 Hz), 9.87 (s, 1H); ^{13}C NMR (CD_3OD) δ: 159.5, 158.6, 155.0, 153.7, 145.7, 143.2, 141.9, 139.7, 135.5, 134.5, 130.8, 130.7, 128.6, 125.8, 121.7, 79.4, 71.6, 66.5, 66.7, 40.8 (2), 40.0, 38.4, 38.3, 38.0, 36.5, 31.6, 31.5, 23.5, 20.0. HRMS: calcd for $C_{30}H_{40}NO_4Cl$ $[M-Cl]^+$ 478.2957, found 478.2954.

*5.7.4. 2-Hydroxy-3-n-butoxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14d**)*

Compound **13** (150 mg , 0.32 mmol) was treated with butyl iodide (36.3 μl , 0.32 mmol), according to the general procedure to give the desired product **14d** as a brown solid, yield: 28%; mp: 71–73 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 0.95 (t, 3H, J = 7.2 Hz), 1.22–1.28 (m, 10H), 1.42–1.51 (m, 4H), 1.73–1.79 (m, 4H), 3.03 (t, 2H, J = 5.6 Hz), 3.33 (s, 2H), 4.08 (s, 6H), 4.78 (s, 2H), 7.09 (s, 1H), 7.23 (s, 1H), 8.17 (s, 2H), 9.31 (s, 1H), 9.85 (s, 1H); ^{13}C NMR (CD_3OD) δ: 149.8, 148.9, 145.2, 144.1, 144.0, 136.0, 133.5, 132.2, 130.0, 125.8, 121.1, 121.0, 118.8, 116.0, 111.9, 67.9, 61.9, 57.3, 56.9, 31.1, 30.5, 30.3, 28.7, 28.6, 28.4, 28.3, 26.8, 21.9, 18.5, 13.8, 13.5. HRMS: calcd for $C_{31}H_{42}NO_4Cl$ $[M-Cl]^+$ 492.3114, found 492.3117.

*5.7.5. 2-Hydroxy-3-benzyloxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14e**)*

Compound **13** (200 mg , 0.41 mmol) was treated with benzyl bromide (49.3 μl , 0.41 mmol), according to the general procedure to give the desired product **14e** as a brown solid, yield: 22%; mp: 113–115 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.13–1.27 (m, 8H), 1.44–1.46 (m, 2H), 1.83 (m, 2H), 3.00 (s, 2H), 3.36 (t, 2H, J = 8.0 Hz), 4.06 (s, 3H), 4.14 (s, 3H), 4.71 (s, 2H), 5.26 (s, 2H), 7.06 (s, 2H), 7.28 (d, 1H, J = 9.2 Hz), 7.34 (m, 3H), 7.46 (d, 1H, J = 7.6 Hz), 8.09 (s, 2H), 9.68 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.0, 148.6, 145.5, 144.2, 144.1, 136.6, 136.0, 133.7, 132.3, 130.0, 128.4 (2), 127.9, 127.7 (2), 125.9, 121.2, 121.1, 119.4, 116.5, 112.7, 69.9, 62.0, 57.3, 56.9, 31.2 (2), 30.5, 28.9, 28.7, 28.4, 26.9, 22.0, 13.9. HRMS: calcd for $C_{34}H_{40}NO_4Cl$ $[M-Cl]^+$ 526.2957, found 526.2942.

*5.7.6. 2,3,9,10-Tetramethoxy-13-n-octylprotoberberine chloride (**14f**)*

Compound **13** (106 mg , 0.22 mmol) was treated with methyl iodide (47 μl , 0.75 mmol), according to the general procedure to give the desired product **14f** as a brown solid, yield: 31%; mp: 89–92 °C; ^{1}H NMR (CD_3OD) δ: 0.84 (t, 3H, J = 6.8 Hz), 1.41–1.46 (m, 10H), 1.87 (t, 2H, J = 7.6 Hz), 3.10 (t, 2H, J = 6.0 Hz), 3.43 (t, 2H, J = 6.4 Hz), 3.87 (s, 3H), 3.90 (s, 3H), 4.07 (s, 3H), 4.16 (s, 3H), 4.74 (s, 2H), 7.08 (s, 1H), 7.34 (s, 1H), 8.10 (s, 2H), 9.73 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.7, 150.0, 147.3, 144.4, 144.2, 135.9, 133.7, 132.3, 132.2, 126.0, 121.2, 121.1, 119.0, 112.7, 111.2, 62.0, 57.2, 57.0 (2), 55.8, 31.2, 30.9, 29.1, 28.7, 28.6, 26.9, 22.1, 22.0, 13.9. HRMS: calcd for $C_{29}H_{38}NO_4Cl$ $[M-Cl]^+$ 464.2801, found 464.2785.

*5.7.7. 2,3-Diethoxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14g**)*

Compound **13** (400 mg , 0.83 mmol) was treated with ethyl bromide (631 μl , 8.3 mmol), according to the general procedure to give the desired product **14g** as a brown solid, yield: 35%; mp: 85–86 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.24–1.29 (m, 8H), 1.38–1.47 (m, 8H), 1.84 (t, 2H, J = 7.2 Hz), 3.08 (t, 2H, J = 5.6 Hz), 3.39 (t, 2H, J = 8.0 Hz),4.06–4.18 (m, 4H), 4.07 (s, 3H), 4.15 (s, 3H), 4.74 (s, 2H), 7.06 (s, 1H), 7.34 (s, 1H), 8.10 (s, 2H), 9.72 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.3, 150.0, 146.5, 144.4, 144.2, 135.9, 133.6, 132.4, 132.3, 126.0, 121.2, 121.1, 118.9, 114.6, 111.2, 64.4, 64.0, 62.0, 57.2, 57.0, 31.2, 30.9, 29.3, 29.1, 28.7, 28.6, 26.9, 22.0, 14.8, 14.6, 13.9. HRMS: calcd for $C_{31}H_{42}NO_4Cl$ $[M-Cl]^+$ 492.3114, found 492.3113.

*5.7.8. 2,3-Di-n-propoxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14h**)*

Compound **13** (150 mg , 0.32 mmol) was treated with propyl iodide (100 μl , 1.03 mmol), according to the general procedure to give the desired product **14h** as a brown solid, yield: 43%; mp: 67–69 °C; ^{1}H NMR (CD_3OD) δ: 0.90 (t, 3H, J = 6.8 Hz), 1.06–1.14 (m, 10H), 1.30–1.34 (m, 5H), 1.52 (m, 2H), 1.81–1.93 (m, 5H), 3.13 (t, 2H, J = 5.6 Hz), 3.43 (t, 2H, J = 8.0 Hz), 3.92–4.10 (m, 4H), 4.12 (s, 3H), 4.21 (s, 3H), 4.80 (t, 2H, J = 5.2 Hz), 7.11 (s, 1H), 7.39 (s, 1H), 8.15 (s, 2H), 9.77 (s, 1H); ^{13}C NMR (CD_3OD) δ: 153.2, 151.7, 149.1, 146.3, 145.2, 137.9, 135.8, 134.6, 133.9, 127.2, 123.1, 122.1, 120.6, 117.2, 113.5, 72.9, 71.7, 62.7, 59.2, 57.5, 33.0, 32.4, 30.9, 30.5, 30.4, 30.3, 28.8, 23.9, 23.7, 23.6, 14.4, 10.9, 10.8. HRMS: calcd for $C_{33}H_{46}NO_4Cl$ $[M-Cl]^+$ 520.3427, found 520.3433.

*5.7.9. 2,3-Di-n-butoxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14i**)*

Compound **13** (150 mg , 0.32 mmol) was treated with butyl iodide (220 μl , 1.94 mmol), according to the general procedure to give the desired product **14i** as a brown solid, yield: 44%; mp: 123–125 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 0.94–0.99 (m, 6H), 1.24–1.29 (m, 8H), 1.46–1.55 (m, 6H), 1.73–1.88 (m, 6H), 3.08 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.0 Hz), 4.02–4.11 (m, 4H), 4.07 (s, 3H), 4.15 (s, 3H), 4.74 (t, 2H, J = 5.6 Hz), 7.06 (s, 1H), 7.34 (s, 1H), 8.10 (s, 2H), 9.72 (s, 1H); ^{13}C NMR (CD_3OD) δ: 153.3, 151.7, 149.1, 146.3, 145.2, 138.0, 135.9, 134.6, 133.9, 127.2, 123.1, 122.1, 120.6, 117.2, 113.5, 71.2, 70.0, 62.7, 59.2, 57.6, 33.1, 32.7, 32.4, 32.3, 30.9 (2), 30.6,

30.5, 28.9, 23.7, 20.4, 20.3, 14.5, 14.2, 14.1. HRMS: calcd for $C_{35}H_{50}NO_4Cl$ $[M-Cl]^+$ 548.3740, found 548.3728.

*5.7.10. 2,3-Dibenzyloxy-9,10-dimethoxy-13-n-octylprotoberberine chloride (**14j**)*

Compound **13** (150 mg , 0.32 mmol) was treated with benzyl bromide (230 μl , 1.94 mmol), according to the general procedure to give the desired product **14j** as a brown solid, yield: 51%; mp: 71–73 °C; ^{1}H NMR (CD_3OD) δ: 0.80 (t, 3H, J = 7.2 Hz), 1.12–1.28 (m, 12H), 1.72 (m, 2H), 3.06 (t, 2H, J = 5.6 Hz), 4.06 (s, 3H), 4.14 (s, 3H), 4.72 (t, 2H, J = 5.6 Hz), 5.18 (s, 2H), 5.24 (s, 2H), 7.19 (s, 1H), 7.27–7.48 (m, 11H), 8.08 (s, 2H), 9.70 (s, 1H); ^{13}C NMR (CD_3OD) δ: 153.0, 151.7, 148.6, 146.3, 145.2, 138.3, 138.0, 137.7, 136.2, 134.5, 134.3, 129.7 (3), 129.6, 129.3 (2), 128.7 (4), 127.2, 123.1, 122.2, 121.2, 118.7, 114.5, 73.1, 72.1, 62.7, 59.0, 57.6, 33.0 (2), 32.3, 30.5 (3), 28.9, 23.7, 14.5. HRMS: calcd for $C_{41}H_{46}NO_4Cl$ $[M-Cl]^+$ 616.3427, found 616.3474.

*5.7.11. 2,3-Ethylenedioxy-9,10-dimethoxy −13-n-octylprotoberberine chloride (**14k**)*

Compound **13** (300 mg , 0.62 mmol) was treated with 1,2-dibromoethane (267 μl , 3.1 mmol), according to the general procedure to give the desired product **14k** as a brown solid, yield: 42%; mp: 110–112 °C; ^{1}H NMR (DMSO-d_6) δ: 0.86 (t, 3H, J = 6.8 Hz), 1.25–1.29 (m, 8H), 1.44–1.47 (m, 2H), 1.79 (m, 2H), 3.05 (t, 2H, J = 5.6 Hz), 3.30 (t, 2H, J = 8.8 Hz), 4.08 (s, 6H), 4.32 (d, 2H, J = 4.8 Hz), 4.36 (d, 2H, J = 4.8 Hz), 4.80 (s, 2H), 7.05 (s, 1H), 7.25 (s, 1H), 8.19 (s, 2H), 9.88 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.2, 145.2, 144.5, 144.2, 142.1, 135.4, 134.1, 132.1, 132.0, 125.9, 121.3, 121.2, 119.8, 118.0, 116.2, 64.6, 64.1, 62.0, 57.3, 57.0, 31.2, 30.4, 28.9, 28.6, 28.5, 28.3, 26.6, 22.0, 13.9. HRMS: calcd for $C_{29}H_{36}NO_4Cl$ $[M-Cl]^+$ 462.2644, found 462.2632.

*5.8. 2,3,9-Trihydroxyl-10-methoxy-13-n-octylprotoberberine chloride (**15**)*

To a stirred solution of **1** (2.0 g , 4.14 mmol) in CH_2Cl_2 (100 mL), $AlCl_3$ (2.43 g , 18.2 mmol) was added. The reaction mixture was stirred at room temperature for 3 days. The solvent was removed by evaporation, and the residue was acidified by 4 N HCl, and then was redissolved in MeOH. The solvent was removed by evaporation, and the residue was purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH as the gradient eluent, affording the title compound **15** (1.5 g , 80%) as a dark brown soild. mp: 128–130 °C; ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.25–1.29 (m, 8H), 1.43 (m, 2H), 1.79 (m, 2H), 2.94 (t, 2H, J = 5.6 Hz), 3.27 (t, 2H, J = 7.2 Hz), 4.03 (s, 3H), 4.71 (s, 2H), 6.84(s, 1H), 7.17 (s, 1H), 7.19, 8.05 (dd, 2H, J = 9.2 Hz), 9.32 (s, 1H), 9.84 (s, 1H), 9.89 (s, 1H), 11.20 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 148.6, 145.5, 145.0, 144.9 (2), 136.3, 133.3, 132.7, 130.9, 125.4, 118.6, 117.8, 117.4, 116.3, 115.5, 57.8, 57.6, 32.0 (2), 31.0, 29.6, 29.5, 29.2, 27.6, 22.8, 14.7. HRMS: calcd for $C_{26}H_{32}NO_4Cl$ $[M-Cl]^+$ 422.2331, found 422.2321.

*5.9. General procedure for the synthesis of compounds **16a–f** (Route C)*

To a stirred solution of **15** (300 mg , 0.66 mmol) and KOH (140 mg , 2.50 mmol) in DMF (10 mL), RX (>2.0 eq) was added. The reaction mixture was stirred at 70 °C for 5–6 h. The solvent was removed by evaporation, the residue was acidified by 2 N HCl, the soild was collected by filtration and then purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH (95:5) as the gradient eluent, affording the title compounds **16a–f**. Compounds **16a–f** were gained following the same procedure using purchased alkyl halide as material.

*5.9.1. 2-Hydroxyl-3,9-diethoxy-10-methoxy-13-n-octylprotoberberine chloride (**16a**)*

Compound **15** (200 mg , 0.44 mmol) was treated with ethyl bromide (300 μl , 4.02 mmol), according to the general procedure to give the desired product **16a** as a brown solid, yield: 35%; mp: 90–92 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 7.2 Hz), 1.24–1.29 (m, 8H), 1.38–1.47 (m, 8H), 1.87 (m, 2H), 3.06 (t, 2H, J = 5.6 Hz), 3.35 (t, 2H, J = 8.0 Hz), 4.04 (s, 3H), 4.07–4.18 (m, 4H), 4.71 (t, 2H, J = 5.6 Hz), 7.05 (s, 1H), 7.33 (s, 1H), 7.82, 7.98 (dd, 2H, J = 8.2 Hz), 9.74 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.1, 146.4, 145.0, 144.6, 144.2, 135.0, 133.0, 132.3, 131.8, 124.7, 119.1, 117.2, 115.7, 114.6, 114.2, 64.4, 64.0, 56.9 (2), 31.2, 30.7, 29.1, 28.7, 28.6, 27.0, 22.0, 15.3, 14.8, 14.6, 13.9. HRMS: calcd for $C_{30}H_{40}NO_4Cl$ $[M-Cl]^+$ 478.2957, found 478.2959.

*5.9.2. 2-Hydroxyl-3,9-di-n-propoxy-10-methoxy-13-n-octylprotoberberine chloride (**16b**)*

Compound **15** (200 mg , 0.44 mmol) was treated with propyl iodide (293 μl, 3.00 mmol), according to the general procedure to give the desired product **16b** as a yellow solid, yield: 45%; mp: 178–179 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 7.2 Hz), 1.02–1.08 (m, 6H), 1.24–1.29 (m, 8H), 1.43–1.47 (m, 2H), 1.81–1.92 (m, 6H), 3.05 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.0 Hz), 4.05 (s, 3H), 4.08 (t, 2H, J = 6.8 Hz), 4.32 (t, 2H, J = 6.8 Hz), 4.75 (t, 2H, J = 5.6 Hz), 7.02 (s, 1H), 7.25 (s, 1H), 8.08 (s, 2H), 9.61 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.0, 149.0, 145.3, 144.0, 143.4, 136.1, 133.6, 132.4, 130.3, 125.9, 121.3, 121.0, 119.0, 116.2, 112.1, 75.8, 69.8, 57.5, 57.0, 31.2 (2), 30.5, 28.8, 28.7, 28.4, 26.9, 22.8, 22.0, 21.9, 13.9, 10.4, 10.2. HRMS: calcd for $C_{32}H_{44}NO_4Cl$ $[M-Cl]^+$ 506.3270, found 506.3266.

*5.9.3. 2-Hydroxyl-3,9-di-n-butoxy-10-methoxy-13-n-octylprotoberberine chloride (**16c**)*

Compound **15** (165 mg , 0.36 mmol) was treated with butyl iodide (340 μl , 3.0 mmol), according to the general procedure to give the desired product **16c** as a yellow solid, yield: 5%; mp: 151–153 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 7.2 Hz), 0.94–1.00 (m, 6H), 1.24–1.29 (m, 8H), 1.45–1.57 (m, 6H), 1.78–1.88 (m, 6H), 3.05 (t, 2H, J = 5.6 Hz), 3.37 (t, 2H, J = 8.0 Hz), 4.06 (s, 3H), 4.08–4.14 (m, 2H), 4.34–4.38 (m, 2H), 4.74 (t, 2H, J = 5.6 Hz), 7.06 (s, 1H), 7.34 (s, 1H), 8.08 (s, 2H), 9.60 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 149.8, 148.0, 144.2, 143.9, 143.3, 136.4, 133.2, 132.5, 130.3, 125.8, 121.2, 120.9, 117.8, 116.5, 114.7, 74.0, 57.5, 57.0 (2), 35.8, 31.5, 31.3, 30.7, 30.6, 28.9 (2), 28.7, 28.5, 26.7, 18.7, 18.5, 14.0, 13.7 (2). HRMS: calcd for $C_{34}H_{48}NO_4Cl$ $[M-Cl]^+$ 534.3588, found 534.3568.

*5.9.4. 2-Hydroxy-3,9-dibenzyloxy-10-methoxy-13-n-octylprotoberberine chloride (**16d**)*

Compound **15** (150 mg , 0.33 mmol) was treated with benzyl bromide (356 μl , 3.0 mmol), according to the general procedure to give the desired product **16d** as a yellow solid, yield: 15%; mp: 171–173 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 6.8 Hz), 1.24–1.29 (m, 8H), 1.41–1.43 (m, 2H), 1.80–1.82 (m, 2H), 2.95 (t, 2H, J = 5.6 Hz), 3.33 (t, 2H, J = 8.0 Hz), 4.10 (s, 3H), 4.58 (t, 2H, J = 5.6 Hz), 5.25 (s, 2H), 5.39 (s, 2H), 7.04 (s, 1H), 7.24–7.47 (m, 11H), 8.10 (s, 2H), 9.38 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.3, 148.7, 145.6, 144.2, 142.5, 136.7, 136.4, 135.9, 133.7, 132.3, 129.9, 128.8, 128.4 (3), 128.3 (3), 127.9, 127.8, 127.4, 125.7, 121.5, 121.4, 119.4, 116.5, 112.7, 75.4, 69.9, 57.5, 57.0, 31.2 (2), 30.5, 28.9, 28.7, 28.4, 26.9, 22.0, 13.9. HRMS: calcd for $C_{40}H_{44}NO_4Cl$ $[M-Cl]^+$ 602.3270, found 602.3252.

*5.9.5. 2,3,9-Triethoxy-10-methoxy-13-n-octylprotoberberine chloride (**16e**)*

Compound **15** (300 mg , 0.66 mmol) was treated with ethyl bromide (485 μl , 6.5 mmol), according to the general procedure to give the desired product **16e** as a brown solid, yield: 39%; mp:

71–73 °C; ^{1}H NMR (CD_3OD) δ: 0.85 (t, 3H, J = 7.2 Hz), 1.24–1.29 (m, 9H), 1.38–1.47 (m, 10H), 1.86 (m, 2H), 3.08 (t, 2H, J = 5.6 Hz), 3.39 (t, 2H, J = 8.0 Hz), 4.06 (s, 3H), 4.07–4.18 (m, 4H), 4.41–4.46 (m, 2H), 4.76 (t, 2H, J = 5.6 Hz), 7.06 (s, 1H), 7.34 (s, 1H), 8.09 (s, 2H), 9.67 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 150.2, 150.1, 146.4, 144.3, 143.1, 135.8, 133.5, 132.3, 132.2, 125.8, 121.5, 121.0, 118.8, 114.5, 112.1, 70.0, 64.4, 64.0, 57.2, 56.9, 31.2, 30.8, 29.2, 29.1, 28.7, 28.6, 26.9, 22.0, 15.3, 14.7, 14.5, 13.9. HRMS: calcd for $C_{32}H_{44}NO_4Cl$ $[M-Cl]^+$ 506.3270, found 506.3258.

*5.9.6. 2,3,9- Tribenzyloxy –10-methoxy-13-n-octylprotoberberine chloride (**16f**)*

Compound **15** (204 mg , 0.45 mmol) was treated with benzyl bromide (336 μl , 4.5 mmol), according to the general procedure to give the desired product **16f** as a dark yellow solid, yield: 28%; mp: 168–170 °C; ^{1}H NMR (DMSO-d_6) δ: 0.79 (t, 3H, J = 6.8 Hz), 1.29–1.45 (m, 10H), 1.88 (s, 2H), 3.07 (s, 2H), 3.25 (s, 2H), 4.10 (s, 3H), 4.79 (s, 2H), 5.20 (s, 2H), 5.27 (s, 2H), 5.35 (s, 2H), 7.30–7.60 (m, 17H), 8.19 (s, 2H), 9.75 (s, 1H); ^{13}C NMR (CD_3OD) δ: 153.0, 152.2, 148.6, 145.0, 144.4, 138.3, 138.0, 137.8, 137.5, 136.1, 134.3, 134.2, 130.3 (2), 129.8, 129.7 (7), 129.6, 129.2, 128.7 (3), 126.8, 123.8, 122.6, 121.1, 118.6, 114.4, 77.1, 73.1, 72.0, 59.1, 57.5, 33.0 (2), 32.2, 30.5 (3), 28.9, 23.7, 14.4. HRMS: calcd for $C_{47}H_{50}NO_4Cl$ $[M-Cl]^+$ 692.3740, found 692.3728.

6. Biological activity

RIF and INH were purchased from Sigma Company. All of 13-n-octylberberine analogs were examined for their activities against the multiplication of drug-susceptible *M. tuberculosis* strain $H_{37}Rv$ with MABA at different concentration. Subsequent two-fold dilutions were performed in 100 μL of 7H9 media in the 48-well microplates, then added 100 μL of bacterial suspension to wells. The amount of bacteria to each well is 4×10^{-4} mg/mL. Plates were incubated at 37 °C. At optimal time, alamar blue solution was added to the entire plate. Results were recorded at 24 h post-reagent addition. Visual MIC was defined as the lowest concentration of drug that prevented a color change.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.03.012.

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