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Synthesis and anticancer activity of a novel series of 9-*O*-substituted berberine derivatives: A lipophilic substitute role

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ABSTRACT

To alter its hydrophobicity, a series of compounds bearing 9-*O*-alkyl- or 9-*O*-terpenyl- substituted berberine were synthesized and evaluated for anticancer activity against human cancer HepG2 and HT29 cell lines. We found that the lipophilic substitute of 9-*O*-alkyl- and 9-*O*-terpenyl berberine derivatives plays a role in inhibiting the human cancer cell growth and its activity could be maximized with the optimized substitute type and chain length. Most strikingly, nonetheless, of the six compounds prepared, sample **8**, a farnesyl 9-*O*-substituted berberine, showed either comparable or better cytotoxic activity against human cancer HepG2 cell line than that of berberine. Compound **8** had also shown a 104-fold antiproliferation activity in compare with berberine against human hepatoma HepG2 cell lines after 48 incubation hours. Further, in Hoechst 33258 and annexin V-FITC/PI staining analyses it induced apoptosis in HepG2 cells at lower concentration than that of berberine for 24 h. Take all; farnesyl 9-*O*-substituted berberine could be a potential candidate for new anticancer drug development.

Millions lives lost to cancer in the world each year and the number is increasing. Through, greater than 30% of cancer is considered preventable[1] and some forms of cancer are curable no matter when they are diagnosed; many others are only curable if they are caught at an early stage. Thus cancer remains a leading life threaten disease and serious peril to humanity health.[2]

Human hepatocellular carcinoma (HCC) and colorectal carcinoma are most common types of malignancy in sub-Saharan Africa and Southeast Asia like Taiwan and China. It is also the fifth most common and lethal cancer worldwide and usually treated by surgically removal followed by chemotherapy.[3,4] We have devoted our research effort in synthesizing and testing high efficient, broad-spectrum and low toxic anticarcinogen compounds for potential new drugs candidates for HCC treatment.

Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. It is found in such plants as *Berberis*, *Berberis aristata*, *Hydrastis canadensis*, *Phellodendron amurense*, *Coptis chinensis, Tinospora cordifolia*, and to a smaller extent in *Argemone mexicana* and *Eschscholzia californica*.[5] It has a wide range of biochemical and pharmacological effects.[6–8] In China and India, berberine was widely in use as an anticancer drug to treat hepatoma,[9] breast cancer,[10] bladder cancer,[11] and colon cancer.[12] In China, especially, this natural alkaloid has been used in the traditional medicine.[13,14] Furthermore, it was reported that berberine possesses significant cytotoxicity against human cancer cell lines, HepG2 and HT-29. It caused a significant reduction of the S phase fraction of HepG2 cells[15] and an arrest of gastric cancer cell in the G2/M phase.[16] Berberine and its derivatives had also been evaluated as inhibitor of topoisomerase I and II and demonstrated with anticancer activity.[17–19] In addition, a recent study reported some effectiveness of 9-*O*-substituted berberine derivatives selectively inhibit acetylcholinesterase (AChE) activity.[20]

However, for its hydrophilic in nature, berberine is absorbed poorly in intestines and thus showed a low inhibiting effect on suppressing cancer cell growth.[21] To enhance its intestine absorption and inhibitory function, we modified berberine analogues and screened its cytotoxicity in vitro on human liver HepG2 and colon HT29 cancer cell lines. Two types of functional groups, terpenyl and alkyl derivatives were investigated for the structure–activity relationship (SAR).

In specific, we synthesized and tested compounds of 9-*O*-alkylberberine derivatives with increased alkyl chain length (butyl, octyl, and dodecyl, samples **3**, **4** & **5**) and terpenyl (isoprenyl,

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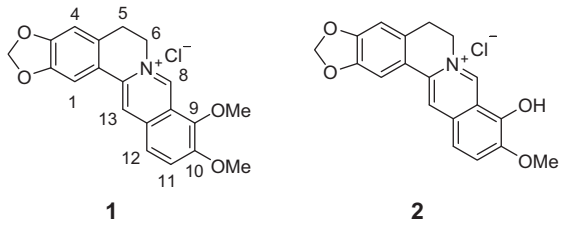
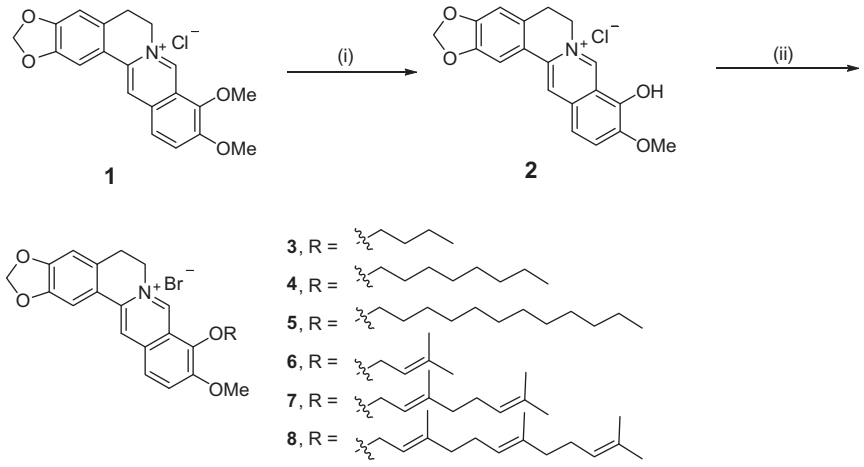


Figure 1. Chemical structures of berberine (**1**) and berberrubine (**2**).

geranyl, and farnesyl, samples **6**, **7** & **8**) substitutes. The effectiveness of anticancer activity of these compounds was carried out by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[22] Data on berberine (sample **1**) and its more hydrophilic derivative, berberrubine (sample **2**, Fig. 1), were used as baseline for evaluating the performance of the synthetic compounds (**3**–**8**). Berberrubine is an isoquinoline alkaloid isolated from the plant *Berberis vulgaris* L.[23] and readily isolated from berberine by pyrolysis.[24] The effects of some potent compounds on human hepatoma HepG2 cells apoptosis were further evaluated.

The synthetic route of lipophilic 9-*O*-substituted berberine derivatives **3**–**8** are outlined in Scheme 1. Berberine chloride (**1**, 5.0 g) was heated at 190 °C under vacuum (20–30 mmHg) for 1–2 h underwent selective demethylation to afford berberrubine (**2**, 3.8 g) in 79% yield.[25] To a solution of berberrubine (**2**, 1 mmol) in dry CH_3CN (10 mL) was added alkylated or terpenylated bromide (1.2 mmol) and the reaction mixture was refluxed for 4–8 h, and the progress of the reaction was monitored by TLC. Then, the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was chromatographed on a neutral Al_2O_3 column and eluted with $CHCl_3$/MeOH (99/1) solvent to afford the desired berberine derivatives **3**–**8** in quantitative yields (Scheme 1). The structures of berberine derivatives **3**–**8** were determined by NMR and LC–MS experiments.[26] Full signal assignment of ^1H and ^{13}C was carried out with NMR techniques including DEPT, COSY, HSQC, and HMBC analyses. All compounds were purified by flash column chromatography and were thoroughly characterized by ^1H and ^{13}C NMR spectroscopy and LC–MS spectroscopy.

The anticancer activity of the compounds studied herein was rated in half maximal inhibitory concentration (IC_{50}), which is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. The data of synthesized berberine derivatives (**3**–**8**), in compare with those of berberine (**1**) and berberrubine (**2**), against two tumor cells HepG2 and HT-29 are summarized in Table 1, with cisplatin data listed as a positive control. First of all, we found that the activities of the 9-*O*-alkyl- and 9-*O*-terpenyl berberine derivatives against human cancer HepG2 and HT-29 cell lines increased with the chain length of the substitute at the C-9 position. In other words, the change in lipophilicity of the



Scheme 1. Reagents and conditions: (i) 20–30 mmHg, 190 °C, 1–2 h; (ii) K_2CO_3, alkyl or terpenyl bromide, acetonitrile, reflux, 4–8 h.

Table 1
Lipophilicity and IC_{50} values for the in vitro screening of berberine **1**, berberrubine **2**, berberine derivatives (**3**–**8**) and cisplatin against human cancer cell lines for 24 h and 48 h

Compound	Clog *P*[a]	HepG2 IC_{50}[b] (μM)		HT-29 IC_{50}[b] (μM)	
		24 h	48 h	24 h	48 h
Berberine (**1**)	−0.771	11.22 ± 1.77	8.32 ± 2.11	>20	8.45 ± 0.35
Berberrubine (**2**)	−0.707	>50	>50	>50	36.93 ± 9.60
3	0.816	1.81 ± 0.70	0.97 ± 0.21	4.41 ± 0.30	0.87 ± 0.11
4	2.932	0.19 ± 0.01	0.10 ± 0.05	0.48 ± 0.06	0.08 ± 0.02
5	5.048	0.26 ± 0.10	0.10 ± 0.03	0.93 ± 0.07	0.28 ± 0.04
6	0.931	4.67 ± 0.54	3.17 ± 0.49	10.76 ± 4.71	1.75 ± 0.63
7	2.961	0.21 ± 0.10	0.17 ± 0.02	1.21 ± 1.14	0.14 ± 0.07
8	4.993	0.21 ± 0.07	0.08 ± 0.02	1.82 ± 0.26	0.27 ± 0.07
Cisplatin[c]	−1.684	82.12 ± 2.31	36.00 ± 3.10	53.28 ± 2.64	24.10 ± 0.10

[a] Calculated log value of partition coefficient by ChemDraw Ultra 8.0.
[b] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%. The values are means ± SD of three experiments conducted in triplicate.
[c] Positive control.