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Note

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Total Synthesis of Tanshinone I

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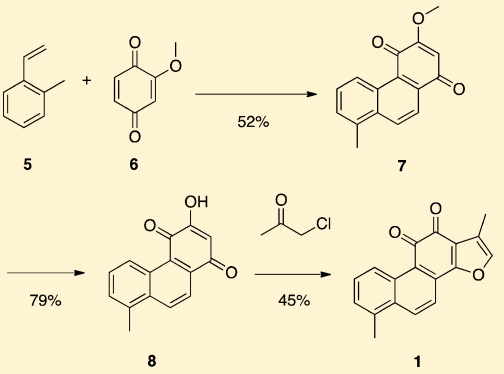
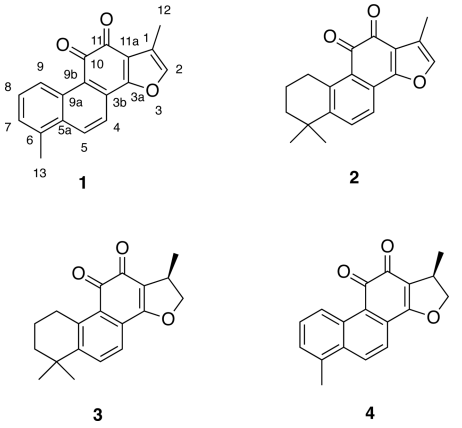
Supporting Information

ABSTRACT: A novel total synthesis of tanshinone I (**1**) via the intermediate 3-hydroxy-8-methyl-1,4-phenanthrenedione (**8**) is described. The low overall yields and the use of expensive reagents in the synthesis process were minimized by the use of the Diels–Alder reaction to directly construct the 1,4-phenanthrenedione scaffold, providing tanshinone I (**1**) in only three steps.



Tan-shen, the Chinese traditional herb *Salvia miltiorrhiza* Bunge, is widely used in the clinic for the treatment of numerous disorders, such as cardiovascular and inflammatory diseases, chronic hepatitis, and liver fibrosis.[1,2] The chemical constituents of tan-shen can be classified as either water-soluble or lipophilic. Among the lipophilic compounds are several abietane-type norditerpenoid quinone pigments, such as tanshinone I (**1**) and tanshinone IIA (**2**).[3] Research on diterpenoids and tanshinones began in the early 1930s and established diverse biological properties, such as antioxidant, anti-inflammatory, antidermatophytic, and antineoplastic effects.[4] Among the various tanshinones, tanshinone I (**1**), tanshinone IIA (**2**), crytotanshinone (**3**), and dihydrotanshinone I (**4**) are the major constituents, and their biological effects, such as cytotoxicity, have been a prime research focus.[5] A previous study has shown that tanshinone I and tanshinone IIA are more potent than DNA topoisomerase II inhibitors such as doxorubicin, etoposide, and etoposide phosphate in inhibiting the growth of a human leukemia cell line and the multi-drug-resistance gene 1-expressing cell line.[6] Another study on the proliferation inhibition of HepG2 cell lines showed that the above-mentioned constituents of the tanshinone family as well as dihydrotanshinone I (**4**) and tanshinone I (**1**), possessing greater potency than tanshinone II (**2**) and crytotanshinone (**3**), respectively, had remarkable inhibitory effects.[7] Thus, a new, efficient, and high-yielding synthesis of tanshinone I (**1**) was considered.

To date, there have been several reports regarding the synthesis of tanshinone I (**1**). The study of the tanshinones began in 1968, with Baillie and Thomson reporting a semisynthesis method using the Feist–Benary reaction to prepare two series of tanshinones from the natural product podocarpic acid.[8] Using the same reaction, Huot and Brassard prepared tanshinone I (**1**) from 3,4-dimethoxy-8-methylphenanthrene (**9**) in 1974.[9] These syntheses were commercially limited by the limited availability of natural raw materials. In 1992, Danheiser et al. synthesized tanshinone I (**1**) via a photochemical reaction.[10] Later, De Koning et al. constructed compound **9** via a Suzuki coupling and a photochemical reaction[11] and also developed a novel synthesis of tanshinones by combining a photochemical reaction with Hout–Brassard's semisynthesis reaction. However, the use of Suzuki couplings still suffered from the problem of intricate cyclizations, and the photochemical steps limited industrial adoption of these approaches. In 2014, Mingkun Jiao et al. developed a novel and mild procedure to synthesize 3-hydroxy-8-methyl-1,4-phenanthrenedione (**8**), a key intermediate through which our group achieved a total synthesis of tanshinone I (**1**).[12] However, the method used to synthesize **8** had the



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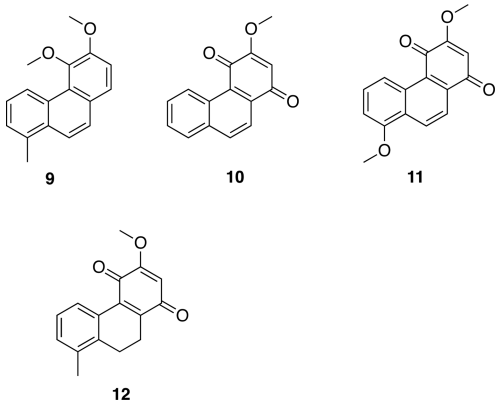
disadvantage of a low overall yield and involved a laborious process handicapped by an especially difficult intermediate purification (by flash chromatography or other means) and the use of expensive and toxic reagents. To establish a scalable process to provide tanshinone I (**1**), we focused on the Diels–Alder reaction to construct the phenanthrene-1,4-dione scaffold.

The Diels–Alder reaction is widely recognized as one of the most useful pericyclic reactions toward a six-membered-ring formation,[13] evidenced by its use in many natural product syntheses. The use of the Diels–Alder reaction in the synthesis of tanshinone I (**1**) had been reported as early as 1968, when Baillie and Thomson coupled diazotized sulfanilic acid with 8-methyl-3-phenanthrol, followed by reduction and oxidation to provide compound **8**.[14] The low yield of the synthesis of the phenanthrene-1,4-dione core limited this approach. In 1969, Inouye and Kakisawa reported a synthesis of tanshinone I (**1**) using the same reaction between 3-methylbenzofuran-4,7-quinone and *o*-methylstyrene, followed by reduction and hydrolysis to construct the phenanthrene-1,4-dione core.[15] This method, which used the Diels–Alder reaction to build the phenanthrene-1,4-dione core, allowed the elaboration of general routes to the entire series of tanshinones, but all three steps suffered from low yields and difficult purifications. Recently, we investigated the Diels–Alder reaction between styrenes and 1,4-benzoquinones to build phenanthrene-1,4-dione. In practice, the addition to substituted 1,4-benzoquinones occurs only at the unsubstituted double bond.[16] Experimentally, the determination of the regioselectivity of substitution (C-2 vs C-3) of the benzoquinone ring is challenging due to the similarity of the chemical shifts in the two regioisomers in both the ^{1}H and ^{13}C NMR spectra.[17] In 1971, Kakisawa and Inouye proved that the Diels–Alder reaction between 2-methoxy-1,4-benzoquinone and styrene produced compound **10**.[16] In 1986, Krohn and Loock determined the structure of 3,8-dimethoxy-1,4-phenanthrenedione (**11**), synthesized via the Diels–Alder reaction between 2-methoxystyrene and 2-methoxy-1,4-benzoquinone (**6**) by X-ray analysis.[18]



The Diels–Alder reaction between equimolar quantities of **5** and **6** afforded two compounds, **7** and **12**, which were difficult to separate by column chromatography or recrystallization. The purification problem was solved by increasing the amount of **6** as oxidant to get compound **7** directly, which was an essential step to increase the overall yields for tanshinone I. Finally, using 2D NMR spectroscopy, the success of this reaction was verified by assigning the accurate structure of compound **7**. Its ^{1}H and ^{13}C NMR data (Table 1) showed the presence of an angular methyl (δ_H 2.73; δ_C 20). The typical signals of a methoxy-1,4-benzoquinone moiety [δ_H 6.14 (1H s H-2), 3.94 (3H s OCH_3); δ_C 182.5 (C-4), 185.4 (C-1)] were observed in the ^{1}H and ^{13}C NMR data of compound **7** (Table 1). The HMBC cross-peaks (Figure 1) from H-2 (δ_H 6.14) to C-10a (δ_C 132.1); from H-10 (δ_H 8.21) to C-1 (δ_C 185.4); from H-9 (δ_H 8.38) to C-10a (δ_C 132.1), C-8 (δ_C 134.9), and C-8a (δ_C 126.4); and from H_3-11 (δ_H 2.73) to C-8a (δ_C 126.4), C-8 (δ_C 134.9), and C-7 (δ_C 129.3) defined the location of the methoxy group.

Table 1. ^{1}H and ^{13}C NMR Data of Compound 7[a]

position	δ_H (J in Hz)	δ_C, type
1		185.4, C
2	6.14 s	107.1, CH
3		161, C
4		182.5, C
4a		135.5, C
4b		130.4, C
5	9.39 d (8.4)	125.7, CH
6	7.61 m	130, CH
7	7.46 d (6.6)	129.3, CH
8		134.9, C
8a		126.4, C
9	8.38 d (9)	131.5, CH
10	8.21 d (8.4)	121.6, CH
10a		132.1, C
11	2.73 s	20, CH_3
OCH_3	3.94 s	56.6, CH_3

[a]Data (δ) were measured in $CDCl_3$ at 600 MHz for protons and 150 MHz for carbons.

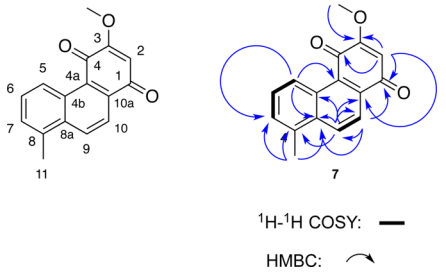
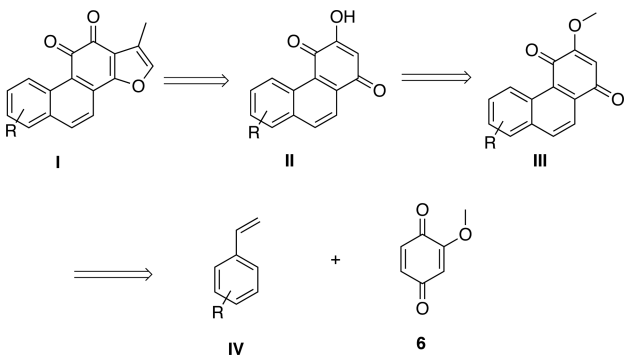


Figure 1. Key COSY and HMBC correlations for compound **7**.

Herein we report a synthesis of tanshinone I and its analogues based on the disassembly (shown in Scheme 1) of the furan ring in compound **I** leading to compound **II**. Compound **II** in turn could be prepared from compound **III**

Scheme 1. Retrosynthetic Analysis of Tanshinone I (1)



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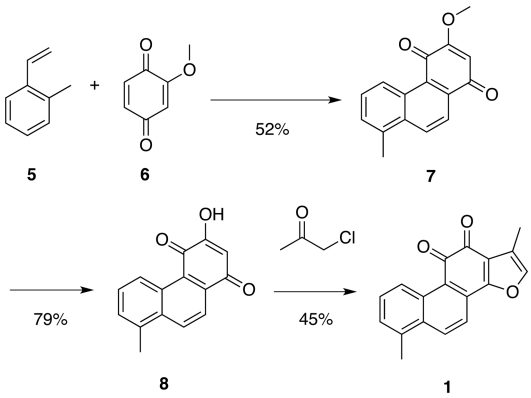
via hydrolysis or demethylation. Compound **III** is considered the crucial intermediate and is derived by a Diels–Alder reaction from the substituted styrene **IV** and the 2-methoxy-1,4-benzoquinone.

Thus, the synthesis of tanshinone I (**1**) starts with the reaction between 2-methylstyrene (**5**) and 2-methoxy-1,4-benzoquinone (**6**). The uncatalyzed Diels–Alder reaction between **5** and **6** in toluene afforded compound **7** in 52% yield. Subsequent demethylation of compound **7** using NaOH in aqueous EtOH afforded compound **8** in 79% yield. In the final step, the Feist–Benary reaction of compound **8** with chloroacetone in HOAc–NH_4OAc gave the target tanshinone I (**1**), in 45% yield. The overall yield for the three-step synthesis was approximately 18.5% (Scheme 2).

Scheme 2. Synthesis of Tanshinone I (1)



In summary, we have developed an efficient three-step total synthesis of tanshinone I (**1**) using three straightforward steps. Furthermore, compound **7** was synthesized in one step through the Diels–Alder reaction between a 1,4-benzoquinone and a styrene, a considerable improvement over current syntheses. The synthesis reduced the number of toxic reagents and lowered production costs through a higher overall yield, less expensive reagents, and only three steps. This readily scaled method should benefit further research on the biological properties of tanshinone I (**1**).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using the X-4 micro melting point apparatus. NMR data were obtained for ^1H at 400 or 600 MHz and for ^{13}C at 100 or 150 MHz in a $CDCl_3$ solution with tetramethylsilane as the internal standard. ESI-HRMS data were obtained with a Waters SYNAPT G2 mass spectrometer. All reagents were obtained from commercial sources and used without further purifications. Column chromatography was carried out on silica gel (200–300 mesh). Thin-layer chromatography was performed using commercially available HSGF 254 precoated plates.

3-Methoxy-8-methyl-1,4-phenanthrenedione (7).[19] A mixture of compound **5** (6.9 g, 50 mmol), compound **6** (35.4 g, 300 mmol), and anhydrous toluene (500 mL) in a sealed autoclave was heated to 200 °C for 6 h. After cooling, the reaction mixture was purified by column chromatography (petroleum ether–CH_2Cl_2, 5:2) and recrystallization using EtOAc to provide compound **7** (7.6 g, 52%) as an orange solid: mp 222–225 °C; ^1H NMR ($CDCl_3$, 600 MHz) δ 9.39 (1H, d, J = 8.4 Hz, H-5), 8.38 (1H, d, J = 9 Hz, H-9), 8.21 (1H, d, J = 8.4 Hz, H-10), 7.63–7.60 (1H, m, H-6), 7.46 (1H, d, J = 6.6 Hz, H-7), 6.14 (1H, s, H-2), 3.94 (3H, s, OCH_3), 2.73 (3H, s, H-11); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.4, 182.5, 161.0, 135.5, 134.9, 132.1, 131.5, 130.4, 130, 129.3, 126.4, 125.7, 121.6, 107.1, 56.6, 20; HRMS (ESI) m/z 275.0686 $[M + Na]^+$ (calcd for $C_{16}H_{12}NaO_3$, 275.0684).

3-Hydroxy-8-methyl-1,4-phenanthrenedione (8). A mixture of compound **7** (5 g, 20 mmol), NaOH (4.2 g, 105 mmol), H_2O (700 mL), and EtOH (150 mL) was stirred at reflux for 1 h. After cooling, the reaction mixture was acidified with 0.5 N HCl and extracted with EtOAc (3 × 200 mL). The combined organic solution was washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to provide crude **8**, which was purified by column chromatography to afford compound **8** (3.8 g, 79%) as a dark red solid: mp 209–211 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.45 (1H, d, J = 8.8 Hz, H-5), 8.46 (1H, d, J = 8.8 Hz, H-9), 8.26 (1H, d, J = 9.2 Hz, H-10), 7.67–7.63 (1H, m, H-6), 7.49 (1H, d, J = 6.8 Hz, H-7), 6.33 (1H, s, H-2), 2.75 (3H, s, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 184.6, 183.4, 161.2, 135.4, 134.2, 123.3, 131.5, 130.6, 129.6, 128.3, 125.4, 124.7, 121.6, 107.6, 19.7; HRMS (ESI) m/z 239.0710 $[M + H]^+$ (calcd for $C_{15}H_{11}O_3$, 239.0708).

Tanshinone I (1). A round-bottomed flask was charged with compound **8** (9.5 g, 40 mmol), toluene (1000 mL), HOAc (12 g, 200 mmol), NH_4OAc (15.4 g, 200 mmol), chloroacetone (18.5 g, 200 mmol), and EtOH (200 mL), and the mixture was refluxed for 2 h in the dark. After cooling, the reaction mixture was diluted with H_2O (200 mL) and extracted with EtOAc (3 × 200 mL). The combined organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a solid residue. The residue was further purified using flash column chromatography (petroleum ether–CH_2Cl_2, 3:1) to afford tanshinone I (**1**) (5.0 g, 45%) as a red solid: mp 228–230 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.21 (1H, d, J = 9.2 Hz, H-9), 8.25 (1H, d, J = 8.8 Hz, H-5), 7.75 (1H, d, J = 8.8 Hz, H-4), 7.54–7.50 (1H, m, H-8), 7.32 (1H, d, J = 6.8 Hz, H-7), 7.28 (1H, s, H-2), 2.66 (3H, s, H-13), 2.27 (3H, s, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.3, 175.5, 161.1, 141.9, 135.1, 133.5, 132.8, 132.6, 130.6, 129.5, 128.2, 124.6, 122.9, 121.7, 120.4, 118.6, 19.8, 8.8; HRMS (ESI) m/z 299.0685 $[M + Na]^+$ (calcd for $C_{18}H_{12}NaO_3$, 299.0684).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00238.

^1H and ^{13}C NMR spectra data for compounds **1** and **7** and 2D NMR spectra data for **7** (PDF)

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Notes

The authors declare no competing financial interest.

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