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Synthesis and Cytotoxicity Evaluation of 3-Substituted Jatrorrhizine and 9-Substituted Berberine Derivatives

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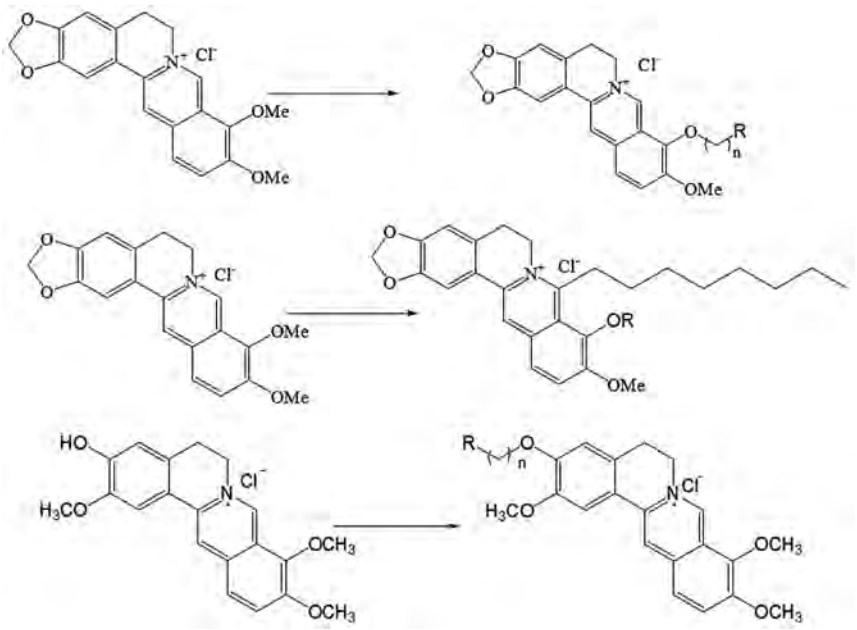
ABSTRACT This paper reports the synthesis of C-3-substituted jatrorrhizine derivatives and C-9-substituted berberine derivatives, by a sequence of reactions. The target compounds were screened for the *in vitro* antitumor activity against NCI-H460 and HeLa cell lines, and compound **14b** exhibited the stronger cytotoxicity with the inhibitory concentration (IC50) values of 3.11 μM against NCI-H460 and 2.88 μM for HeLa, and compound **26c** displayed the highest cytotoxic activity against NCI-H460 and HeLa cell lines with IC50 values of 1.50 μM and 1.04 μM, respectively.



KEY WORDS Berberine derivatives, Cytotoxic activity, Jatrorrhizine derivatives, Structure-activity relationship.

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INTRODUCTION

Coptis Chinensis Franch is a plant of traditional Chinese medicine which is mainly cultivated in Southern China. Jatrorrhizine [**Figure 1**] and berberine [**Figure 2**] are quaternary protoberberine alkaloids (QPA) considered as the active compounds of *C. chinensis* Franch.[1,2] Berberine possesses various biological activities such as antibacterial[3,4] antifungal,[5] anticancer,[6,7] anti-Alzheimer's disease,[8] and hypoglycemic effect,[9] whereas jatrorrhizine has hypoglycemic,[10] antimicrobial,[11] and antioxidant activity.[12] Substituted products of QPA in the A, C, or D ring system showed alterations in their biological activity. Previous studies have shown that dioxymethylene substitution at the C-2 and C-3 position of the A ring,[13] and 8-Alkyl- and 13-Alkyl-substitution increase their antibacterial activity.[14] However, 13-Hydroxy substituent decreases antibacterial activity[15] and 13-Alkyl groups substitution increase cytotoxicity.[16] Park *et al.* reported that when the benzyl group was connected to C-13 of berberine and berberrubine, their antifungal activities were increased.[5] The antibacterial activity of 8-alkylberberine substitutions was increased as the alkyl chain elongated, but decreased gradually when the alkyl chain was more than eight carbon atoms.[17] Many benzothiazole, benzoxazole, and paeonol derivatives have been reported as antitumor agents.[18-21] Zhang *et al.* reported that the cytotoxic activity of 13-n-alkyl berberine and palmatine derivatives were better than berberine and palmatine.[16] In addition, different derivatives with various chain lengths and terminal amino groups were prepared, and the DNA-binding affinity or G-quadruplex stabilizing ligands were studied.[22,23] Based on the above reports, jatrorrhizine and berberine, which have similar skeletal structure belonging to isoquinoline type alkaloid, attracted our attention. Even though many derivatives with their bioactivities were investigated, there is no reported research on the synthesis of jatrorrhizine derivatives with their cytotoxic activity tests *in vitro* yet. In this work, we present a synthesis of new jatrorrhizine and berberine derivatives anticipated to enhance the cytotoxic activity *in vitro* with their structure-activity relationships.

RESULTS AND DISCUSSION

Synthesis

Jatrorrhizine was purified from fibrous root of Rhizoma Coptidis according to reference,[24] and the purity of jatrorrhizine was more than 98% as determined by high-performance liquid chromatographic. A series of new jatrorrhizine and berberine derivatives were synthesized by means of the introducing benzothiazole, benzoxazole, and paeonol linked to the 3-position of jatrorrhizine, and introduction of vanillin and paeonol linked to the 9-position of berberine, respectively, and by substituting alkoxy in C-9 and octyl to C-8 of berberine. The newly designed compounds were synthesized as outlined in **Scheme 1** (for jatrorrhizine derivatives), **Scheme 2 (**berberine derivatives**),** and **Scheme 3 (**9-alkoxy-8-octylberberine derivatives**)**.

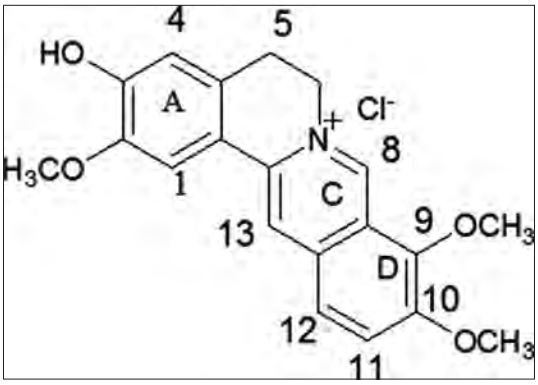


Figure 1: Jatrorrhizine

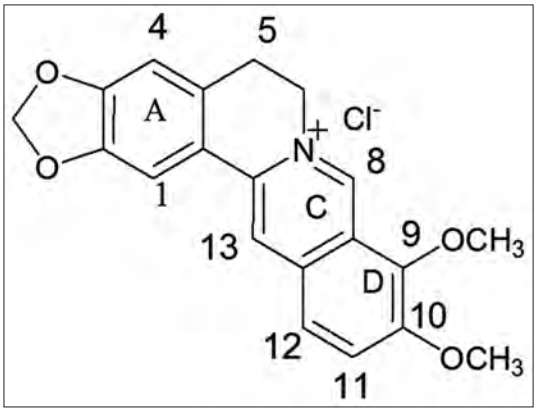
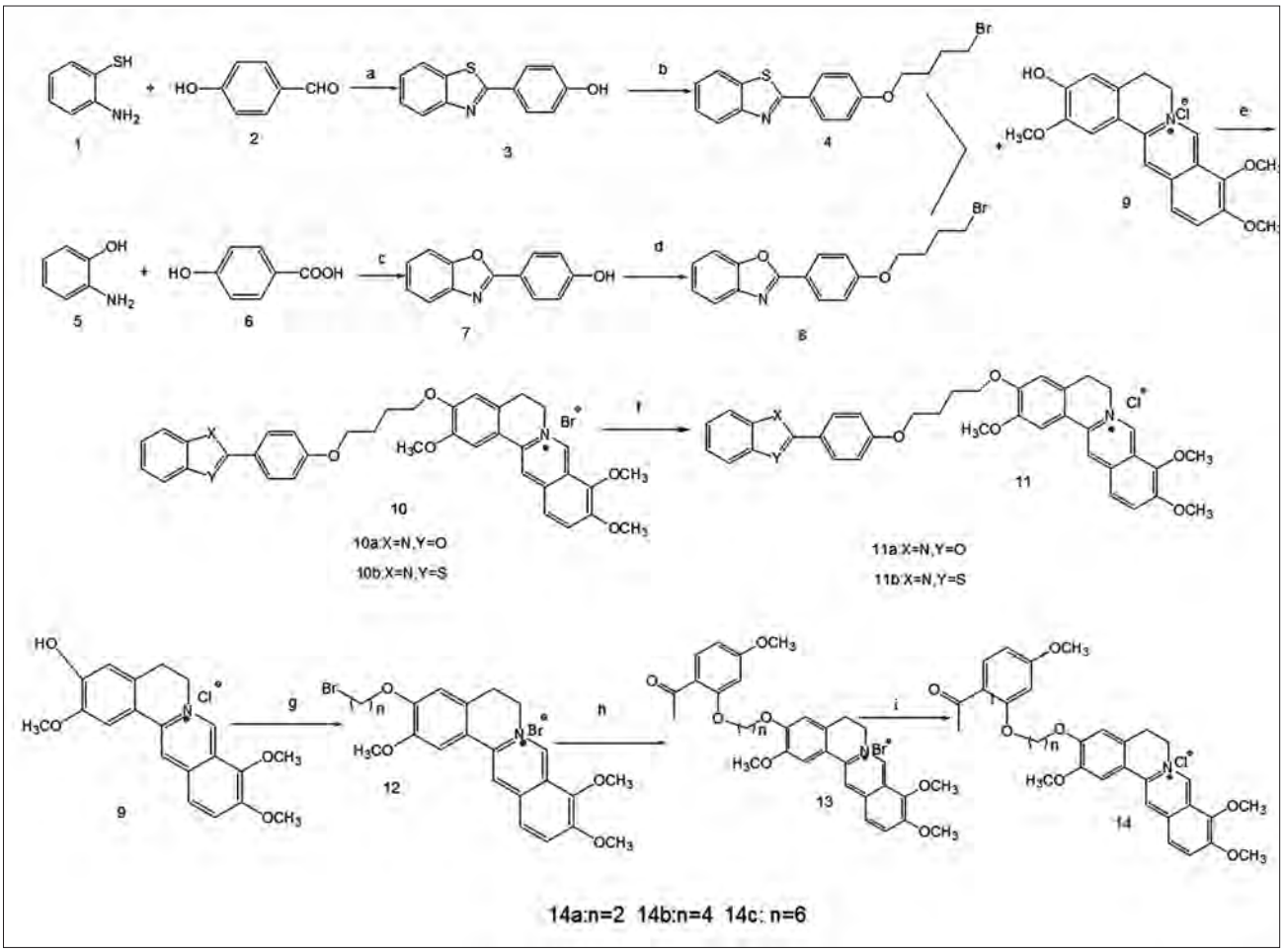


Figure 2: Berberine

Cytotoxicity study

The cytotoxic activities of new compounds were tested in the NCI-H460 and HeLa cell lines. The results, expressed as inhibitory concentration (IC50) values, are given in **Table 1**. Most of berberine and jatrorrhizine derivatives displayed stronger cytotoxic activity than berberine and jatrorrhizine in both NCI-H460 and HeLa cell lines. The hybrid compound of jatrorrhizine and paeonol (**14b**) exhibited good cytotoxic activity with the IC50 value of 3.11 μM for NCI-H460 and 2.88 μM for HeLa, being 4–8 fold greater cytotoxic than jatrorrhizine. Hybridizing berberine with paeonol gave compound **19a**, which exhibited strong cytotoxic activity with the IC50 values of 3.21 μM for NCI-H460 and 3.14 μM for HeLa, which is 9–15-fold stronger cytotoxic activity than berberine. These results indicated that the use of antitumor pharmacophore at C-3-position of jatrorrhizine and C-9-position of berberine greatly improved the cytotoxic activity showing that introducing the antitumor active group to some natural products to increase antitumor activity is feasible. Jatrorrhizine with substituted paeonol linked using four carbons (**14b**) showed more potent activity against both NCI-H460 and HeLa cell lines (IC50 = 3.11, 2.88 μM) than using two carbons (**14a**, IC50 = 4.13, 5.21 μM) and six carbons (**14c**, IC50 = 4.41, 3.84 μM), suggesting substituents



Scheme 1: The synthetic routes of jatrorrhizine derivatives. Reagents and conditions: (a) toluene, reflux, overnight, (b) Br(CH$_2$)4 Br, K$_2$CO$_3$, acetone, reflux, (c) p-TsOH, xylene, reflux, 12 h, (d) Br (CH$_2$)4Br, K$_2$CO$_3$, acetone, reflux, (e) K$_2$CO$_3$, N$_2$, DMF, 80°C, (g) K$_2$CO$_3$, Br (CH$_2$) nBr, CH$_3$CN, reflux, (h) CH$_3$CN, K$_2$CO$_3$, paeonol, 80°C, (i) AgCl, CH$_3$OH,50°C

Table 1 Cytotoxicity of jatrorrhizine and berberine derivatives in cancer cells

Compounds	IC$_{50}$ (μM)	
	NCI-H460	HeLa
Doxorubicin	0.64±0.032	2.86±0.12
Jatrorrhizine	12.5±0.35	22.49±0.2
Berberine	28.01±0.89	46.12±1.32
11a	8.01±0.35	10.13±0.35
11b	9.21±0.65	14.08±0.85
14a	4.13±0.15	5.21±0.09
14b	3.11±0.24	2.88±0.13
14c	4.41±0.22	3.84±0.14
19a	3.21±0.26	3.14±0.55
22a	22.01±0.16	35.01±1.26
26a	3.13±0.26	4.12±0.16
26b	1.52±0.05	1.59±0.03
26c	1.50±0.02	1.04±0.02
26d	4.25±0.13	3.05±0.08
26e	4.9±0.21	3.29±0.09

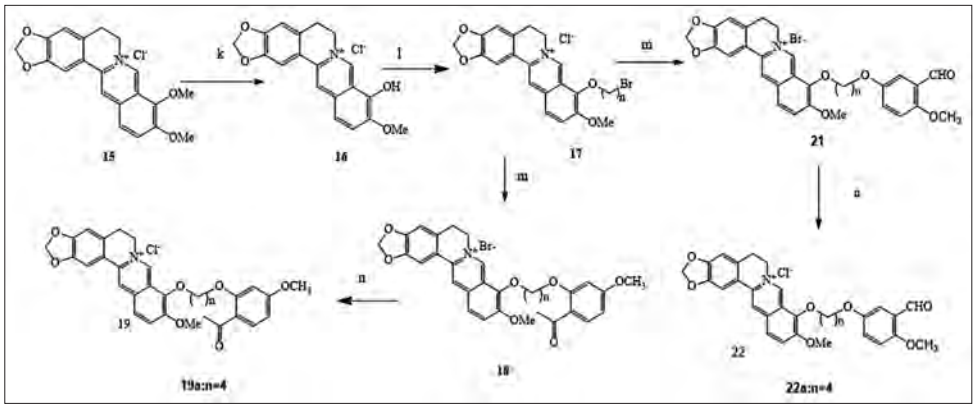
[a]IC$_{50}$ values in μM are means standard deviations obtained from at least two (mostly three) independent experiments, and doxorubicin was used as a positive control

connection with the moderate length of carbon chain enhanced the cytotoxic activity. Similarly, the cytotoxic activity of compounds **26a-c** increased from on the elongation of aliphatic chain to six carbons, but decreased for compounds **26d (**IC50 = 4.25 μM**)** and **26e (**IC50 = 4.9 μM**)** as the length of aliphatic chain was increased to 8 and 10 carbon atoms, respectively. Compounds **14b, 19a,** and **26a** exhibited nearly similar cytotoxic activity. Among all compounds examined, although the 9-octyloxy-8-octylberberine derivatives **26a-e** showed good antitumor activity, **26c** displayed the highest cytotoxic activity against both NCI-H460 and HeLa cell lines with the IC50 values of 1.50 μM and 1.04μM, respectively, which is 19–44-fold stronger cytotoxic activity than berberine. This suggested that connecting hydrophobic groups with moderate carbon chain length alkyl groups to berberine improved the cytotoxic activity.

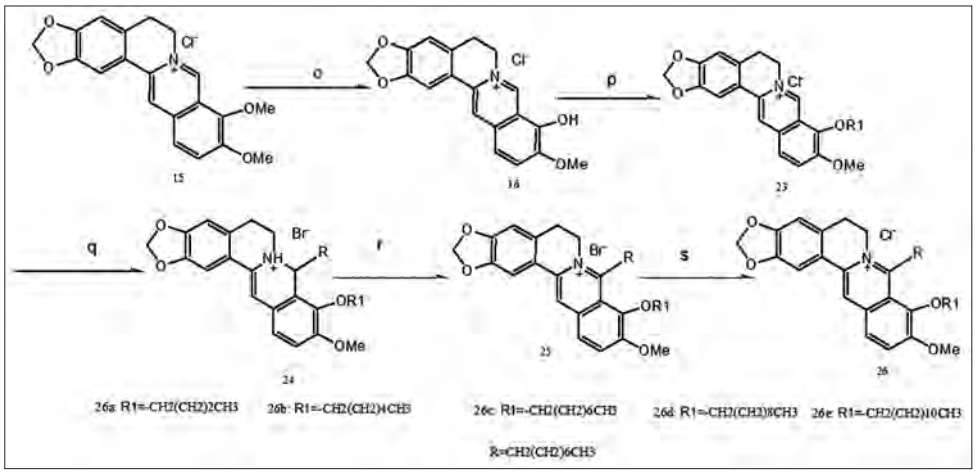
EXPERIMENTAL SECTION

Chemistry

All chemical reagents and solvents were A.R. grade and were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. Melting points were determined on an RD-2C electrothermal melting point apparatus and are



Scheme 2: The synthetic routes of berberine derivatives. Reagents and conditions: (k) DMF, 190°C, (l) Br (CH_2)4Br, K_2CO_3, acetone, reflux, (m) CH_3CN, K_2CO_3, vanillin, 80°C, (n) CH_3CN, K_2CO_3, paeonol, (o) AgCl, CH_3OH,50°C



Scheme 3: The synthetic routes of 9-Alkoxy-8-octylberberine derivatives. Reagents and conditions: (o) DMF, 190°C, (p) K_2CO_3, acetone, R1Br, (q) RMgBr, THF, (r) Br_2, CH_3COOH, 50°C, (s) AgCl,CH_3OH,50°C

uncorrected. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 (400 MHz) using tetramethylsilane as the internal standard and CD_3OD and dimethyl sulfoxide (DMSO)-*d*6 as a solvent. Mass spectra were recorded on 1100 series LC/MSD instrument. Thin-layer chromatography analysis was used to explore the condition of the lotion, which was performed on silica gel-GF254 thin layers and developed with eluents C_6H_6/EtOAc/MeOH/C_3H_7OH/NH_3 (6:3:1.5:1.5:0.5).

Synthesis of the intermediate 2-(4-(4-bromobutoxy)phenyl) benzo[d]thiazole (4)

The synthesis of intermediates **3** and **7** was carried out according to a report in the previous literature.[25] To a stirred suspension of compound **3 (**5 mmol) and K_2CO_3 (15 mmol) in acetone (35 mL), dibromoalkanes (20 mmol) was added. The mixture was heated to reflux for 12–24 h and then filtered. The filtrate was evaporated under vacuum to afford the crude product, which was purified by chromatography on a silica gel column with ethyl acetate/petroleum ether (1:6) as eluent to give a desired product **4**, yield 40%.^1H NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, J = 8.8 Hz, 3H), 7.87 (d, J = 7.8 Hz, 1H), 7.47 (t, J = 7.5 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 6.98 (d, J = 8.7 Hz, 2H), 4.06 (t, J = 6.2 Hz, 2H), 3.50 (t, J = 6.2 Hz,2H), 2.01–2.10 (m, 4H).

Synthesis of the intermediate 2-(4-(4-bromobutoxy)phenyl) benzo[d]oxazole (8)

The synthetic method was similar to that described for compound **4**, except that the intermediate is compound **7** instead of **3.** Accordingly product **8**, yield 38%.^1H NMR (400 MHz, $CDCl_3$) δ: 8.17 (d, J = 8.8 Hz, 3H), 7. 72 (d,J = 7.8 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.32 (t, J = 7.5 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 4.06 (t, J = 6.2 Hz, 2H), 3.56 (t, J = 6.2 Hz,2H), 1.99–2.09 (m, 4H).

General procedures for the preparation of 11a and 11b

Compounds **4** or **8 (**2 mmol) were added to a magnetically stirred suspension of Jatrorrhizine **9 (**2 mmol) and K_2CO_3 (6 mmol) in DMF (15 mL) under nitrogen atmosphere. The reaction mixture was allowed to heat in reflux for 24 h, and monitored by TLC. Then, the mixture was cooled to room temperature, filtered, and evaporated under vacuum. The crude product was purified by column chromatography, eluted with $CHCl_3$/MeOH (9:1) to afford the target compounds **10a** and **10b**. Then, these compounds were added into hot MeOH solution which contains AgCl to obtain their corresponding chlorides **11a** and **11b**.

3-(4-(4-(benzo[d]thiazol-2-yl)phenoxy)butoxy)-2,9,10-trimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium chloride (11a)

Yellow solid, yield 42%, mp 210–211°C; MS (*m/z*) 620.3 $[M-Cl]^+$; ^{1}H NMR (400 MHz, DMSO-*d*6) *δ*: 9.86(s, 1H)9.00 (s, 1H)8.16 (d*J* = 7.2 Hz, 1H), 8.08 (d, *J* = 7.2 Hz, 1H), 8.01 (d,*J* = 7.2 Hz, 4H), 7.67 (s, 1H), 7.49 (t, *J* = 6.8 Hz, 1H), 7.39 (t, *J* = 6.8 Hz, 1H), 7.12 (s, 1H), 7.10 (d, *J* = 6.8Hz, 2H), 4.93 (t, *J* = 6.8 Hz, 2H), 4.17 (m, 4H), 4.08 (s, 3H, -OCH_3-), 4.05 (s, 3H, -OCH_3-), 3.93 (s, 3H, -OCH_3-), 3.20 (t, *J* = 6.8 Hz, 2H), 1.95 (m, 4H, -CH_2-);^{13} C NMR *δ*167.1, 161.2, 153.7, 150.8, 150.3, 148.9, 145.4, 143.6, 137.7, 134.2, 133.1, 130.6, 128.9, 128.6, 126.7, 126.6, 125.4, 125.2, 123.5, 122.5, 122.2, 121.4, 119.9, 118.9, 115.2, 114.3, 108.9, 68.2, 67.6, 61.9, 57.1, 56.2, 26.0, 25.4, 25.2.

3-(4-(4-(benzo[d]oxazol-2-yl)phenoxy)butoxy)-2,9,10-trimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium chloride (11b)

Yellow solid, yield 42%, mp 208–209°0; MS (m/z) 604.1 $[M-Cl]^+$; 1H NMR (400 MHz, DMSO-*d*6) δ:9.87 (s1H)8.99 (s1H), 8.19 (d, *J* = 7.2 Hz, 1H), 8.13 (d, *J* = 7.2 Hz, 2H), 8.00 (d, *J* = 7.2 Hz, 1H), 7.71–7.55 (m, 2H), 7.68 (s, 1H), 7.34–7.39 (m, 2H), 7.19 (d, *J* = 7.2 Hz, 2H), 7.09 (s, 1H), 4.93 (t, *J* = 6.8 Hz, 2H), 4.16 (m, 4H), 4.08 (s, 3H, -OCH_3-), 4.05 (s, 3H, -OCH_3-), 3.93 (s, 3H, -OCH3-), 3.20 (t, *J* = 6.8 Hz, 2H), 1.94 (m, 4H, -CH_2-);^{13}C NMR *δ*: 161.9, 161.1, 150.2, 149.6, 148.3, 144.9, 143.1, 141.2, 137.2, 132.6, 128.7, 128.1, 125.0, 124.5, 124.2, 123.5, 122.9, 120.7, 119.2, 118.9, 118.4, 118.2, 114.8, 110.2, 108.3, 105.1, 101.8, 61.4, 66.9, 67.2, 56.6, 55.7, 54.9, 25.5, 24.9, 24.6.

General procedures for the preparation of 14

Intermediates **12** were synthesized based on previous literature.[26] To a stirred suspension of Jatrorrhizine (5 mmol) and K_2CO_3 (15 mmol) in CH_3CN (35 mL), dibromoalkanes (20 mmol) was added. The reaction mixture was heated to reflux for 12–24 h, and then filtered. The filtrate was evaporated under reduced pressure to afford the crude product, which was further purified by chromatography on an Al_2O_3 column using CH_3OH/CH_3Cl (9:1) as eluent to give the intermediates **12**. Compound **13** was prepared by reacting intermediate **12 (**0.1 mol) with commercially available paeonol (0.011 mol) in DMF in the presence of K_2CO_3 (0.02 mol) as catalyst. All the target compounds were purified by chromatography on an Al_2O_3 column using CH_3OH/CH_3Cl (9:1) as eluent to give **13a-c**, then these compounds (0.1 mmol) were added into hot MeOH solution which contains AgCl (0.11 mmol) to obtain their corresponding chlorides (**14a-c**).

3-(2-(2-acetyl-5-methoxyphenoxy)ethoxy)-2,9,10-trimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium chlorides (14a)

Yellow solid, yield 45%, mp 210–211°C; MS (*m/z*) 529.9 $[M-Cl]^+$; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 9.77 (s, 1H), 8.78 (s, 1H), 8.05 (dd, *J* = 8.7, 2.3 Hz, 2H), 7.76 (d, *J* = 9.1 Hz, 1H), 7.63 (s, 1H), 7.08 (s, 1H), 6.64 (s, 1H), 6.58 (d, *J* = 9.1 Hz, 1H), 4.96 (t, *J* = 6.8 Hz, 2H), 4.55 (br s, 4H), 4.23 (s, 3H), 4.12 (s, 3H), 3.99 (s, 3H), 3.89 (s, 3H), 3.28 (t, *J* = 6.8 Hz, 2H), 2.57 (s, 3H); ^{13}C NMR *δ*:199.3, 165.1, 160.4, 151.3, 150.5, 149.9, 144.8, 144.3, 138.2, 133.8, 132.3, 128.2, 126.8, 123.2, 122.0, 120.7, 120.4, 119.7, 112.7, 108.8, 106.0, 99.1, 67.5, 67.0, 61.6, 56.6, 56.0, 55.2, 31.3, 26.6.

3-(4-(2-acetyl-5-methoxyphenoxy)butoxy)-2,9,10-trimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium chlorides (14b)

Yellow solid, yield 41%, mp 200–201°C: MS (*m/z*) 557.5 $[M-Cl]^+$; ^{1}H NMR(400 MHz, CD_3OD) *δ*: 9.75 (s, 1H), 8.68 (s, 1H), 8.05 (dd, *J* = 8.7, 2.3 Hz, 2H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.58 (s, 1H), 6.95 (s, 1H), 6.52–6.56 (m, 2H), 4.95 (t, *J* = 6.8 Hz, 2H), 4.25 (t, *J* = 6.8 Hz, 2H), 4.24 (s, 3H), 4.22 (t, *J* = 6.8 Hz, 2H), 4.12 (s, 3H), 3.98 (s, 3H), 3.87 (s, 3H), 3.26 (t, *J* = 6.8 Hz, 2H), 2.57 (s, 3H), 2.12–2.15 (m, 4H); ^{13}C NMR *δ*:199.1, 165.0, 160.8, 151.7, 150.4, 149.8, 144.6, 144.4, 138.4, 133.8, 132.4, 128.0, 127.0, 123.1, 121.9, 120.5, 120.2, 118.8, 111.9, 108.5, 105.2, 98.8, 68.7, 68.1, 61.7, 56.8, 55.2, 53.4, 31.3, 26.8, 26.0, 25.7.

3-((6-(2-acetyl-5-methoxyphenoxy)hexyl)oxy)-2,9,10-trimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium chlorides (14c)

Yellow solid, yield 35%, mp 188–189°C; MS (*m/z*) 585.9 $[M-Cl]^+$; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 9.76 (s, 1H), 8.74 (s, 1H), 8.05 (dd, *J* = 8.7, 2.3 Hz, 2H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.58 (s, 1H), 6.96 (s, 1H), 6.52–6.56 (m, 2H), 4.95 (t, *J* = 6.8Hz, 2H), 4.24 (s, 3H), 4.17 (t, *J* = 6.8 Hz, 2H), 4.12 (s, 3H), 4.10 (t, *J* = 6.8 Hz, 2H), 4.00 (s, 3H), 3.86 (s, 3H), 3.26 (t, *J* = 6.8 Hz, 2H), 2.56 (s, 3H), 1.94 (m, 4H), 1.64 (m,4H); ^{13}C NMR *δ*:199.0, 165.1, 161.0, 152.0, 150.4, 149.7, 144.7, 144.3, 138.4, 133.9, 132.3, 128.2, 126.8, 123.2, 121.9, 120.3, 120.1, 118.8, 111.9, 108.7, 105.3, 98.6, 68.8, 68.3, 61.6, 56.2 (overlapped), 55.1, 31.4, 28.8, 28.7, 26.7, 25.7, 25.4.

General procedure for the preparation of 19 and 22

Partial demethylation of berberine **15** at 190°C under vacuum for 15 min gave berberrubine **16**, with a 68% yield.[26] The alkylations of berberrubine with 1, 4-dibrombutane in DMF was done according to our previous report[27] affording **17**. Compounds **18** and **21,** were prepared by reacting intermediates **17 (**0.01 mol) with commercially available paeonol (0.011 mol) and vanillin (0.011 mol), respectively, in DMF with K_2CO_3 (0.03 mol) which gave a 58–61% yield. Both of the target compounds were purified by chromatography on an Al_2O_3 column with CH_3OH/CH_3Cl (9:1) eluent, then these compounds (0.1 mmol) were added into hot MeOH solution which contained AgCl (0.11 mmol) to obtain their corresponding chlorides **19a** and **22a.**

3-(4-(2-acetyl-5-methoxyphenoxy)butoxy)-2,9,10-trimethoxy-5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium chloride (19a)

Yellow solid, yield 58%, mp 178–179°C; MS (*m/z*) 542.3 $[M-Cl]^+$; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 9.71 (s, 1H), 8.71 (s, 1H), 8.13 (d, *J* = 9.1Hz, 1H), 8.01 (d, *J* = 9.1Hz,1H), 7.73 (d, *J* = 9.1Hz, 1H), 7.69 (s, 1H), 6.99 (s,1H), 6.53–6.60 (m, 2H), 6.13 (m, 2H), 4.95 (t, *J* =7.8 2H), 4.57 (t, *J* = 5.7 Hz, 2H), 4.28 (t, *J* = 5.7 Hz, 2H), 4.12 (s, 3H), 3.83 (s, 3H), 3.27 (t, *J* = 7.8 Hz, 2H), 2.61(s, 3H), 1.97 (t, *J* = 5.7 Hz,

2H), 1.68 (t, *J* = 5.7 Hz, 2H); ^{13}C NMR *δ*: 198.6, 165.1, 160.7, 150.8, 150.4, 148.5, 144.8, 143.3, 138.2, 133.8, 132.1, 130.5, 126.5, 123.0, 122.1, 120.5, 120.4, 120.2, 108.0, 105.17, 105.15, 102.3, 98.6, 73.9, 68.1, 56.2, 55.8, 54.7, 30.7, 26.8, 26.7, 25.6.

9-(2-(3-formyl-4-methoxyphenoxy)ethoxy)-10-methoxy-5,6-dihydro-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a] isoquinolin-7-ium chloride (22a)

Yellow solid, yield 61%, mp 174–175°C; MS (*m/z*) 499.7 [M-Cl]$^+$; ^{1}H NMR (400 MHz, CD3OD) *δ*: 9.77 (s, 1H), 9.71 (s, 1H), 8.70 (s, 1H), 8.14 (d, *J* = 9.1 Hz, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.67 (s, 1H), 7.52 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.40 (d, *J* = 9.1 Hz, 1H), 7.14–7.16 (d, *J* = 9.1Hz, 1H), 6.97 (s, 1H), 6.13 (s, 2H), 4.92 (t, *J* = 8.8 Hz, 2H), 4.58 (t, *J* = 5.7 Hz, 2H), 4.29 (t, *J* = 7.8 Hz, 2H), 4.11 (s, 3H), 3.81(s, 3H), 3.25 (t, *J* = 7.8 Hz, 2H), 2.17 (t, *J* = 7.8 Hz, 2H), 1.29 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR *δ*:191.4, 154.1, 150.8, 150.8, 149.7, 148.5, 144.9, 143.4, 138.2, 133.8, 130.4, 130.0, 126.6, 126.2, 122.9, 122.1, 120.4, 120.1, 111.6, 109.5, 108.0, 105.1, 102.3, 73.9, 68.6, 56.2, 55.8, 55.0, 26.8, 26.7, 25.3.

General procedure for synthesis of compounds 26a-e

Compound **16** (berberrubine, 5 mmol) was dissolved in acetone (50 mL) in the presence of K_2CO_3 (15 mmol) followed by addition of appropriate n-alkyl bromide groups (20 mmol). The reaction mixture was stirred and heated to reflux for 12 h. After cooling to the room temperature, it was filtered and the filtrate was evaporated under vacuum to afford the crude product, which was purified by column chromatography using CH_3OH/CH_3Cl (9:1) as eluent to give **23a-e**. The 9-alkoxyl-8-octyloxy berberine substitutions **24a-e** were synthesized according to reported paper.[28] The Grignard reagent synthesized from magnesium powder (8.8 mmol) and n-octyl bromide (8.0 mmol) dissolved in anhydrous THF (10 mL) was slowly added to the suspension of 23a-e (2.0 mmol) under N_2 at 0. A mixture of **24a-e**, Br_2 (1.9 mmol) and THF (20 mL), was heated at 50°C and no change in the composition of the reaction mixture was verified by TLC. The solution was cooled and the precipitate was filtered and washed with 15% $Na_2S_2O_5$ solution and H_2O. The crude **25a-c** was obtained after crystallization from MeOH, and then added into hot MeOH solution which contains AgCl (1.8 mmol) to afford target compounds **26a-e**.

9-butoxy-8-octyloxyberberine(26a)

Yellow solid, 39% yield, mp236–238°C; electrospray ionization mass spectrometry (ESI-MS) *m/z* 490.5 [M-Cl]$^+$; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.79 (1H, s, H-13), 8.19 (1H, d, *J* = 9.0 Hz, H-11), 8.02 (1H, d, *J* = 9.0 Hz, H-12), 7.73 (1H, s, H-1), 7.11 (1H, s, H-4), 6.16 (2H, s, -OCH_2O-), 4.82 (2H, t, *J* = 6.0 Hz, H-6), 4.18 (2H, t, *J* = 6.0 Hz, -OCH2), 4.05 (3H, s, -OCH_3), 3.84 (2H, br s, ArCH_2), 3.15 (2H, t, *J* = 6.0 Hz, H-5), 1.80–1.84 (2H, m, ArCCH_2), 1.72–1.75 (2H, m, ArCCCH2), 1.47–1.52 [4H, m, $(CH_2)2$], 1.14–1.28 [8H, m, $(CH_2)4$], 0.97 (3H, t, *J* = 6.0 Hz, -CH_2), 0.86 (3H, t, *J* = 6.0 Hz, -CH3); ^{13}C NMR *δ*:161.2, 152.60, 149.7, 147.7, 144.9, 137.8, 132.7, 130.9, 125.1, 124.8, 121.5, 121.3, 120.4, 107.8, 105.9, 102.1, 74.0, 57.1, 49.7, 31.8, 31.7, 31.3, 29.3, 28.9, 28.7, 28.1, 26.7, 22.1, 18.7, 14.0, 13.8.

9-hexyloxy-8-octyloxyberberine (26b)

Yellow solid, yield 34%, mp 230–231°C; ESI-MS *m/z*518.2 [M-Cl]$^+$; ^{1}HNMR (400 MHz,CD_3OD) *δ*: 8.80 (1H, s, H-13), 8.17 (1H, d, *J* = 9.0 Hz, H-11), 8.02 (1H, d, *J* = 9.0 Hz, H-12), 7.73 (1H, s, H-1), 7.12 (1H, s, H-4), 6.17 (2H, s, -OCH_2O-), 4.81(2H, t, *J* = 6.0 Hz, H-6), 4.15(2H, t, *J* = 6.0 Hz, -OCH_2), 4.05 (3H, s, -OCH3), 3.82 (2H, br s, ArCH_2), 3.14 (2H, t, *J* = 6.0Hz, H-5), 1.85–1.87 (2H, m, ArCCH_2), 1.72–1.74 (2H, m, ArCCCH$_2$), 1.48–1.55 [4H, m, $(CH_2)2$], 1.28–1.36 [16H, m, $(CH_2)2$, $(CH_2)6$], 0.86–0.87 (6H, m, 2-CH_3); ^{13}C NMR *δ*:161.1, 152.5, 149.6, 147.6, 144.8, 137.7, 132.6, 130.8, 125.1, 124.7, 121.4, 121.1, 120.2, 107.7, 105.8, 102.0, 74.2, 57.0, 49.5, 31.7, 31.3 (overlapped), 29.5, 29.3, 28.9 (overlapped), 28.7, 28.7, 28.1, 26.6, 25.4, 22.1 (overlapped), 13.9 (overlapped).

9-octyloxy-8-octylberberine (26c)

Yellow solid, yield 33%,mp 210–211°C; ESI-MS *m/z* 546.3 [M-Cl]$^+$; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.80 (1H, s, H-13), 8.17 (1H, d, *J* = 9.0 Hz, H-11), 8.02 (1H, d, *J* = 9.0Hz, H-12), 7.73 (1H, s, H-1), 7.12 (1H, s, H-4), 6.17 (2H, s, -OCH_2O-), 4.81 (2H, t, *J* = 6.0 Hz, H-6), 4.15 (2H, t, *J* = 6.0 Hz, -OCH_2), 4.05 (3H, s, -OCH_3), 3.82 (2H, br s, ArCH2), 3.14 (2H, t, *J* = 6.0 Hz, H-5), 1.85–1.87 (2H, m, ArCCH_2), 1.72–1.74 (2H, m, ArCCCH$_2$), 1.48–1.55 [4H, m, $(CH_2)2$], 1.28–1.36 [16H, m, $(CH_2)2$, $(CH_2)6$], 0.86–0.87 (6H, m, 2-CH3); ^{13}C NMR *δ*: 161.1, 152.5, 149.6, 147.6, 144.8, 137.7, 132.6, 130.8, 125.1, 124.7, 121.4, 121.2, 120.3, 107.7, 105.8, 102.0, 74.2, 57.0, 49.5, 31.7, 31.3 (overlapped), 29.5, 29.3, 28.9 (overlapped), 28.7, 28.7, 28.1, 26.6, 25.4, 22.1 (overlapped), 13.9 (overlapped).

9-decyloxy-8-octylberberine (26d)

Yellow solid, yield 30%, mp 210–211°C; ESI-MS *m/z* 574.2 [M-Cl]$^+$; ^{1}HNMR (400 MHz,CD_3OD) *δ*: 8.80 (1H, s, H-13), 8.17 (1H, d, *J* = 9.0 Hz, H-11), 8.02 (1H, d, *J* = 9.0Hz, H-12), 7.73 (1H, s, H-1), 7.12 (1H, s, H-4), 6.17 (2H, s, -OCH_2O-), 4.80 (2H, t, *J* = 6.0 Hz, H-6), 4.15 (2H, t, *J* = 6.0 Hz, -OCH_2), 4.05 (3H, s, -OCH_3), 3.84 (2H, br s, ArCH2), 3.14 (2H, t, *J* = 6.0 Hz, H-5), 1.85–1.87 (2H, m, ArCCH2), 1.72–1.74 (2H, m, ArCCCH$_2$), 1.48–1.55 [4H, m, (CH2)2], 1.28–1.37 [20H, m, $(CH_2)2$, $(CH_2)8$], 0.86–0.87 (6H, m, 2-CH_3); ^{13}C NMR *δ*: 161.1, 152.5, 149.6, 147.6, 144.5, 137.7, 132.6, 130.8, 125.1, 124.7, 121.4, 121.3, 120.2, 107.7, 105.7, 102.0, 74.2, 57.0, 49.6, 31.7, 31.3 (overlapped), 29.5, 29.4, 29.0 (overlapped), 28.9 (overlapped), 28.7, 28.7, 28.1, 26.6, 25.4, 22.1 (overlapped), 13.9 (overlapped).

9-dodecyloxy-8-octylberberine (26e)

Yellow solid, yield 28%,mp 199–201°C; ESI-MS *m/z* 602.4 [M-Cl]$^+$; ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.80 (1H, s, H-13), 8.17 (1H, d, *J* = 9.0 Hz, H-11), 8.02 (1H, d, *J* = 9.0 Hz, H-12), 7.73 (1H, s, H-1), 7.12 (1H, s, H-4), 6.17 (2H, s, -OCH_2O-), 4.80 (2H, t, *J* = 6.0 Hz, H-6), 4.16 (2H, t, *J* = 6.0 Hz, -OCH2), 4.05 (3H, s, -OCH_3), 3.83 (2H, br s, ArCH_2), 3.15 (2H, t, *J* = 6.0 Hz, H-5), 1.85–1.87 (2H, m, ArCCH_2), 1.72–1.74 (2H, m, ArCCCH2), 1.49–1.55 [4H, m, $(CH_2)2$], 1.25–1.32 [24H, m, $(CH_2)2$, (CH2)10], 0.84–0.87 (6H, m, 2-CH_3); ^{13}C NMR *δ*: 161.0, 152.5, 149.6, 147.6, 144.8,

137.7, 132.6, 130.8, 125.1, 124.7, 121.4, 121.2, 120.3, 107.7, 105.7, 102.0, 74.2, 57.0, 49.6, 31.7, 31.3 (overlapped), 29.5, 29.4, 28.99 (overlapped), 28.89 (overlapped), 28.8, 28.7, 28.1, 26.6, 25.4, 22.1 (overlapped), 13.9 (overlapped).

Cytotoxicity assay

The cytotoxic activities of all target compounds were evaluated against human lung cancer cell line (NCI-H460), human cervical cancer cell line (HeLa) using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) colorimetric assay method.[29] Cells were plated at 1×10^4 cells per well in 96 well microtiter plates and incubated for 48 h at 37°C, 5% CO2. Each tumor cell line was treated with each test compounds at various concentrations in triplicate incubating for 48 h; doxorubicin, purchased from Shanghai Bo'ao Biotech Co. Ltd., Shanghai/ China, was used as a positive control. After 10 µL MTT reagent (5 mg/ml) was added, and the incubation continued at 37 ºC for 4 h, the MTT reagent was removed, and DMSO (150 µL) was added to dissolve the formazan crystals to measure the absorbance at 570 nm in a microplate reader (Bio-Rad 680). MTT solution in DMSO (without cells and medium) was used as a blank control. The half-maximum IC50 values were calculated by software statistical product and service solutions 16.0 from the reduction of absorbance in the control assay. The assay was performed in triplicate, and the data were presented as mean ± standard deviation.

CONCLUSION

We explored the effect of a series of pharmacophore groups at the carbon-9 of berberine and carbon-3 of jatrorrhizine on cytotoxic activity against NCI-H460 and HeLa cell lines. All synthesized compound displayed better cytotoxic activity against both NCI-H460 and HeLa cell lines *in vitro* than berberine and jatrorrhizine. Among lead compounds of this class, **26c** was identified to have a greatest cytotoxic activity *in vivo*. In general, the finding of this study can be used as a baseline for improvement and further development of antitumor drugs from natural products.

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