Regioselective semi-synthesis of 6-isomers of 5,8-O-dimethyl ether of shikonin derivatives via an ‘intramolecular ring-closing/ring-opening’ strategy as potent anticancer agents

Li Zhou¹, Xu Zhang² and Wen Zhou³*

Abstract
Synthesis of 6-isomer of 5,8-O-dimethyl ether of shikonin (13), a promising anticancer scaffold, always remains a huge challenge. Herein a key intermediate for 13, 2-(1-hydroxyl-4-methyl-3-pentenyl)-1,4,5,8-tetramethoxynaphthalene (10), was obtained on the large-scale synthesis. A ring-closing/ring-opening strategy was applied to avoid the undesired reactivity posed by the side chain and racemization of the chiral centre. Incorporation of bulky substituent 4-((tert-butoxycarbonyl)amino)phenyl to hydroxyl group in the side chain redistributed electron density of naphthalene core (10), overwhelmingly favoring the generation of 13 when oxidized by cerium(IV) ammonium nitrate followed by hydrolysis. As a result, three 6-isomers (14a–14c) with very potent antitumor activity were easily synthesized. This study opened an novel avenue to selectively prepare 6-isomers of 5,8-dimethoxy1,1,4-naphthaquinones, bearing the synthetically challenging side chain such as 2-hydroxyl-5-methylpentenyl group.

Keywords: 6-isomer of 5,8-O-dimethyl ether of shikonin, Ring-closing/ring-opening strategy, Bulky substituent, Semi-synthesis, Shikonin, Anticancer scaffold

Background
The medical application of Lithospermum erythrorhizon extract as an effective therapy for inflammation [1], infectious diseases [2], cancer [2] and atherosclerosis [2, 3] has been known very well for centuries. Its active ingredients, shikonin and its derivatives, have been extensively explored using various semi-synthetic or total-synthetic methodologies. Compounds with different substituents, such as hydroxalkyl [4], acyl [5], or hydroxyliminoalkyl [6], on C-6 (6-isomer, 1) or C-2 (2-isomer, 2) of 5,8-dimethoxy1,4-naphthaquinone (DMNQ) scaffold (Fig. 1), showed promising potency in the inhibition of DNA topoisomerase-I. They displayed high reactivity in conjugation with glutathione, which was responsible for their cytotoxicity. Their inhibitory effects against L1210 cells were also demonstrated [2]. Interestingly, when a double bond contained in the side chain was incorporated to naphthaquinone core, its cytotoxicity to normal cells was reduced while its bioactivity kept unchanged [2]. Moreover, in combination with our previous report [8], 6-isomers were found to exhibit better anticancer activity than the corresponding 2-isomers. Unfortunately, researches on DMNQ with double bond contained in the side chain had been largely impeded, mainly lacking an efficient synthetic methodology to prepare such derivatives. Later on, we found that synthesis of 2-isomer of 5,8-O-dimethyl ether of shikonin was accessible through the direct methylation of shikonin [9], while its corresponding 6-isomer was formidable to be prepared. To

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acquire natural product shikonin with high optical purity, asymmetric synthesis and chiral resolution were proposed to prepare crucial intermediates, 5,8-0-dimethyl ether of shikonin derivatives, in our group [10, 11]. However, the reaction conditions of asymmetric synthesis were harsh and difficult to be controlled and its catalytic agents were so expensive. In the process of chiral resolution two enantiomers were too close to be separated and this operation was time-consuming. Based on the issues mentioned above, we took our efforts to develop an efficient synthetic approach to semi-synthesize an more excellent antitumor scaffold, 6-isomer of 5,8-dimethoxy-1,4-naphthaquinones, bearing the synthetically challenging side chain such as 2-hydroxy-5-methylpentenyl group (13).

Modification of shikonin (3) was limited by its tendency to polymerize in the presence of acid, base, heat or temperature [2, 12–14]. Synthesis of compound 13 via direct methylation of shikonin failed as previously reported [2]. Selective preparation of compound 13 was ever pushed ahead when methoxymethyl was used as a protecting group, however, its application and scale were confined to deprotection and in situ oxidation. It was widely accepted that compound 13 could be synthesized in the form of mixture by oxidative demethylation of compound 10 [15]. Although 1,4,5,8-tetramethoxynaphthaquinones could be obtained from 5,8-dihydroxyl-1,4-naphthaquinones using proper reducing agents and methylating ones [16], the presence of hydroxyl-containing side-chain on tetrahydroxynaphthalene posed synthetically preparation of compound 10 a huge challenge [2, 17, 18] (Scheme 1). Therefore, to minimize its interference on the chemical behavior of the rest of the molecule, the side chain to be hidden was an appropriate approach to synthesise compound 10. Previous researches on shikonin and its derivatives had demonstrated that cycloshikonin (4) was more stable than shikonin itself toward Lewis acid, strong base or high temperatures [19, 20].

![Scheme 1](image1.png)

The structure of cycloshikonin had been confirmed by Sankawa et al. [7] as 5,8-dihydroxy-2-(5,6-dimethyl-2-tetrahydrofuranyl)-1,4-naphthaquinone. Although exposure to light, air or even high temperatures had little effect on racemization of shikonin as it existed in the solid form [21], little reports provided evidence for stability of chiral centre in the preparation for shikonin. Cyclization of the side chain of shikonin stood for a practical strategy for the preparation of compound 10. We speculated that cycloshikonin would survive the reaction conditions where compound 4 could be converted into 5 while leaving R-configuration intact. In this paper, we described a targeting semi-synthesis of 6-isomers of 5, 8-O-dimethoxyl ether of shikonin via an ‘intra-molecular ring-closing/ring-opening’ strategy, coupled with introduction of a bulky substituent for regulating distribution of electron density on naphthaquinone scaffold. This methodology is being applied to explore and obtain a variety of more potential shikonin derivatives in search of promising candidate drugs for anticancer therapy.

### Results and discussion

A facile synthesis of 2-(1-hydroxyl-4-methyl-3-pentenyl)-1,4,5,8-tetramethoxynaphthalene (10) is illustrated in Scheme 2. Cyclization of the side chain of shikonin (3) to form cycloshikonin (4) had been well demonstrated by previous investigators [2, 22]. Cyclization of shikonin could proceed in the presence of p-toluenesulfonic acid (PTSA) within 24 h, but the yield was low [22]. An alternative method that stannic chloride anhydrous was in place of PTSA gave compound 4 with the yield of 95% in 30 min. Noticeably, in the process of cyclization, shikonin with R-configuration didn’t change and e.e. value kept consistent, this was supported by the evidence that S-enantiomer of cycloshikonin analyzed with chiral HPLC didn’t appear (Additional file 1: Fig. S24).

Treatment of 4 with Na2S2O4 in a mixture of water and THF under N2 atmosphere provided the reduced cycloshikonin. Tetrabutylammonium bromide, NaOH and (CH3)2SO4 were subsequently added to a solution of the reduced cycloshikonin [17]. The ratio of NaOH to (CH3)2SO4 was found to be critical to the yield, and 4:1 was optimal. The above reaction mixture was stirred for 24 h under reflux to afford compound 5 with good repeatability in a more than 90% yield. Addition of tetrabutylammonium bromide, a phase transfer catalyst, was used to improve the solubility of the anion of the reduced shikonin, and then significantly increased the yield of compound 5. However, a few alternative reductive methylation conditions failed to provide compound...
For instance, the most commonly used methylating agent CH₃I in the presence of Ag₂O failed to convert compound 4 to compound 5. Reduced cycloshikonin was likely to be oxidized by Ag₂O back to compound 4, thus leading to the above observation. Treatment of reduced cycloshikonin with (CH₃)₂SO₄ in the presence of K₂CO₃ and (CH₃)₂CO under various temperatures proved to be problematic as well. This could be due to reaction of cycloshikonin with (CH₃)₂CO to form 1,8-bridged or 4,5-bridged cycloshikonin, and then hampering further conversion [23]. Other reaction conditions including CH₂N₂, trimethylsilyldiazomethane (TMSCHN₂) did not succeed in producing compound 5, either.

Opening of furan ring of compound 5 was a crucial step, which was carried out with PTSA in Ac₂O at low temperature to produce diacetyl 6 in an 88% yield. Higher temperature (> −16 °C) or room temperature resulted in yielding compound 15, which is an isomer of compound 9 (Scheme 2). The amount of compound 15 increased with reaction temperature rising. Deprotection of acyl group from compound 6 by 1 N NaOH readily produced diol 7 with a yield of 99%. Subsequent acetylation of compound 7 with acetic anhydride in pyridine gave ester 8. However, addition of 4-dimethylaminopyridine (DMAP) in this reaction gave rise to the undesired compound 6. Compound 9 was produced from ester 8 in the presence of pyridine and thionyl chloride. Subsequently, treated with 1 N NaOH, compound 9 was hydrolyzed to compound 10 in a 94% yield. Since all the reaction conditions for synthesizing compound 10 were totally defined, several reactions were reasonably combined into one pot to spare reaction time and simplify purification operation. As demonstrated in Scheme 2, a concise synthetic route toward more efficient preparation of compound 10 was optimized from seven-step to three-step using “one-pot” strategy, the yield increased by 15%.

As we known, oxidative demethylation of compound 10 in a solution of cerium(IV) ammonium nitrate (CAN) afforded the mixture of 13 and its positional isomer [2, 14]. In terms of the mechanism of CAN-mediated oxidative demethylation [24], introduction of a bulky substituent to 1-hydroxyl of the side chain to increase electron density of B ring contributed to its selective oxidation. Accordingly, esterification of compound 10 with a bulky group, 4-((tertbutoxycarbonyl)amino)benzoic acid in the presence of dicyclohexylcarbodiimide (DCC) and DMAP, gave rise to yield ester 11 in a 91% yield, which was selectively oxidative demethylated with CAN to compound 12. The latter was hydrolyzed to target compound 13 in the presence of K₂CO₃ in a 92% yield. Finally, various 6-isomer ester derivatives (14a–14c) [8] with very potent antitumor activities were taken as representative examples to demonstrate the advantageous application of the method (Scheme 3 and “Experimental Section”).

Conclusions
In summary, we have developed selective semi-synthesis of 5,8-dimethoxyl-6-(1-hydroxyl-4-methylpentyl)-1,4-naphthaquinones (13) from natural product shikonin. The ring-closing/ring-opening strategy for
obtaining the key intermediate, 2-(1-hydroxyl-4-methyl-3-pentenyl)-1,4,5,8-tetramethoxynaphthalene (10), was demonstrated to be effective, and the synthetic route was reasonably combined and optimized from seven-step to three-step. Cyclization of the side chain was applied to avoid the influence of hydroxyl-containing side-chain on reaction of its naphthaquinone core, and to ensure stereochemical retention of the configuration. A bulky-substituent-mediated oxidative demethylation was used to control the regioselective direction of 1,4,5,8-tetramethoxynaphthalin derivatives. This work has provided a new targeting semi-synthetic route toward biologically important 6-isomer derivatives starting from shikonin.

**Experimental section**

**General** Melting points (m.p.) were determined on a SGWX-4 micro-melting point apparatus and are uncorrected. NMR spectra were recorded on Varian Mercury-300 spectrometer (300 MHz for 1H and 75 MHz for 13C) or Varian Mercury-400 spectrometer (400 MHz for 1H and 100 MHz for 13C), chemical shifts of 1H and 13C spectra were recorded with tetramethylsilane as internal standard (CDCl3 δ H 7.26, δ C 77.2), and coupling constants were reported in hertz. Mass spectra were obtained on a ZAB-2F or JEOLDX-300 spectrometer. Optical rotations were measured on WZZ-3 polarimeter calibrated at the sodium D line (598 nm). Reactions where exclusion of water was necessary were performed according to Ref. [25]. TLC was carried out on silica gel (GF254) under UV light. Column chromatography was run on silica gel (200–300 mesh) or alumina from Qingdao Ocean Chemical Factory.

**Shikonin (3)**

Shikonin was extracted from Lithospermum erythrorhizon according to the procedure described by Birch [26].

Red-brownish needles, m.p. 145–146 °C (from CH3OH) (lit. m.p. 146–147 °C [27]); [α]D25 + 126.5° (c 0.2, C6H6), (lit. +138° [2]).

(R)-5,8-dihydroxyl-2-(5,5-dimethyl-2-tetrahydrofuranyl)-1,4-naphthaquinone, (+) cycloshikonin (4)

Cycloshikonin was prepared from shikonin by the method proposed previously [2]. Yield: 98%. Solid, m.p. 78–80 °C (from CH3OH) (lit. m.p. 79–80 °C [2]); [α]D25 + 156.6° (c 0.33, CHCl3). 1H NMR (300 MHz, CDCl3) δ: 12.53 (s, 1H, ArOH), 12.52 (s, 1H, ArOH), 7.23–7.19 (m, 3H, ArH, Quinone H), 5.17 (dd, 1H, J = 6.3, 5.7 Hz, CH), 2.66–2.62 (m, 1H, CH2), 1.93–1.91 (m, 1H, CH2), 1.90–1.89 (m, 1H, CH2), 1.88–1.74 (m, 1H, CH2), 1.38 (s, 3H, CH3), 1.35 (s, 3H, CH3). 13C NMR (75 MHz, CDCl3) δ: 182.5, 181.5, 164.2, 163.7, 133.1, 132.0, 131.5, 131.4, 112.3, 111.9, 82.3, 74.7, 38.9, 33.7, 28.9, 28.0. MS (EI, m/z): 288 [M]+, 255, 232, 219.

(R)-2-(5,5-dimethyl-2-tetrahydrofuranyl)-1,4,5,8-tetramethoxynaphthalene (5)

To a solution of 4 (5 g, 17.3 mmol) and tetrabutylammonium bromide (1.0 g) in THF (160 mL) and water (80 mL) was added sodium dithionite (15.1 g, 86.3 mmol). After stirring for 15 min, NaOH (13.9 g, 0.35 mol) was added at room temperature. Dimethyl sulfate (21 mL) was added dropwise in 10 min, and the mixture was refluxing for 24 h. The product was separated by partitioning between water and DCM. The crude product was purified by column chromatography over silica gel with ethyl acetate/petroleum ether (1/4, v/v) to give 5.46 g of pale-yellow oil. Yield: 91%. [α]D25 +139.2° (c 0.2, CHCl3); 1H NMR (300 MHz, CDCl3) δ: 7.12 (s, 1H, ArH), 6.80 (s, 2H, ArH), 5.52 (m, 1H, CH), 3.99 (s, 3H, OCH3), 3.95 (s, 3H, OCH3), 3.93 (s, 3H, OCH3), 3.75 (s, 3H, OCH3), 2.54–2.48 (m, 5H, ArH).
(R)-2-(1,4-diacetoxy-4-methylpentyl)-1,4,5,8-tetramethoxynaphthalene (6) and 2-(4-acetoxyl-4-methyl-2-pentenyl)-1,4,5,8-tetramethoxynaphthalene (15)

A mixture of 5 (2 g, 5.8 mmol) and p-toluenesulfonic acid monohydrate (1.14 g, 6 mmol) in acetic anhydride was allowed to stir overnight at −16 °C, and then the reaction mixture was diluted with methanol to quench excess acetic anhydride and extracted with ethyl acetate. After the usual work-up, the residue was purified by column chromatography over silica gel with ethyl acetate/petroleum ether (1/3, v/v) as an eluent to give 1.23 g of pale-yellow oil. Yield: 88%. [α] D 25° +142.2° (c 0.2, CHCl3). 1H NMR (300 MHz, CDCl3) δ: 6.85 (s, 1H, ArH), 6.83 (s, 2H, ArH), 6.32 (t, 1H, J = 7.8 Hz, CH3), 3.94 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 2.12 (s, 3H, COOC), 1.93–1.71 (m, 5H, CH2, COCH3), 1.41 (s, 3H, CH3), 1.39 (s, 3H, CH3).

13C NMR (75 MHz, CDCl3) δ: 150.7, 147.1, 130.9, 122.9, 121.1, 109.2, 108.1, 105.1, 81.9, 71.1, 62.7, 58.2, 57.7, 57.1, 37.1, 30.8, 26.2, 26.0, 22.6, 21.5. MS (ESI, %): 471 (M+Na+, 100), 503 (M2+NaOCH3, 31) and no parent peak was observed. HRMS (ESI) calc'd for C22H31O7Na+: 449.2170 [M+H]+, found: 449.2166.

The same operation as compound 6 was done at room temperature, major by-product 15 could be obtained as pale-yellow oil. 1H NMR (300 MHz, CDCl3) δ: 6.99 (s, 1H, ArH), 6.90 (d, 1H, J = 15.6 Hz, CH=CH2), 6.83 (s, 2H, ArH), 6.28 (m, 1H, CH=CH2), 4.00 (s, 3H, OCH3), 3.95 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 3.73 (s, 3H, OCH3), 2.78 (d, 2H, J = 6.6 Hz, CH2), 2.02 (s, 3H, COCH3), 1.52 (s, 6H, CH3). 13C NMR (75 MHz, CDCl3) δ: 171.2, 153.6, 151.3, 150.5, 147.2, 131.0, 122.2, 119.1, 109.5, 105.8, 105.3, 81.8, 71.0, 62.4, 58.0, 57.5, 57.3, 37.0, 30.6, 26.3, 21.6, 22.7. MS (ESI, %): 411 (M+Na+, 100), 443 (M2+NaOCH3, 38) and no parent peak was observed. HRMS (ESI) calc'd for C22H31O7Na+: 449.2170 [M+H]+, found: 449.2166.
OCH$_3$), 2.59–2.54 (m, 2H, CH$_2$), 2.10 (s, 3H, OCOCH$_3$), 1.65 (s, 3H, CH$_3$). $^{13}$C NMR (75 MHz, CDCl$_3$) δ: 170.4, 153.5, 151.6, 150.8, 147.1, 134.8, 130.9, 122.9, 120.9, 119.4, 109.0, 108.2, 105.6, 71.1, 62.7, 58.1, 57.7, 57.3, 34.8, 25.9, 21.5, 18.1. MS (ESI, %): 411 (M$^+$+Na$^+$, 100), 443 (M$^+$+NaOCH$_3$, 18) and no parent peak was observed. HRMS (ESI) calcld. for C$_{22}$H$_{28}$O$_6$Na$^+$: 411.1778 [M+Na$^+$], found: 411.1776.

(R)-2-(1-hydroxy-4-methyl-3-pentenyl)-1,4,5,8-tetramethoxyoxynaphthalene (10)
Hydrolysis of 9 (1 g, 2.6 mmol) in 1 N sodium hydroxide (100 mL) and methanol (50 mL) was stirred at 0–5 °C for 12 h under a nitrogen atmosphere. Ethyl acetate was added to dilute the reactive mixture. Organic layer was washed with water and saturated brine, and dried over anhydrous MgSO$_4$, and then evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel with ethyl acetate/petroleum ether (1/4, v/v) to obtain 839.2 mg of desirable compound. Yield: 91%. $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.94 (d, J = 0.8 Hz, 2H, ArH), 7.42 (d, J = 0.8 Hz, 2H, ArH), 7.23 (s, 1H, ArH). $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.42 (d, J = 0.8 Hz, 2H, ArH), 7.23 (s, 1H, ArH), 6.70 (s, 2H, QuinoneH), 6.62 (t, J = 4.0 Hz, 1H, CH$_2$), 5.14 (t, J = 6.8 Hz, 1H, CH$_2$), 3.91 (s, 3H, OCH$_3$), 3.80 (s, 3H, OCH$_3$), 2.59–2.64 (m, 1H, CH$_2$), 2.49–2.56 (m, 1H, CH$_2$), 1.61 (s, 3H, CH$_3$), 1.50 (s, 3H, CH$_3$), 1.44 (s, 9H, CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 184.8, 184.3, 165.3, 156.1, 152.2, 150.6, 144.9, 143.2, 138.9, 137.8, 135.8, 130.8, 125.2, 123.9, 120.1, 118.2, 117.5, 116.6, 81.3, 71.2, 62.0, 56.6, 34.1, 28.2, 25.8, 17.9. HRMS (ESI) calcld. for C$_{39}$H$_{54}$NO$_8$+: 536.2279 [M+H$^+$]+, found: 536.2284.

(R)-5,8-dimethoxy-6-(1-hydroxy-4-methylpentyl)-1,4-naphthoquinones (13)
A solution of K$_2$CO$_3$ (6.6 g, 48.0 mmol) was added dropwise to a stirred solution of 12 (12.9 g, 24.0 mmol) dissolved in THF (250 mL) at ice-bath. The reaction mixture was stirred for 2 h at the same temperature. The progress was monitored by TLC. After completion, the mixture was neutralized with saturated NH$_4$Cl solution, and then diluted with water and ethyl acetate. Organic layer was separated and aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with saturated brine (150 mL), and dried over anhydrous Na$_2$SO$_4$, and then concentrated under reduced pressure. The residue was purified by column chromatography with ethyl acetate/petroleum ether (1/1, v/v) to give 3.1 g of compound 12 as yellow oil. Yield: 91%. $^1$H NMR (400 MHz, CDCl$_3$) δ: 7.94 (d, J = 0.8 Hz, 2H, ArH), 7.42 (d, J = 0.8 Hz, 2H, ArH), 7.23 (s, 1H, ArH). 

$^{1}$H NMR (400 MHz, CDCl$_3$) δ: 7.14 (s, 1H, ArH), 6.70 (s, 2H, QuinoneH), 6.62 (t, J = 4.0 Hz, 1H, CH$_2$), 5.14 (t, J = 6.8 Hz, 1H, CH$_2$), 3.91 (s, 3H, OCH$_3$), 3.80 (s, 3H, OCH$_3$), 2.59–2.64 (m, 1H, CH$_2$), 2.49–2.56 (m, 1H, CH$_2$), 1.61 (s, 3H, CH$_3$), 1.50 (s, 3H, CH$_3$), 1.44 (s, 9H, CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 184.8, 184.3, 165.3, 156.1, 152.2, 150.6, 144.9, 143.2, 138.9, 137.8, 135.8, 130.8, 125.2, 123.9, 120.1, 118.2, 117.5, 116.6, 81.3, 71.2, 62.0, 56.6, 34.1, 28.2, 25.8, 17.9. HRMS (ESI) calcld. for C$_{39}$H$_{54}$NO$_8$+: 536.2279 [M+H$^+$]+, found: 536.2284.
139.2, 137.9, 136.9, 125.1, 68.8, 62.4, 56.9, 37.2, 26.1,
18.2. MS (ESI, %): 317 (M+H, 12.5), 339 (M+Na, 30), 371 (M+NaOCH3, 100). HRMS (ESI) calcd. for C18H20O5Na+: 339.1203 [M+Na]+, found: 339.1207.

(R)-1-(1,4-dimethoxy-5,8-dioxo-5,8-dihyronaphthalen-2-yl)-4-methylpent-3-en-1-yl 3-hydroxy-3-methylbutanoate (14a)
To a stirred solution of 13 (3.16 g, 10.0 mmol) and 3-hydroxy-3-methylbutanoic acid (1.30 g, 11.0 mmol) in anhydrous DCM were added DCC (2.27 g, 11.0 mmol) and DMAP (350 mg, 2.9 mmol). TLC was applied to monitor the progression. After completion, petroleum ether was added into the reaction mixture to facilitate precipitation at 4 °C, and filtered to remove the insoluble precipitates at 4 °C, and filtered to remove the insoluble precipitates. The residue was purified by flash chromatography to afford 2.54 g of 14a as yellow oil. Yield: 61%. $[α]_{D}^{25} +59.3^\circ$ (c 0.4, CHCl3). $^1$H NMR (300 MHz, CDCl3) δ: 7.24 (d 1H, J = 3.0 Hz, ArH), 6.78 (d, 2H, J = 8.1 Hz, CH), 3.95 (s, 3H, OCH3), 3.94 (s, 3H, OCH3), 2.58–2.38 (m, 4H, 2 × CH2), 1.68 (s, 3H, CH3), 1.55 (s, 3H, CH3), 1.29 (s, 3H, CH3), 1.26 (s, 3H, CH3). $^{13}$C NMR (75 MHz, CDCl3) δ: 187.6, 186.5, 173.2, 152.1, 138.7, 134.2, 132.0, 124.1, 119.7, 115.2, 114.3, 70.9, 70.0, 62.3, 55.4, 42.1, 32.4, 29.2, 24.4, 18.1. HRMS (ESI) calcd. for C23H29O7+: 417.1908 [M+H]+; found: 417.1902. These data were in accordance with the literature [8].

Chiral HPLC analysis conditions for shikonin and its derivatives
The chiral HPLC column applied (150 × 4.6 mm) was Sino-Chiral OD [No. 0A02014-C (Packing cellulose-tris (3,5-dimethylphenyl carbamate)], which was purchased from FunSea Beijing Technology Co. Ltd (Beijing). All the separations were performed at ambient temperature. The mobile phase, hexane–isopropanol (80:20, v/v) was degassed before application. To obtain sufficient resolution of shikonin, alkannin and their derivatives, the flow rate of mobile phase was adjusted to 0.65 mL/min and injection volume was set at 5 μL.

Additional file

Additional file 1. Additional figures.

Authors’ contributions
LZ performed the experiments, analyzed the data and write part of the paper; XZ conducted some of the experiments and contributed reagents and materials; WZ conceived and designed the experiments, and wrote part of the paper. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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