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SYNTHESIS AND *IN VITRO* TRIGLYCERIDE-LOWERING ACTIVITY OF 2,3-DISUBSTITUTED BERBERINE DERIVATIVES

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Abstract – Twenty-six 2,3-disubstituted berberine derivatives were designed，synthesized, and evaluated for their triglyceride-lowering activities *in vitro*. The results showed that most 2,3-disubstituted berberine derivatives had triglyceride-lowering activities in HepG2 cells. Among these compounds, compound **3c** had the highest triglyceride-lowering activity with an inhibition rate of 63%, much higher than berberine. Therefore, we believed that forming an octatomic ring at R^1 and R^2 in berberine might be beneficial for triglyceride-lowering activity, which will provide a new idea for the development of berberine-like lipid-lowering agents with new structures.

INTRODUCTION

In recent years, cardiovascular diseases (CVDs) have seriously threatened human health and become one of the main causes of global mortality. It is reported that hyperlipidemia is a major factor leading to CVDs.[1] At present, the drugs used to reduce blood lipids in clinic are mainly divided into five categories, including statins,[2] nicotinic acids,[3] fibrous acid derivatives,[4] bile acid chelators and cholesterol absorption inhibitors.[5,6] However, these lipid-lowering drugs have certain side effects, such as rhabdomyolysis or gastrointestinal discomfort.[7-9] Therefore, the development of new lipid-lowering drugs has always been the research hotspot of medical workers all over the world.

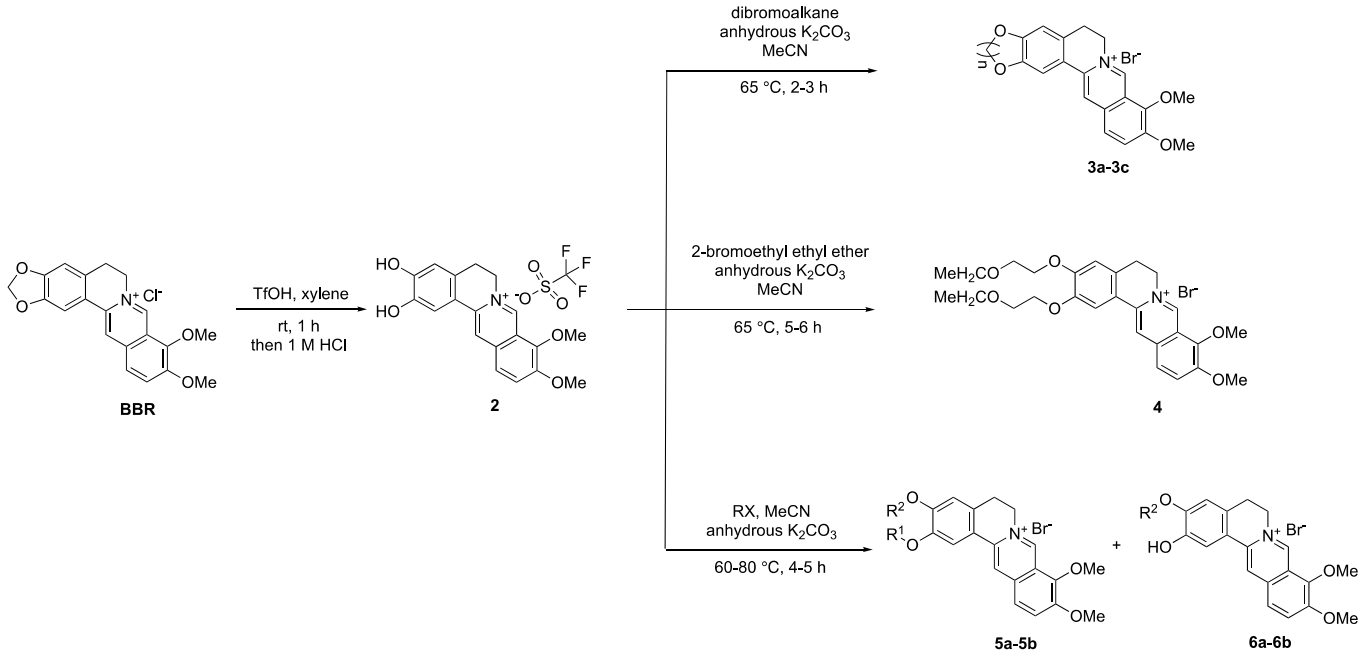
Berberine is a naturally isoquinoline alkaloid, which exists in many medicinal plants, such as *Coptidis rhizoma*, *Phellodendron amurense* and *Berberis aristate*.[10,11] In China, berberine is commonly used to treat diarrhea and gastrointestinal disorders.[12] In recent years, studies have shown that berberine has a wide range of pharmacological activities, including blood lipids regulation, antibacterial, antiviral, antitumor, neuroprotection, anti-inflammatory.[13,14] Especially, berberine has a very broad prospect as a

lipid-lowering agent. The reduction of triglyceride is an important manifestation of berberine's blood lipid lowering effect.[15]

However, the clinical application of berberine is greatly limited due to the poor solubility and low bioavailability.[16] Therefore, the structural modification of berberine has become a research hotspot of many researchers, in order to solve the shortcomings of berberine and improve its curative effect. In the past, most structural modifications mainly focused on the C8, C9, C12, C13 positions of berberine. There were few reports on the structural modification of berberine C2 and C3, so it was very necessary to perform the SAR study on C2 and C3 positions of berberine. In this paper, we designed and synthesized a series of 2,3-disubstituted berberine derivatives, and briefly evaluated their triglyceride-lowering activities *in vitro*, of which eighteen compounds were synthesized for the first time.

RESULTS AND DISCUSSION

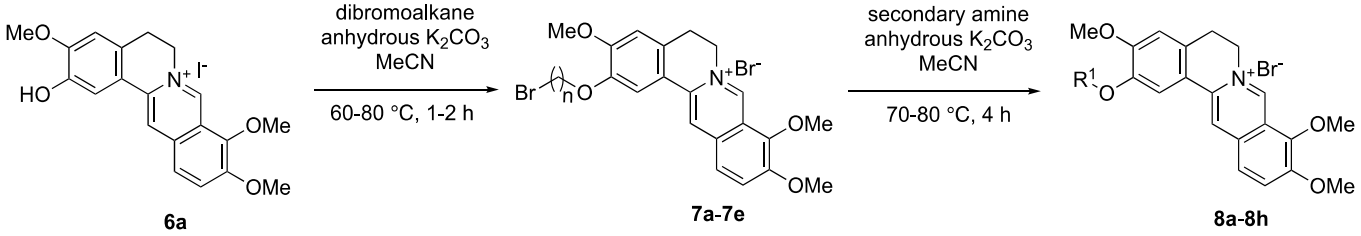
All 2,3-disubstituted berberine derivatives were prepared as displayed in Schemes 1-3, taking commercially available berberine as the starting material. As shown in Scheme 1, compound **2** was obtained by treating berberine with TfOH in xylene.[17] Then, the desired compounds **3a-3c**, **4**, **5a-5b** and **6a-6b** were obtained through nucleophilic substitution of compound **2** with dibromoalkane, 2-bromoethyl ethyl ether, and RX (X=Br, I) respectively in yields of 22%-98%, in which anhydrous K_2CO_3 was used as the base and MeCN as the solvent.[18]



Scheme 1

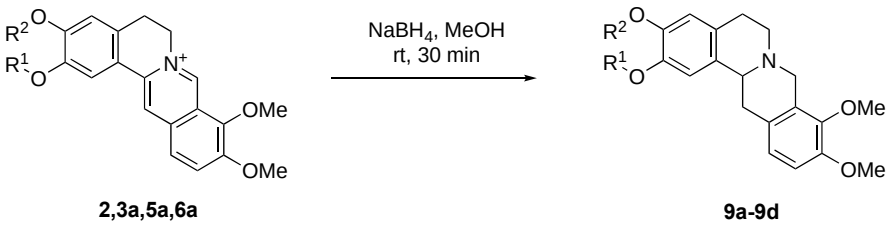
Next, as shown in Scheme 2, the intermediates **7a-7e** were obtained through nucleophilic substitution of compound **6a** with different dibromoalkanes respectively in the mixture of MeCN and anhydrous K_2CO_3.

Then, intermediates **7a-7e** reacted with different secondary amines to obtain compounds **8a-8h** in yields of 17%-51%, in which anhydrous K_2CO_3 was used as the base and MeCN as the solvent.[19]



Scheme 2

Finally, as shown in Scheme 3, the compounds **9a-9d** were obtained by reduction reaction, in which $NaBH_4$ was used as a reducing agent and MeOH as the solvent, with a yield of 40%-87%.[20]



Scheme 3

The cytotoxic activities of all 2,3-disubstituted berberine derivatives were tested by MTT assay using HepG2 cells cultured in DMEM medium. The drug concentration was set at 10 μM. The cell survival rates of all 2,3-disubstituted berberine derivatives are shown in Figure 1, and berberine is a positive molecule. It is obvious that most of 2,3-disubstituted berberine derivatives showed very low toxicity in HepG2 cells when compared with the blank control and berberine. However, compounds **7c-7e** showed certain cytotoxicity in HepG2 cells, which was higher than berberine. The cell survival rates of compounds **7d** and **7e** were less than 50%. Therefore, we selected 2,3-disubstituted berberine derivatives with high cell survival rates for triglyceride-lowering activity assay.

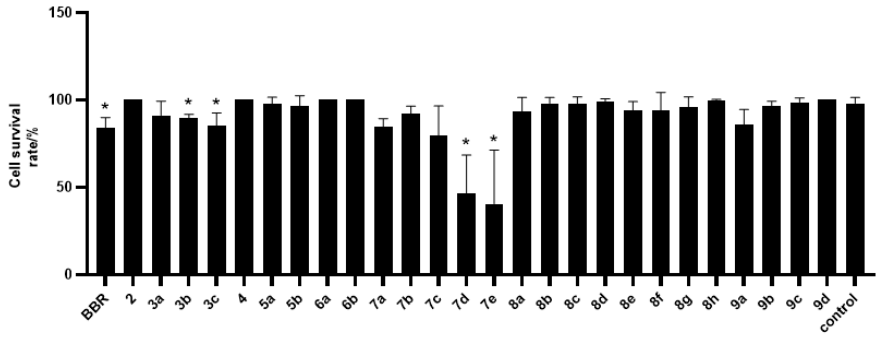
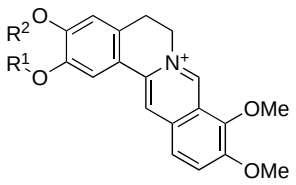


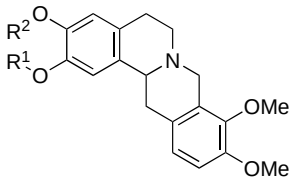
Figure 1. The cell survival rates of all 2,3-disubstituted berberine derivatives (10 μM) in HepG2 cells (*P<0.05, n=3)

In the present study, HepG2 cells were used as a screening tool to evaluate the triglyceride-lowering activities of 2,3-disubstituted berberine derivatives with high cell survival rates. The expression level of triglycerides in HepG2 cells was measured by commercial reagent kit according to the standard protocol provided by the manufacturer. The drug concentration was set at 10 μM. Berberine was used as reference drug. The structures and triglyceride inhibition rates of all 2,3-disubstituted berberine derivatives are shown in Table 1.

Table 1. The structures and triglyceride inhibition rates of all 2,3-disubstituted berberine derivatives (10 μM) in HepG2 cells

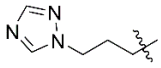
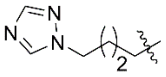
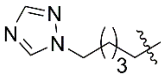
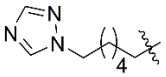
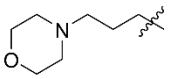
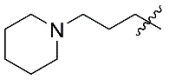
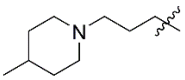
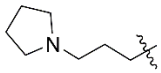


BBR, 2, 3a-3c, 4, 5a-5b, 6a-6b, 7a-7e, 8a-8h



9a-9d

Compound	R^1	R^2	Inhibition rate (%)
BBR	CH_2		50
2	H	H	52
3a	$(CH_2)_2$		57
3b	$(CH_2)_3$		57
3c	$(CH_2)_4$		63
4	$MeCH_2OCH_2CH_2$	$MeCH_2OCH_2CH_2$	61
5a	Me	Me	58
5b	$MeCH_2$	$MeCH_2$	50
6a	H	Me	50

6b	H		$MeCH_2$	61
7a	Br $(CH_2)_2$		Me	0
7b	Br $(CH_2)_3$		Me	9
7c	Br $(CH_2)_4$		Me	NT
7d	Br $(CH_2)_5$		Me	NT
7e	Br $(CH_2)_6$		Me	NT
8a			Me	28
8b			Me	34
8c			Me	34
8d			Me	52
8e			Me	14
8f			Me	28
8g			Me	47
8h			Me	31
9a	H		H	54
9b		$(CH_2)_2$		49
9c	Me		Me	18
9d	H		Me	42

NT=Not Tested.

The hydrogen, ethylidene, propylidene, butylidene, 2-ethoxyethyl, methyl or ethyl was attached at R^1 and R^2, respectively, with which seven 2,3-disubstituted berberine derivatives were generated and tested. Compounds **2**, **3a-3c**, **4**, and **5a-5b** showed strong triglyceride-lowering activities, and the inhibition rates reached 50%. It was noteworthy that compound **3c** had the most triglyceride-lowering activity, and its inhibition rate could reach 63%, which was much higher than berberine. It seemed that forming an octatomic ring at R^1 and R^2 in berberine might be beneficial for triglyceride-lowering activity.

Secondly, when R^1 was hydrogen and R^2 was substituted by methyl or ethyl respectively, generated two 2,3-disubstituted berberine derivatives. The results showed that compounds **6a** and **6b** exhibited strong

triglyceride-lowering activities, and the inhibition rate of compound **6b** could reach 61%. Therefore, R^1 was hydrogen and R^2 was substituted by a suitable substituent, especially an ethyl, which could have high triglyceride-lowering activity.

Next, R^2 was methyl and R^1 was 2-bromoethyl, 3-bromopropyl, 3-(1,2,4-triazol-1-yl)propyl, 4-(1,2,4-triazol-1-yl)butyl, 5-(1,2,4-triazol-1-yl)pentyl, 6-(1,2,4-triazol-1-yl)hexyl, 3-(morpholin-1-yl)propyl, 3-(piperidin-1-yl)propyl, 3-(4-methylpiperidin-1-yl)propyl or 3-(tetrahydropyrrol-1-yl)propyl respectively, generated and tested ten 2,3-disubstituted berberine derivatives. The inhibition rate of triglyceride of compound **8d** was 52%, slightly higher than that of berberine. Compounds **7b**, **8a-8c** and **8e-8h** showed general triglyceride-lowering activities. Compound **7a** had no triglyceride-lowering activity. Therefore, R^2 was methyl and R^1 was other substituents, which seemed to be unfavorable to the improvement of triglyceride-lowering activity.

Finally, the compounds **9a-9d** were obtained by reduction reaction. The results showed that the four products showed triglyceride-lowering activities, and the triglyceride-lowering activity of compound **9a** was slightly higher than that of berberine. Therefore, it could be inferred that the structural reduction of quaternary amine in berberine derivatives might contribute to the reduction of triglyceride in cells.

In conclusion, we designed, synthesized twenty-six 2,3-disubstituted berberine derivatives and evaluated their triglyceride-lowering activities *in vitro*. The results showed that most 2,3-disubstituted berberine derivatives had triglyceride-lowering activities in HepG2 cells. Compound **3c** had the most triglyceride-lowering activity, and its inhibition rate was 63%, which was much higher than berberine. We inferred that forming an octatomic ring at R^1 and R^2 in berberine might be beneficial for triglyceride-lowering activity. Therefore, berberine with an octatomic ring at R^1 and R^2 deserve further study, which will provide a new idea for the development of berberine-like lipid-lowering agents with new structures.

EXPERIMENTAL

All reagents are from commercial suppliers without further purification. Silica gel column chromatography was carried out on 200-300 mesh silica gel. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV 600 MHz spectrometer in DMSO-d_6 with Me_4Si as the internal standard. Mass spectrometry (MS) spectra were collected in ESI model on an Agilent 6430 Mass instrument.

Demethyleneberberine (2). To a stirred suspension of berberine (10.00 g, 26.90 mmol) in xylene (100 mL) was added trifluoromethanesulfonic acid (TfOH) (14.28 mL, 161.37 mmol), and the reaction mixture was stirred at room temperature for 1 h. Then 1 M HCl was added to the resulting mixture at 0 °C until the precipitate was not generated. The mixture was filtered and the precipitate was washed with water and

petroleum ether. The resulting precipitate was dried in a vacuum drying oven to obtain compound **2** (11.15 g, 87%) as yellow powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.82 (overlap, 3H), 8.74 (s, 1H), 8.16 (d, *J* = 9.0 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 7.50 (s, 1H), 6.81 (s, 1H), 4.89 (t, *J* = 5.6 Hz, 2H), 4.09 (s, 3H), 4.06 (s, 3H), 3.12 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 150.00, 149.17, 145.57, 145.12, 143.47, 138.26, 133.31, 127.23, 126.68, 123.49, 121.20, 119.10, 117.81, 114.85 (overlap), 112.69, 61.85, 57.05, 55.56, 25.77; ESI-MS *m/z*: 324.2 $(M\text{-}O_3SCF_3)^+$.

General procedure for the synthesis of compounds 3a-3c. To a stirred solution of compound **2** (1.00 g, 2.11 mmol) and anhydrous K_2CO_3 (1.46 g, 10.57 mmol) in MeCN (100 mL), dibromoalkane (10 eq) was added. The reaction mixture was stirred at 65 °C for 2-3 h. Then, the reaction mixture was washed with distilled water and the aqueous layer was extracted 3 times with DCM: MeOH (30:1). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (35:1) as the gradient eluent gave the title compounds **3a-3c**.

2,3-Ethylenedioxy-10,11-dimethyoxyprotoberberine bromide (3a). Compound **2** (1.00 g, 2.11 mmol) was treated with 1,2-dibromoethane (1.82 mL, 21.13 mmol) according to the general procedure to give the desired product **3a** as a yellow solid. Yield: 98%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.91 (s, 1H), 8.18 (d, *J* = 9.1 Hz, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.72 (s, 1H), 6.98 (s, 1H), 4.92 (t, *J* = 6.1 Hz, 2H), 4.36 (t, *J* = 4.3 Hz, 2H), 4.33 (t, *J* = 4.3 Hz, 2H), 4.09 (s, 3H), 4.06 (s, 3H), 3.18 (t, *J* = 6.1 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 150.35, 146.15, 145.40, 143.59, 143.37, 137.33, 132.98, 128.66, 126.69, 123.51, 121.43, 120.13, 119.94, 116.57, 114.33, 64.63, 64.14, 61.89, 57.03, 55.43, 25.69; ESI-MS *m/z*: 350.2 $(M\text{-}Br)^+$.

2,3-Propylenedioxy-10,11-dimethyoxyprotoberberine bromide (3b). Compound **2** (1.00 g, 2.11 mmol) was treated with 1,3-dibromopropane (2.15 mL, 21.13 mmol) according to the general procedure to give the desired product **3b** as a yellow solid. Yield: 86%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.99 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.85 (s, 1H), 7.08 (s, 1H), 4.94 (t, *J* = 6.1 Hz, 2H), 4.26 (t, *J* = 5.4 Hz, 2H), 4.23 (t, *J* = 5.4 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.20 (t, *J* = 6.1 Hz, 2H), 2.19 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 153.43, 150.64, 150.53, 145.54, 143.66, 136.93, 132.89, 130.58, 126.72, 123.63, 121.86, 121.55, 121.11, 120.53, 118.98, 70.71, 70.62, 61.90, 57.05, 55.32, 31.03, 25.61; ESI-MS *m/z*: 364.2 $(M\text{-}Br)^+$.

2,3-Butylenedioxy-10,11-dimethyoxyprotoberberine bromide (3c). Compound **2** (1.00 g, 2.11 mmol) was treated with 1,4-dibromobutane (2.56 mL, 21.13 mmol) according to the general procedure to give the desired product **3c** as a yellow solid. Yield: 46%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.97 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.88 (s, 1H), 7.04 (s, 1H), 4.94 (t, *J* = 6.2 Hz, 2H), 4.49 (t, *J* = 5.5 Hz, 2H), 4.29 (t, *J* = 5.4 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.20 (t, *J* = 6.2 Hz, 2H),

1.94 (m, 2H), 1.81 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 153.23, 150.42, 147.00, 145.44, 143.63, 137.10, 132.95, 131.33, 126.76, 123.55, 121.49, 121.32, 121.24, 120.48, 120.29, 74.05, 71.09, 61.90, 57.05, 55.29, 27.40, 25.70, 24.44; ESI-MS *m/z*: 378.2 $(M\text{-}Br)^+$.

2,3-Bis(2-ethoxyethoxy)-10,11-dimethyoxyprotoberberine bromide (4). To a stirred solution of compound **2** (1.00 g, 2.11 mmol) and anhydrous K_2CO_3 (1.46 g, 10.57 mmol) in MeCN (100 mL), 2-bromoethyl ethyl ether (2.38 mL, 21.13 mmol) was added. The reaction mixture was stirred at 65 °C for 5-6 h. Then, the reaction mixture was washed with distilled water and the aqueous layer was extracted 3 times with DCM: MeOH (30:1). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (30:1) as the gradient eluent gave the title compounds **4** as a yellow solid. Yield: 76%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.99 (s, 1H), 8.21 (d, *J* = 9.0 Hz, 1H), 8.00 (d, *J* = 9.0 Hz, 1H), 7.74 (s, 1H), 7.12 (s, 1H), 4.94 (t, *J* = 5.9 Hz, 2H), 4.27 (t, *J* = 4.0 Hz, 2H), 4.21 (t, *J* = 4.0 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.78 (t, *J* = 4.3 Hz, 2H), 3.75 (t, *J* = 4.3 Hz, 2H), 3.55 (m, 4H), 3.21 (t, *J* = 5.9 Hz, 2H), 1.15 (q, *J* = 6.8 Hz, 6H); ^{13}C NMR (151 MHz, DMSO) δ 151.09, 150.25, 148.07, 145.43, 143.63, 137.63, 133.06, 128.92, 126.80, 123.36, 121.36, 119.90, 119.14, 112.83, 110.85, 68.80, 68.39, 68.35, 68.14, 65.81, 61.89, 57.03, 55.36, 54.90, 25.95, 15.13, 15.10; ESI-MS *m/z*: 468.3 $(M\text{-}Br)^+$.

General procedure for the synthesis of compounds 5a-5b. To a stirred solution of compound **2** (2.00 g, 4.23 mmol) and anhydrous K_2CO_3 (1.46 g, 10.56 mmol) in MeCN (100 mL), RX (2.5 eq) was added. The reaction mixture was stirred at 60-80 °C for 4-5 h. Then, the reaction mixture was washed with 1 M HCl and the aqueous layer was extracted 3 times with DCM: MeOH (30:1). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (80:1 to 30:1) as the gradient eluent gave the title compounds **5a-5b**.

Palmatine (5a). Compound **2** (2.00 g, 4.23 mmol) was treated with methyl iodide (658 μL, 10.56 mmol) according to the general procedure to give the desired product **5a** as a yellow solid. Yield: 51%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.99 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.69 (s, 1H), 7.09 (s, 1H), 4.95 (t, *J* = 5.8 Hz, 2H), 4.11 (s, 3H), 4.07 (s, 3H), 3.94 (s, 3H), 3.87 (s, 3H), 3.23 (t, *J* = 5.8 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.50, 150.23, 148.73, 145.43, 143.62, 137.69, 133.07, 128.62, 126.78, 123.37, 121.34, 119.82, 118.89, 111.28, 108.68, 61.89, 57.02, 56.12, 55.85, 55.38, 25.97; ESI-MS *m/z*: 352.2 $(M\text{-}I)^+$.

2,3-Diethoxy-10,11-dimethyoxyprotoberberine bromide (5b). Compound **2** (2.00 g, 4.23 mmol) was treated with 1-bromoethane (795 μL, 10.56 mmol) according to the general procedure to give the desired product **5b** as a yellow solid. Yield: 57%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.96 (s, 1H),

8.19 (d, *J* = 9.1 Hz, 1H), 8.00 (d, *J* = 9.1 Hz, 1H), 7.68 (s, 1H), 7.07 (s, 1H), 4.93 (t, *J* = 6.3 Hz, 2H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.14 (q, *J* = 7.0 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.21 (t, *J* = 6.3 Hz, 2H), 1.39 (m, 6H); ^{13}C NMR (151 MHz, DMSO) δ 151.03, 150.22, 147.99, 145.37, 143.61, 137.75, 133.12, 128.65, 126.78, 123.39, 121.34, 119.79, 118.79, 112.26, 110.17, 64.46, 64.07, 61.90, 57.04, 55.40, 26.00, 14.70, 14.60; ESI-MS *m/z*: 380.3 $(M\text{-}Br)^+$.

General procedure for the synthesis of compounds 6a-6b. To a stirred solution of compound **2** (1.00 g, 2.11 mmol) and anhydrous K_2CO_3 (730 mg, 5.28 mmol) in MeCN (50 mL), RX (1 eq) was added. The reaction mixture was stirred at 60-70 °C for 4-5 h. Then, the reaction mixture was washed with 1 M HCl and the aqueous layer was extracted 3 times with DCM: MeOH (30:1). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (30:1) as the gradient eluent gave the title compounds **6a-6b**.

Columbamine (6a). Compound **2** (1.00 g, 2.11 mmol) was treated with methyl iodide (132 μL, 2.11 mmol) according to the general procedure to give the desired product **6a** as a yellow solid. Yield: 22%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.38 (s, 1H), 8.80 (s, 1H), 8.17 (d, *J* = 9.1 Hz, 1H), 8.04 (d, *J* = 9.1 Hz, 1H), 7.53 (s, 1H), 7.05 (s, 1H), 4.93 (t, *J* = 5.9 Hz, 2H), 4.09 (s, 3H), 4.06 (s, 3H), 3.89 (s, 3H), 3.20 (t, *J* = 5.9 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 150.64, 150.21, 146.40, 145.35, 143.56, 137.80, 133.16, 127.08, 126.69, 123.58, 121.35, 119.59, 119.13, 112.24, 111.41, 61.90, 57.07, 55.92, 55.56, 25.98; ESI-MS *m/z*: 338.2 $(M\text{-}I)^+$.

3-Ethoxy-2-hydroxy-10,11-dimethoxyprotoberberine bromide (6b). Compound **2** (1.00 g, 2.11 mmol) was treated with 1-bromoethane (159 μL, 2.11 mmol) according to the general procedure to give the desired product **6b** as a yellow solid. Yield: 34%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.27 (s, 1H), 8.79 (s, 1H), 8.17 (d, *J* = 9.1 Hz, 1H), 8.05 (d, *J* = 9.1 Hz, 1H), 7.54 (s, 1H), 7.03 (s, 1H), 4.92 (t, *J* = 6.3 Hz, 2H), 4.15 (q, *J* = 7.0 Hz, 2H), 4.09 (s, 3H), 4.06 (s, 3H), 3.18 (t, *J* = 6.3 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ 150.20, 149.88, 146.53, 145.33, 143.55, 137.85, 133.17, 127.08, 126.69, 123.58, 121.34, 119.54, 118.99, 112.26, 112.21, 64.12, 61.89, 57.06, 55.56, 25.96, 14.59; ESI-MS *m/z*: 352.2 $(M\text{-}Br)^+$.

General procedure for the synthesis of compounds 7a-7e. To a stirred solution of compound **6a** (1.00 g, 2.15 mmol) and anhydrous K_2CO_3 (1.49 g, 10.75 mmol) in MeCN (80 mL), dibromoalkane (10 eq) was added. The reaction mixture was stirred at 60-80 °C for 1-2 h. Then, the reaction mixture was washed with distilled water and the aqueous layer was extracted 3 times with DCM: MeOH (30:1). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (30:1) as the gradient eluent gave the title compounds **7a-7e**.

2-Bromoethoxy-3-methoxy-10,11-dimethyoxyprotoberberine bromide (7a). Compound **6a** (1.00 g, 2.15 mmol) was treated with 1,2-dibromoethane (1.85 mL, 21.50 mmol) according to the general procedure to give the desired product **7a** as a yellow solid. Yield: 47%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.99 (s, 1H), 8.21 (d, *J* = 9.1 Hz, 1H), 8.00 (d, *J* = 9.1 Hz, 1H), 7.74 (s, 1H), 7.13 (s, 1H), 4.95 (t, *J* = 5.9 Hz, 2H), 4.49 (t, *J* = 5.6 Hz, 2H), 4.11 (s, 3H), 4.07 (s, 3H), 3.90 (overlap, 5H), 3.24 (t, *J* = 5.9 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.74, 150.27, 147.23, 145.45, 143.65, 137.54, 133.02, 129.44, 126.80, 123.35, 121.36, 119.96, 118.97, 111.75, 110.72, 69.12, 61.89, 57.02, 55.97, 55.33, 31.15, 26.00; ESI-MS *m/z*: 445.1 $(M\text{-}Br)^+$.

2-Bromopropyloxy-3-methoxy-10,11-dimethyoxyprotoberberine bromide (7b). Compound **6a** (1.00 g, 2.15 mmol) was treated with 1,3-dibromopropane (2.19 mL, 21.50 mmol) according to the general procedure to give the desired product **7b** as a yellow solid. Yield: 72%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.99 (s, 1H), 8.21 (d, *J* = 9.1 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.75 (s, 1H), 7.11 (s, 1H), 4.95 (t, *J* = 6.3 Hz, 2H), 4.25 (t, *J* = 5.9 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.89 (s, 3H), 3.72 (t, *J* = 6.4 Hz, 2H), 3.23 (t, *J* = 6.3 Hz, 2H), 2.33 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.79, 150.25, 147.72, 145.43, 143.62, 137.60, 133.05, 129.09, 126.77, 123.39, 121.35, 119.89, 118.97, 111.57, 110.33, 66.70, 61.89, 57.02, 55.95, 55.36, 31.96, 31.33, 26.01; ESI-MS *m/z*: 459.1 $(M\text{-}Br)^+$.

2-Bromobutyloxy-3-methoxy-10,11-dimethyoxyprotoberberine bromide (7c). Compound **6a** (1.00 g, 2.15 mmol) was treated with 1,4-dibromobutane (2.60 mL, 21.50 mmol) according to the general procedure to give the desired product **7c** as a yellow solid. Yield: 82%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.97 (s, 1H), 8.21 (d, *J* = 9.0 Hz, 1H), 8.02 (d, *J* = 9.0 Hz, 1H), 7.71 (s, 1H), 7.09 (s, 1H), 4.95 (t, *J* = 5.4 Hz, 2H), 4.18 (t, *J* = 5.9 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.88 (s, 3H), 3.67 (t, *J* = 6.5 Hz, 2H), 3.23 (t, *J* = 5.4 Hz, 2H), 2.03 (m, 2H), 1.92 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.74, 150.23, 147.91, 145.44, 143.62, 137.66, 133.05, 128.78, 126.78, 123.35, 121.34, 119.81, 118.90, 111.47, 110.03, 68.02, 61.88, 57.02, 55.92, 55.38, 34.86, 29.18, 27.37, 25.98; ESI-MS *m/z*: 473.1 $(M\text{-}Br)^+$.

2-Bromopentyloxy-3-methoxy-10,11-dimethyoxyprotoberberine bromide (7d). Compound **6a** (1.00 g, 2.15 mmol) was treated with 1,5-dibromopentane (2.93 mL, 21.50 mmol) according to the general procedure to give the desired product **7d** as a yellow solid. Yield: 76%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.97 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.69 (s, 1H), 7.09 (s, 1H), 4.94 (t, *J* = 6.2 Hz, 2H), 4.14 (t, *J* = 6.4 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.88 (s, 3H), 3.60 (t, *J* = 6.7 Hz, 2H), 3.23 (t, *J* = 6.2 Hz, 2H), 1.92 (m, 2H), 1.82 (m, 2H), 1.60 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.73, 150.21, 148.04, 145.40, 143.60, 137.68, 133.06, 128.66, 126.76, 123.36, 121.32, 119.81, 118.88, 111.43, 109.89, 68.69, 61.88, 57.01, 55.89, 55.38, 35.11, 31.95, 27.87, 25.98, 24.40; ESI-MS *m/z*: 487.2 $(M\text{-}Br)^+$.

2-Bromohexyloxy-3-methoxy-10,11-dimethyoxyprotoberberine bromide (7e). Compound **6a** (1.00 g, 2.15 mmol) was treated with 1,6-dibromohexane (3.31 mL, 21.50 mmol) according to the general procedure to give the desired product **7e** as a yellow solid. Yield: 90%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.97 (s, 1H), 8.20 (d, *J* = 7.7 Hz, 1H), 8.02 (d, *J* = 7.7 Hz, 1H), 7.69 (s, 1H), 7.09 (s, 1H), 4.94 (t, *J* = 6.1 Hz, 2H), 4.10 (overlap, 5H), 4.07 (s, 3H), 3.88 (s, 3H), 3.56 (t, *J* = 6.7 Hz, 2H), 3.23 (t, *J* = 6.1 Hz, 2H), 1.86 (m, 2H), 1.80 (m, 2H), 1.49 (m, 4H); ^{13}C NMR (151 MHz, DMSO) δ 151.75, 150.21, 148.07, 145.41, 143.61, 137.69, 133.07, 128.65, 126.77, 123.37, 121.33, 119.82, 118.89, 111.43, 109.88, 68.70, 61.88, 57.02, 55.89, 55.38, 35.12, 32.18, 28.59, 27.32, 25.98, 24.75; ESI-MS *m/z*: 501.2 $(M\text{-}Br)^{+}$.

General procedure for the synthesis of compounds 8a-8h. To a stirred solution of 2-bromoalkyloxy-3-methoxy-10,11-dimethoxyprotoberberine bromide (300 mg) and anhydrous K_2CO_3 (1.8-2.0 eq) in MeCN (30 mL), secondary amine (1.8-2.0 eq) was added. The reaction mixture was stirred at 70-80 °C for 4 h. Then, the reaction mixture was washed with distilled water and the aqueous layer was extracted 3 times with DCM. The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (30:1 to 20:1) as the gradient eluent gave the title compounds **8a-8h**.

2-(3-(1,2,4-Triazol-1-yl)propyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8a). Compound **7b** (300 mg, 0.56 mmol) was treated with 1,2,4-triazole (69 mg, 1.00 mmol) according to the general procedure to give the desired product **8a** as a yellow solid. Yield: 42%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.94 (s, 1H), 8.56 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 8.00 (overlap, 2H), 7.70 (s, 1H), 7.11 (s, 1H), 4.94 (t, *J* = 6.1 Hz, 2H), 4.41 (t, *J* = 6.8 Hz, 2H), 4.14 (t, *J* = 5.8 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.90 (s, 3H), 3.23 (t, *J* = 6.1 Hz, 2H), 2.33 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.88, 151.49, 150.25, 147.72, 145.44, 144.20, 143.63, 137.61, 133.04, 129.14, 126.79, 123.38, 121.35, 119.84, 118.95, 111.59, 110.49, 66.00, 61.89, 57.03, 55.96, 55.36, 45.70, 29.11, 26.01; ESI-MS *m/z*: 447.2 $(M\text{-}Br)^{+}$.

2-(4-(1,2,4-Triazol-1-yl)butyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8b). Compound **7c** (300 mg, 0.54 mmol) was treated with 1,2,4-triazole (66 mg, 0.96 mmol) according to the general procedure to give the desired product **8b** as a yellow solid. Yield: 38%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.95 (s, 1H), 8.57 (s, 1H), 8.20 (d, *J* = 9.1 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.98 (s, 1H), 7.69 (s, 1H), 7.09 (s, 1H), 4.94 (t, *J* = 5.8 Hz, 2H), 4.31 (t, *J* = 6.8 Hz, 2H), 4.16 (t, *J* = 6.1 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.88 (s, 3H), 3.23 (t, *J* = 5.8 Hz, 2H), 2.00 (m, 2H), 1.74 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.74, 151.42, 150.23, 147.91, 145.45, 144.03, 143.63, 137.66, 133.05, 128.79, 126.79, 123.36, 121.34, 119.80, 118.90, 111.46, 110.00, 68.36, 61.89, 57.02, 55.91, 55.38, 48.25, 26.31, 25.99, 25.63; ESI-MS *m/z*: 461.2 $(M\text{-}Br)^{+}$.

2-(5-(1,2,4-Triazol-1-yl)pentyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8c). Compound **7d** (300 mg, 0.53 mmol) was treated with 1,2,4-triazole (65 mg, 0.94 mmol) according to the general procedure to give the desired product **8c** as a yellow solid. Yield: 31%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.96 (s, 1H), 8.53 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.96 (s, 1H), 7.68 (s, 1H), 7.08 (s, 1H), 4.94 (t, J = 5.6 Hz, 2H), 4.24 (t, J = 6.8 Hz, 2H), 4.10 (overlap, 5H), 4.07 (s, 3H), 3.87 (s, 3H), 3.22 (t, J = 5.6 Hz, 2H), 1.89 (m, 2H), 1.81 (m, 2H), 1.41 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.71, 151.32, 150.22, 148.03, 145.41, 143.94, 143.62, 137.69, 133.07, 128.66, 126.78, 123.37, 121.33, 119.82, 118.89, 111.43, 109.85, 68.60, 61.88, 57.02, 55.88, 55.38, 48.43, 29.01, 28.15, 25.98, 22.55; ESI-MS *m/z*: 475.3 $(M\text{-}Br)^{+}$.

2-(6-(1,2,4-Triazol-1-yl)hexyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8d). Compound **7e** (300 mg, 0.52 mmol) was treated with 1,2,4-triazole (71 mg, 1.03 mmol) according to the general procedure to give the desired product **8d** as a yellow solid. Yield: 17%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.97 (s, 1H), 8.52 (s, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.95 (s, 1H), 7.68 (s, 1H), 7.08 (s, 1H), 4.94 (t, J = 6.1 Hz, 2H), 4.19 (t, J = 6.1 Hz, 2H), 4.10 (overlap, 5H), 4.07 (s, 3H), 3.87 (s, 3H), 3.22 (t, J = 6.1 Hz, 2H), 1.83 (m, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.31 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.75, 151.29, 150.22, 148.06, 145.41, 143.89, 143.61, 137.69, 133.08, 128.65, 126.77, 123.38, 121.34, 119.81, 118.89, 111.43, 109.86, 68.67, 61.88, 57.02, 55.88, 55.38, 48.50, 29.19, 28.55, 25.98, 25.58, 25.01; ESI-MS *m/z*: 489.3 $(M\text{-}Br)^{+}$.

2-(3-(Morpholin-1-yl)propyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8e). Compound **7b** (300 mg, 0.56 mmol) was treated with morpholine (87 μL, 1.00 mmol) according to the general procedure to give the desired product **8e** as a yellow solid. Yield: 51%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.98 (s, 1H), 8.20 (d, J = 9.1 Hz, 1H), 8.02 (d, J = 9.1 Hz, 1H), 7.70 (s, 1H), 7.09 (s, 1H), 4.94 (t, J = 6.2 Hz, 2H), 4.18 (t, J = 6.1 Hz, 2H), 4.10 (s, 3H), 4.07 (s, 3H), 3.88 (s, 3H), 3.60 (br, 4H), 3.23 (t, J = 6.2 Hz, 2H), 2.41 (br, 6H), 1.97 (m, 2H); ^{13}C NMR (151 MHz, DMSO) δ 151.78, 150.23, 148.00, 145.42, 143.62, 137.68, 133.07, 128.77, 126.78, 123.38, 121.34, 119.84, 118.92, 111.48, 110.06, 67.16, 66.10 (overlap2), 61.88, 57.02, 55.90, 55.38, 54.90, 53.34 (overlap2), 26.00 (overlap2); ESI-MS *m/z*: 465.3 $(M\text{-}Br)^{+}$.

2-(3-(Piperidin-1-yl)propyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8f). Compound **7b** (300 mg, 0.56 mmol) was treated with piperidine (111 μL, 1.11 mmol) according to the general procedure to give the desired product **8f** as a yellow solid. Yield: 27%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.97 (s, 1H), 8.21 (d, J = 8.9 Hz, 1H), 8.01 (d, J = 8.9 Hz, 1H), 7.75 (s, 1H), 7.13 (s, 1H), 4.94 (t, J = 6.7 Hz, 2H), 4.23 (t, J = 5.0 Hz, 2H), 4.11 (s, 3H), 4.08 (s, 3H), 3.89 (s, 3H), 3.54 (br, 2H), 3.25 (overlap, 4H), 2.96 (br, 2H), 2.21 (m, 2H), 1.85 (m, 2H), 1.70 (m, 4H); ^{13}C NMR (151 MHz, DMSO) δ 151.72, 150.30, 147.56, 145.58, 143.70, 137.58, 133.02, 129.27, 126.85, 123.33, 121.38,

119.80, 118.96, 111.57, 110.37, 66.60, 61.90, 57.04, 55.97, 55.38, 53.86, 52.34 (overlap2), 26.00, 23.60, 22.76 (overlap2), 21.27; ESI-MS *m/z*: 463.3 $(M-Br)^+$.

2-(3-(4-Methylpiperidin-1-yl)propyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8g). Compound **7b** (300 mg, 0.56 mmol) was treated with 4-methylpiperidine (132 μL, 1.11 mmol) according to the general procedure to give the desired product **8g** as a yellow solid. Yield: 27%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.97 (s, 1H), 8.22 (d, *J* = 9.1 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.75 (s, 1H), 7.14 (s, 1H), 4.95 (t, *J* = 6.2 Hz, 2H), 4.23 (t, *J* = 5.5 Hz, 2H), 4.11 (s, 3H), 4.08 (s, 3H), 3.89 (s, 3H), 3.55 (br, 2H), 3.24 (overlap, 4H), 2.96 (br, 2H), 2.21 (br, 2H), 1.85 (m, 2H), 1.64 (br, 1H), 1.33 (m, 2H), 0.95 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (151 MHz, DMSO) δ 151.71, 150.30, 147.56, 145.58, 143.70, 137.58, 133.02, 129.26, 126.85, 123.32, 121.38, 119.80, 118.96, 111.57, 110.35, 66.58, 61.90, 57.04, 55.96, 55.38, 54.90, 53.90, 52.20, 31.12 (overlap2), 28.07, 26.00 (overlap2), 21.08; ESI-MS *m/z*: 477.3 $(M-Br)^+$.

2-(3-(Tetrahydropyrrol-1-yl)propyloxy)-3-methoxy-10,11-dimethyoxyprotoberberine bromide (8h). Compound **7b** (300 mg, 0.56 mmol) was treated with tetrahydropyrrole (84 μL, 1.00 mmol) according to the general procedure to give the desired product **8h** as a yellow solid. Yield: 36%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.97 (s, 1H), 8.21 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 7.74 (s, 1H), 7.13 (s, 1H), 4.94 (t, *J* = 6.0 Hz, 2H), 4.24 (t, *J* = 5.7 Hz, 2H), 4.11 (s, 3H), 4.08 (s, 3H), 3.89 (s, 3H), 3.64 (br, 2H), 3.36 (overlap, 2H), 3.24 (t, *J* = 6.0 Hz, 2H), 3.09 (br, 2H), 2.19 (m, 2H), 1.98 (m, 4H); ^{13}C NMR (151 MHz, DMSO) δ 151.71, 150.30, 147.56, 145.58, 143.70, 137.58, 133.02, 129.26, 126.85, 123.33, 121.39, 119.80, 118.97, 111.56, 110.29, 66.48, 61.91, 57.04, 56.00, 55.38, 53.49 (overlap2), 51.81, 26.00, 25.43, 22.59 (overlap2); ESI-MS *m/z*: 449.3 $(M-Br)^+$.

General procedure for the synthesis of compounds 9a-9d. To a stirred solution of berberine derivatives (**2**, **3a**, **5a**, **6a**) (300 mg) in MeOH (30 mL), $NaBH_4$ (3.0-5.0 eq) was added. The reaction mixture was stirred at room temperature for 30 min. Then, the reaction mixture was washed with distilled water and the aqueous layer was extracted 3 times with DCM: MeOH (30:1). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and volatiles were removed under reduced pressure to yield the crude products. Finally, purification via flash chromatography over silica gel using DCM: MeOH (80:1 to 40:1) as the gradient eluent gave the title compounds **9a-9d**.

Tetrahydrodemethyleneberberine (9a). Compound **2** (300 mg, 0.63 mmol) was treated with $NaBH_4$ (120 mg, 3.17 mmol) according to the general procedure to give the desired product **9a** as a white solid. Yield: 40%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.60 (s, 1H), 6.87 (s, 2H), 6.64 (s, 1H), 6.46 (s, 1H), 4.04 (d, *J* = 15.7 Hz, 1H), 3.77 (s, 3H), 3.72 (s, 3H), 3.36 (overlap, 2H), 3.16 (m, 1H), 3.06 (br, 1H), 2.84 (m, 1H), 2.54 (m, 1H), 2.48 (overlap, 1H), 2.43 (m, 1H); ^{13}C NMR (151 MHz, DMSO) δ 149.85, 144.39, 143.59, 143.56, 128.36, 127.66, 127.64, 124.73, 123.75, 114.97, 112.47, 111.17, 59.57, 58.65, 55.73, 53.47, 51.22, 35.91, 28.25; ESI-MS *m/z*: 328.2 $(M-H)^+$.

2,3-Ethylenedioxy-10,11-dimethyoxytetrahydroprotoberberine (9b). Compound **3a** (300 mg, 0.70 mmol) was treated with $NaBH_4$ (132 mg, 3.49 mmol) according to the general procedure to give the desired product **9b** as a white solid. Yield: 47%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.86 (q, J = 8.4 Hz, 7.0 Hz, 2H), 6.79 (s, 1H), 6.58 (s, 1H), 4.20 (s, 4H), 4.05 (d, J = 15.7 Hz, 1H), 3.77 (s, 3H), 3.72 (s, 3H), 3.36 (overlap, 2H), 3.25 (m, 1H), 3.07 (m, 1H), 2.87 (m, 1H), 2.56 (m, 1H), 2.52 (overlap, 1H), 2.42 (m, 1H); ^{13}C NMR (151 MHz, DMSO) δ 149.84, 144.38, 141.67, 141.60, 130.75, 128.26, 127.56, 127.19, 123.74, 116.06, 113.79, 111.15, 64.10, 64.07, 59.57, 58.57, 55.71, 53.39, 50.95, 35.70, 28.28; ESI-MS *m/z*: 354.2 $(M-H)^+$.

Tetrahydropalmatine (9c). Compound **5a** (300 mg, 0.63 mmol) was treated with $NaBH_4$ (71 mg, 1.88 mmol) according to the general procedure to give the desired product **9c** as a white solid. Yield: 87%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.88 (s, 2H), 6.87 (s, 1H), 6.68 (s, 1H), 4.06 (d, J = 15.7 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.39 (m, 2H), 3.36 (overlap, 1H), 3.10 (m, 1H), 2.93 (m, 1H), 2.61 (m, 1H), 2.54 (m, 1H), 2.46 (m, 1H); ^{13}C NMR (151 MHz, DMSO) δ 149.84, 147.20, 147.18, 144.40, 129.67, 128.32, 127.70, 126.38, 123.68, 111.69, 111.17, 109.39, 59.58, 58.82, 55.73, 55.69, 55.41, 53.44, 50.95, 35.69, 28.59; ESI-MS *m/z*: 356.2 $(M-H)^+$.

Tetrahydrocolumbamine (9d). Compound **6a** (300 mg, 0.65 mmol) was treated with $NaBH_4$ (122 mg, 3.23 mmol) according to the general procedure to give the desired product **9d** as a white solid. Yield: 80%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (s, 1H), 6.87 (s, 2H), 6.69 (s, 1H), 6.64 (s, 1H), 4.04 (d, J = 15.7 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.72 (s, 3H), 3.37 (overlap, 2H), 3.18 (m, 1H), 3.08 (m, 1H), 2.90 (m, 1H), 2.56 (m, 1H), 2.52 (overlap, 1H), 2.45 (m, 1H); ^{13}C NMR (151 MHz, DMSO) δ 149.86, 146.06, 144.65, 144.41, 129.87, 128.37, 127.62, 124.75, 123.77, 112.43, 111.83, 111.17, 59.58, 58.60, 55.73, 55.54, 53.45, 51.14, 35.84, 28.54; ESI-MS *m/z*: 342.2 $(M-H)^+$.

MTT assay. The HepG2 cells were seeded at a density of 1×10^3-1×10^5 cells/well in 96-well plates. After 12 h, all the target compounds dissolved in DMSO were added to HepG2 cells and incubated in an incubator at 37 ℃, 5% CO_2 and saturated humidity for 24 h. Three parallel wells were set up for each target compound with a concentration of 10 μM. After incubation, the cells were treated with 20 μL 5mg/mL MTT for 4 h at 37 ℃. Subsequently, the medium was removed, 150 μL DMSO was added to dissolve the purple formazan crystals, and the absorbance at 490 nm was measured using a CMax Plus.[21]

Triglyceride-lowering activity assay. The HepG2 cells were seeded at a density of 1×10^4-1×10^5 cells/well in 24-well plates. After 12 h, the oleic acid solution and all target compounds dissolved in DMSO were added to HepG2 cells and incubated in an incubator at 37 ℃, 5% CO_2 and saturated humidity for 48 h. Three parallel wells were set up for each target compound with a concentration of 10 μM. After incubation, the medium was removed, and HepG2 cells on 24-well plates were rinsed twice with PBS. Then the HepG2 cells were isolated by enzymatic digestion, washed once with PBS, and

centrifuged to obtain HepG2 cells. The HepG2 cells were incubated in 100 μL isopropanol on ice for 2 h, centrifuged to obtain the supernatant, and the absorbance at 510 nm was measured using the Triglyceride Assay Kit. The remaining HepG2 cells were lysed in 40 μL Denature Lysis Buffer on ice for 30 min, and the protein concentrations were measured using the Enhanced BCA Protein Assay Kit.

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