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Further evidence for the participation of primary carbon-centered free radicals in the antimalarial action of the qinghaosu (artemisinin) series of compounds

Dong-Ye Wang,[a] Yu-Lin Wu,*[a] Yikang Wu,[a] Jie Liang[b] and Ying Li*[b]

[a] *State Key Laboratory of Bio-organic & Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China. E-mail: ylwu@pub.sioc.ac.cn*
[b] *Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, China. E-mail: yli@mail.shcnc.ac.cn*

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Friedel–Crafts alkylation of 1,3-dimethoxybenzene with *O*-acetyldihydroqinghaosu gave a pair of 11-epimers of 12-(2,4-dimethoxyphenyl)deoxoqinghaosu **2** and **2′**. The antimalarial activity of 11β-epimer **2** was comparable with that of artemether. Compound **2** reacted smoothly with ferrous ions or L-cysteine and catalytic amounts of ferrous ions to give a series of products derived from the previously postulated C-centered free radical, in particular the primary C-centered free radical. However, 11α-epimer **2′** was almost inert to ferrous ions and also showed low antimalarial activity. The identification of free radical mediated products and the correlation between chemical reactivity and bio-activity once again provided evidence for the key role of primary carbon-centered free radicals in the antimalarial activity of the qinghaosu series of compounds.

Introduction

The incidence of malaria is dramatically increasing as many *Plasmodium falciparum* strains have now become resistant to the widely-used drugs like chloroquine. It is imperative that novel antimalarial compounds are developed to treat the disease. The most promising compound at present is qinghaosu (**1**, QHS, artemisinin), which was isolated from the Chinese medicinal herb qinghao (*Atremisia annua* L.) in 1971.[1] QHS is a sesquiterpene lactone bearing an endoperoxide function which has been proved to be essential for antimalarial activity. The mechanism of action of QHS probably involves the cleavage of the endoperoxide function, leading to C-centered radicals derived from qinghaosu, which act as alkylation agents or free radical transfer agents. Alkylation of heme and specific parasite proteins by QHS has been reported,[2] but the results do not provide a full explanation of the antimalarial mechanism of QHS.

A number of research groups including ours have reported on the mechanism of the cleavage of QHS and its derivatives by Fe(II) and certified the presence of C-centered radicals derived from the cleavage of the endoperoxide function.[3] The intermolecular interaction—abstraction of a hydrogen atom from cysteine and attachment of it to the cysteine sulfur atom—of the primary C-centered radicals involved in the degradation of QHS has been found in our previous work.[4]

Recent studies of the chemistry of the digestive vacuole (pH 5.0–5.4) within *Plasmodium falciparum* have revealed a defined metabolic pathway for the degradation of hemoglobin. *Plasmodium* has a limited capacity for *de novo* amino acid synthesis, so hemoglobin proteolysis may be essential for its survival. However, hemoglobin degradation alone appears insufficient for the parasite's metabolic needs since it is a poor source of methionine, cysteine, glutamine, and glutamate and contains no isoleucine. On the other hand, as pointed out by Fracis *et al.*,[5] a number of experiments show that cysteine protease has a key role in the hemoglobin degradation pathway; it has even been hypothesized that the plasmepsins generate hemoglobin fragments that cannot be further catabolized without cysteine protease action. Meshnick *et al.*[6] have concluded that the binding between artemisinin and albumin probably involves thiol and amino groups *via* both iron-dependent and -independent reactions, but they have not isolated and confirmed the presence of such covalent adducts.

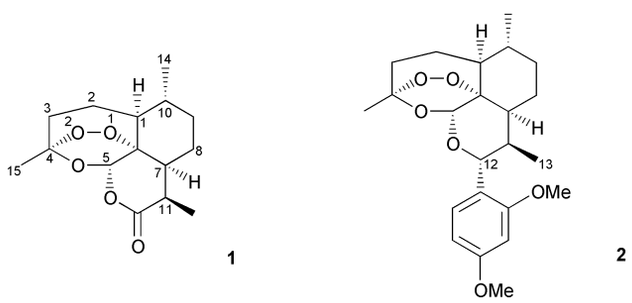

Fig. 1

Therefore inspection of the interaction of cysteine with the C-centered radical produced from qinghaosu and its derivatives will provide interesting data on the molecular level, which may provide useful insight into the antimalarial mechanism.

In our previous paper[7] the synthesis and iron(II)-induced cleavage of an aromatic derivative of qinghaosu have been reported. Experimental evidence on the relationship of peroxide bond cleavage and antimalarial potency was also presented. Herewith we would like to report a further example in this respect: the synthesis and iron(II)-catalyzed cleavage of 12-(2,4-dimethoxyphenyl)deoxoqinghaosu (**2**) and the identification of the adduct from L-cysteine and the C-centered free radical produced from its peroxide bond cleavage (Fig. 1).

Results and discussion

The synthesis of 12-(2,4-dimethoxyphenyl)deoxoQHS was

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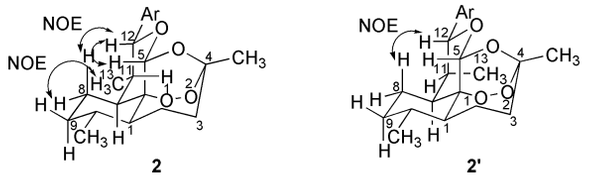
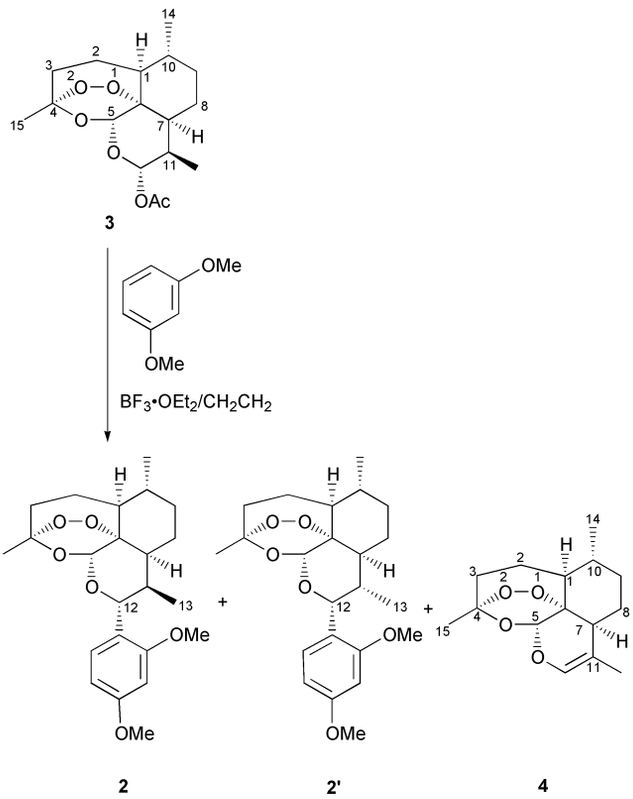


Fig. 2



Scheme 1

achieved as shown in Scheme 1, which was similar to the synthesis of naphthyl-deoxoQHS described in our previous paper.[7] The Friedel–Crafts alkylation of 1,3-dimethoxybenzene using compound **3**[8] (an activated acetal) as alkylation agent proceeded under the catalysis of BF_3·OEt_2, giving products **2** and **2′** in 26% and 9% yield respectively. The major undesired product was dehydrated dihydroQHS **4**. Recently Posner[9] has reported another method for the synthesis of compound **2** in rather good yield, without obtaining the interesting isomer **2′** (*vide infra*).

The structural assignments of **2** and **2′** were made on the basis of the spectroscopic data. The H-12 in compound **2** appears as a doublet with a *J*-value of about 10.5 Hz, indicating a nearly 180° dihedral angle (*trans*) with respect to H-11. However the H-12 in compound **2′** has a *J*-value of 2.3 Hz. To assign the absolute configuration at C-11 and C-12, DQF-COSY and NOESY experiments were performed. Compound **2** showed several cross-peaks (H^{β}-8/H-12, H-12/H-5, and H^{α}-8 with Me at C-11) in the NOESY spectrum (Fig. 2). Compound **2′** showed cross-peaks correlating H^{β}-8/H-12 and H-5/H-12, but there was absolutely no NOE at all between H^{α}-8 and the Me at C-11. So the methyl group at C-11 and the aryl group at C-12 are in *cis* arrangement with the methyl group at C-11 having a different configuration from compound **2**. Compound **2′** is believed to be produced *via* enol ether **4**.

Cleavage experiments on **2** and **2′** with $FeSO_4$ (Scheme 2) were carried out in aq. acetonitrile at 37 °C.

In the case of **2**, after 48 hours' reaction the starting material was almost fully consumed to give two products **5** and **6** in 20% and 40% yield respectively. Compound **2′** was much less reactive than **2**. This lower reactivity was attributed to the steric congestion around the peroxy bridge especially near the O-1 atom. In the case of **2′**, after 3 days of reaction the starting material was recovered in 90% yield along with a small amount (2%) of **6′**.

In the presence of L-cysteine and a catalytic amount of ferrous ions QHS could be cleaved to give the C-centered free radical, its hydrogen trapped product and its adduct with cysteine *via* a carbon–sulfur bond, the presence of which was proved by a further reaction product.[4] A much clearer picture of the reaction was obtained, when 12-(2,4-dimethoxyphenyl)-deoxoQHS **2** was used as the substrate in the reaction with L-cysteine and a catalytic amount of ferrous ions. Thus **2** was added to a reaction mixture of L-cysteine (2 equiv.) and ferrous sulfate (0.01 equiv.) in aqueous acetonitrile, and the mixture was reacted for 4.5 h at room temperature. In addition to the normal cleavage products **5** and **6**, two hydrogen trapped products **7** and **8** from primary C-centered free radicals and a stable adduct **9** with cysteine were also separated from the organic phase after work-up of the reaction mixture (Scheme 3). Compound **8** was sensitive to acid and was completely transformed into compound **7**. The absolute conformation of product **7** was determined by NOESY (NOE between H-12 and HC=O). Compound **9** has high polarity, but it is more soluble in EtOAc than in water. The situation was quite different from the water soluble adduct in the case of QHS as the substrate. Compound **9** was visible on TLC by spraying with 0.5% ninhydrin solution in EtOH as a pink spot. Its ESI mass spectrum (CH_3OH–H_2O) exhibited peaks at m/z = 549 (M + Na), 1052 (2(M + 1)) and 1599 (3M + Na + 1), and all protons in the 600 MHz ^{1}H NMR spectrum could be assigned.

All the results mentioned above confirmed once again our previous deduction about the C-centered free radical mediated reaction of QHS and its derivatives with ferrous ions. In the presence of a thiol compound even catalytic amounts of ferrous ion could cleave QHS type compounds giving the cleavage products, hydrogen trapped product and C–S bonded adduct. The results seem to indicate that the primary free radical was more active than the secondary free radical in the chemical reaction and probably more important in the antimalarial activity. Usually only the adduct from the primary free radical could be isolated. An interesting experimental result was that the C-11-epimer **2′** was almost inert to ferrous ions, except for the formation of 2% of the 3-hydroxy product produced from the secondary C-centered free radical and showed much lower antimalarial activity in the *in vivo* test on mice against *Plasmodium berghei* K_{173} strain, especially in its ED_{90}-value. In comparison with this result the normal QHS derivative **2** was much more reactive and gave predominantly those products deduced from the primary C-centered radical. In parallel with the chemical behavior compound **2** also showed high antimalarial activity in the *in vivo* test[10] (Table 1).

Table 1 The ED_{50}- and ED_{90}-values against *Plasmodium berghei* K_{173} strain (administered orally to mice as suspensions in Tween 80)

Compound	ED_{50}/mg kg^{-1}	ED_{90}/mg kg^{-1}
2	1.27	5.27
2′	4.18	76.27
Artemether	1	3.1

Summary

The present work describes the synthesis of 12α-(2,4-dimethoxyphenyl)deoxoQHS and its reactions with ferrous ions as well as L-cysteine and catalytic amounts of ferrous ions. Isolation and identification of these reaction products provided further

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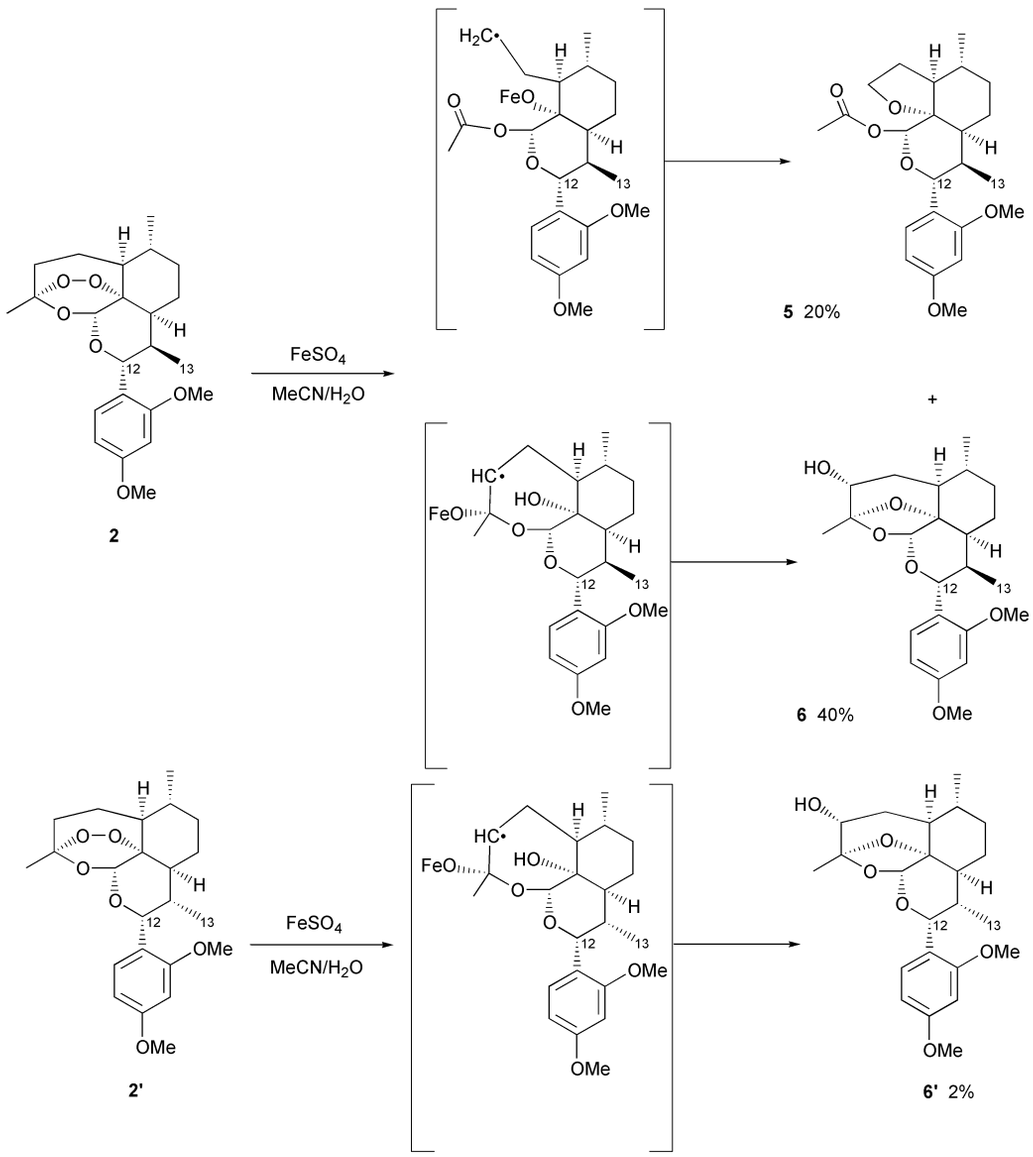

Scheme 2

evidence on the participation of the primary carbon-centered free radicals, which could abstract hydrogen and couple with L-cysteine through a carbon–sulfur bond. However, the 11-epimer of 12α-(2,4-dimethoxyphenyl)deoxoQHS obtained as a by-product in the Friedel–Crafts reaction showed very low reactivity with ferrous ions and also low antimalarial activity *in vivo*. This significant correlation between reactivity *in vitro* and bio-activity *in vivo* may present further evidence for the participation of primary carbon-centered free radicals in the antimalarial activity of the qinghaosu (artemisinin) series of compounds.

Experimental

Melting points were determined on a ZMD-2 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 300 or Varian INOVA-600 instruments. Detailed signal assignments in the ^{13}C and ^{1}H NMR are based on COSY, HMQC, and DEPT experiments. Mass spectra were obtained using HP 5989A or QUATTRO (ESI) instruments. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Thin layer chromatography (TLC) was performed on silica gel 60 F-254, and column chromatography was performed on silica gel (300–400 mesh) or RP-C18.

Compounds 2 and 2′

To a solution of *O*-acetyldihydroQHS **3**[8] (1.30 g, 4.0 mmol) and 1,3-dimethoxybenzene (0.78 mL, 6 mmol) in dry dichloromethane (100 mL) was added boron trifluoride–diethyl ether (0.08 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and then was quenched with distilled water and the resultant mixture was washed in turn with aq. $NaHCO_3$ and brine. The organic solution was dried over sodium sulfate and concentrated under reduced pressure. The resultant oil was purified by column chromatography (petroleum ether (PE)–EtOAc) to provide compound **2** (424 mg, 26%), and compound **2′** (139 mg, 9%).

Compound 2. Mp: 138–140 °C (ethyl acetate–petroleum); $[\alpha]^{15}_{D}$ = +92.8 (*c* = 1.03, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): 7.54 (d, 1H, *J* = 7.8 Hz, Ar-H), 6.55 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.2 Hz, Ar-H), 6.39 (d, 1H, *J* = 2.2 Hz, Ar-H), 5.40 (s, 1H, H-5), 4.95 (d, 1H, *J* = 10.0, H-12), 3.97 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.52 (m, 1H, H-11), 2.40 (td, 1H, J_1 = 13.7 Hz, J_2 = 4.1 Hz, H-3α), 2.03 (ddd, J_1 = 14.4 Hz, J_2 = 4.80 Hz, J_3 = 3.0 Hz, 1H, H-3β), 1.89 (m, 1H, H-2α), 1.80–1.72 (m, 2H, H-8α, H-9β), 1.66–1.58 (m, 2H, H-8β and H-7), 1.55 (m, 1H, H-2β), 1.42 (m, 4H, CH_3 at C-4 and H-10), 1.29 (td, 1H, J_1 = 11.4 Hz, J_2 = 6.7 Hz, H-1), 1.60 (m, 1H, H-9α), 0.98 (d, 3H,

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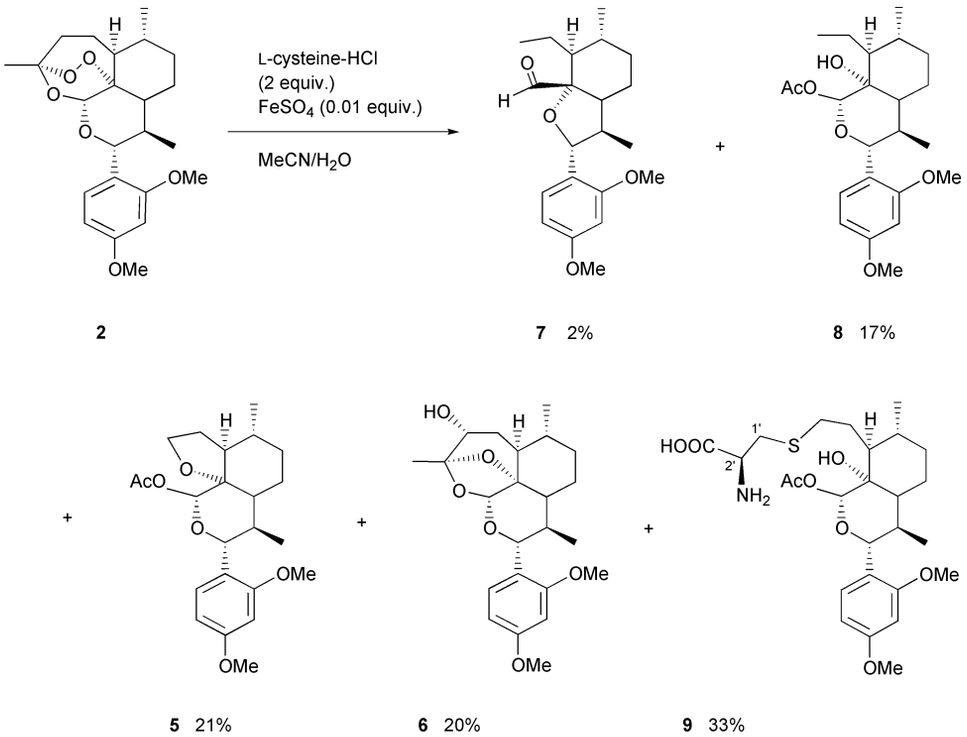

Scheme 3

J = 6.8 Hz, CH_3 at C-10), 0.57 (d, 3H, J = 7.4 Hz, CH_3 at C-11); IR (KBr): 2973, 2944, 2921, 2855, 1612, 1587, 1506, 1462, 1207, 1065, 1042, 879, 828 cm^{-1}; EIMS: 404 (M^+), 195, 179, 178, 167, 165, 151, 121, 43; Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.43; H, 7.96%.

Compound 2′. Mp: 134–136 °C; $[\alpha]^{14}_D$ = +152.3 (c = 1.04, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 7.50 (d, 1H, J = 8.5 Hz, Ar-H), 6.49 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.3 Hz, Ar-H), 6.38 (d, 1H, J = 2.3 Hz, Ar-H), 5.32 (s, 1H, H-5), 5.15 (d, 1H, J = 2.3 Hz, H-12), 3.78 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 2.35 (m, 1H, H-11), 1.46 (s, 3H, CH_3 at C-4), 0.95 (d, 3H, J = 6.0 Hz, CH_3 at C-10), 0.94 (d, 3H, J = 7.4 Hz, CH_3 at C-11); EIMS: 404 (M^+), 359, 358, 195, 178, 167, 165, 151, 43; IR (KBr): 2934, 2873, 1617, 1590, 1506, 1458, 1208, 1133, 1036, 934, 832 cm^{-1}; Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.04; H, 8.02%.

The reaction of compound 2 or 2′ with ferrous sulfate

With compound 2. To a solution of **2** (734 mg, 1.8 mmol) in MeCN (22 mL) was added a mixture of $FeSO_4 \cdot 7H_2O$ (750 mg, 2.7 mmol) in water (18 mL). The mixture was stirred at 37 °C under a nitrogen atmosphere for 48 h. The mixture was concentrated under reduced pressure to remove MeCN. The residue was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (PE–EtOAc) to give compounds **5** (131 mg, 20%) and **6** (278 mg, 40%).

Compound 5. Mp: 157–160 °C; $[\alpha]^{15}_D$ = −29.8 (c = 1.04, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 7.47 (d, 1H, J = 8.5 Hz, Ar-H), 6.49 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.4 Hz, Ar-H), 6.39 (d, 1H, J = 2.4 Hz, Ar-H), 6.25 (s, 1H, H-5), 5.06 (d, 1H, J = 11.0 Hz, H-12), 4.34 (td, 1H, J_1 = 8.6, J_2 = 1.7 Hz, H-4), 3.95 (m, 1H, H-3), 3.78 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.37 (m, 1H, H-11), 2.09 (s, 3H, Ac), 0.95 (d, 3H, J = 6.3 Hz, CH_3 at C-10), 0.59 (d, 3H, J = 7.1 Hz, CH_3 at C-11); IR (KBr): 2952, 2893, 2849, 1753, 1614, 1590, 1509, 1232, 1209, 1157, 1051 cm^{-1}; EIMS: 179, 178, 167, 151, 149, 147, 138, 121; Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.30; H, 8.16%.

Compound 6. Mp: 198–200 °C; $[\alpha]^{14}_D$ = −57.9 (c = 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (d, 1H, J = 8.5 Hz, Ar-H), 6.52 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.4 Hz, Ar-H), 6.39 (d, 1H, J = 2.3 Hz, Ar-H), 5.34 (s, 1H, H-5), 4.96 (d, 1H, J = 10.7 Hz, H-12), 3.79 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.60 (d, 1H, J = 2.4 Hz, H-3), 2.46 (m, 1H, H-11), 1.65 (s, 3H, CH_3 at C-4), 0.90 (d, 3H, J = 6.1 Hz, CH_3 at C-10), 0.58 (d, 3H, J = 7.2 Hz, CH_3 at C-11); IR (KBr): 3511, 2934, 2912, 2871, 1613, 1591, 1207, 1036, 1019, 930, 829 cm^{-1}; EIMS: 344, 180, 179, 178, 167, 165, 151, 149, 121; Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.28; H, 8.16%.

With compound 2′. To a solution of **2′** (101 mg, 0.25 mmol) in MeCN (2.5 mL) was added a mixture of $FeSO_4 \cdot 7H_2O$ (750 mg, 2.7 mmol) in water (18 mL). The mixture was stirred at 37 °C under a nitrogen atmosphere for 48 h. Work-up as above gave product **6′** (2 mg, 2%) and recovered **2′** (90%).

Compound 6′. Mp: 81–83 °C; $[\alpha]^{15}_D$ = +18.5 (c = 0.96, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 7.40 (d, 1H, J = 8.5 Hz, Ar-H), 6.50 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.2 Hz, Ar-H), 6.40 (d, 1H, J = 2.2 Hz, Ar-H), 5.27 (s, 1H, H-5), 5.12 (d, 1H, J = 1.9 Hz, H-12), 3.80 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.60 (d, 1H, H-3), 1.64 (s, 3H, CH_3 at C-4), 0.92 (d, 3H, J = 7.4 Hz, CH_3 at C-10), 0.88 (d, 3H, J = 5.8 Hz, CH_3 at C-11); IR (KBr): 3500, 2931, 1616, 1591, 1508, 1208, 1052, 1033, 935, 881 cm^{-1}; EIMS: 404, 344, 300, 262, 218; HRMS for $C_{23}H_{32}O_6$ 404.2199. Found: 404.2222.

The reaction of compound 2 with L-cysteine hydrochloride

L-Cysteine·HCl·H_2O (176 mg, 1 mmol), $NaCO_3$ (106 mg, 1 mmol) and $FeSO_4 \cdot 7H_2O$ (1.4 mg, 0.005 mmol) were dissolved in water (4 mL). To the solution was added **2** (202 mg, 0.5 mmol) in MeCN (6 mL). After the mixture was stirred at 15 °C under an argon atmosphere for 4.5 h, the starting material was consumed completely. The mixture was concentrated under reduced pressure to remove MeCN. The residue was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography

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(PE–EtOAc) to give compounds **5** (42 mg, 21%), **6** (40 mg, 20%), **7** (5 mg, 2%), **8** (35 mg, 17%) and **9** (86 mg, 33%).

Compound 7. $[\alpha]^{14}_{D}$ = +44.1 (*c* = 0.97, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): 10.19 (s, 1H, -COH), 7.39 (d, 1H, *J* = 8.4 Hz, Ar-H), 6.49 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.4 Hz, Ar-H), 6.44 (d, 1H, *J* = 1.8 Hz, Ar-H), 5.03 (d, 1H, *J* = 3.0, H-12), 3.80 (s, 6H, 2OCH_3), 2.03 (m, 1H, H-11), 1.98–1.90 (m, 3H, H-9β, H-10, H-1), 1.84 (qd, 1H, J_1 = 12.9, J_2 = 3.3 Hz, H-8β), 1.61 (m, 1H, H-2), 1.50 (m, 1H, H-8α), 1.46–1.40 (m, 2H, H-2 and H-7), 1.17 (qd, J_1 = 13.5 Hz, J_2 = 4.8 Hz, 1H, H-9α), 1.06 (t, 3H, *J* = 7.2 Hz, CH_3 at C-2), 0.98 (d, 3H, *J* = 6.6 Hz, CH_3 at C-10), 0.96 (d, 3H, *J* = 7.2 Hz, CH_3 at C-11); ^{13}C NMR (75 MHz, $CDCl_3$) broadband decoupling (BB): 209.7, 159.8, 157.0, 125.9, 125.5, 103.6, 98.2, 91.1, 83.3, 55.9, 55.4, 55.2, 52.5, 43.0, 36.3, 33.3, 22.3, 20.9, 20.2, 16.1, 14.2; DEPT 135° (CH + CH_3): 209.7, 125.9, 103.6, 98.2, 83.3, 55.9, 55.4, 55.2, 52.5, 43.0, 33.3, 20.2, 16.1, 14.2; (CH_2): 36.3, 22.3, 20.9; IR (KBr) 1726, 1614, 1591, 1506, 1465, 1209, 1157, 1073 cm^{-1}; MS (ESI) 369 (M^+ + Na); Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.79; H, 8.73. Found: C, 72.88; H, 8.99%.

Compound 8. Mp: 95–97 °C; $[\alpha]^{17}_{D}$ = +22.6 (*c* = 1.06, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): 7.24 (d, 1H, Ar-H), 6.48 (dd, 1H, J_1 = 8.1 Hz, J_2 = 1.8 Hz, Ar-H), 6.43 (d, 1H, *J* = 1.8 Hz, Ar-H), 6.30 (s, 1H, H-5), 4.82 (m, 1H, H-12), 3.79 (s, 6H, 2OCH_3), 2.63 (m, 1H, H-11), 2.20 (m, 1H, H-2), 2.07 (s, 3H, AcO), 1.83 (m, 1H, H-9β), 1.75–1.70 (m, 3H, H-8β, H-8α, H-7), 1.48 (m, 1H, H-10), 1.11 (m, 1H, H- 9α), 1.04 (m, 1H, H-1), 0.99 (t, 3H, *J* = 6.6 Hz, CH_3 at C-2), 0.96 (d, 3H, *J* = 6.6 Hz, CH_3 at C-10), 0.92 (m, 1H, H-2), 0.59 (d, 3H, *J* = 7.2 Hz, CH_3 at C-11); ^{13}C NMR (75 MHz, $CDCl_3$) BB: 169.1, 160.5, 158.2, 128.9, 120.8, 104.7, 98.8, 91.9, 77.2, 73.7, 55.8, 55.6, 55.3, 49.7, 35.7, 34.8, 33.7, 21.8, 21.5, 21.0, 20.7, 17.4, 13.5; DEPT 90° (CH): 128.9, 104.7, 98.8, 91.9, 77.2, 55.8, 49.7, 34.8, 33.7; DEPT 135° (CH + CH_3): 128.9, 104.7, 98.8, 91.9, 77.2, 55.8, 55.6, 55.3, 49.7, 34.8, 33.7, 21.5, 21.0, 17.4, 13.5; (CH_2): 35.7, 21.8, 20.7; ^{13}C NMR (150 MHz, $CDCl_3$): 169.1 (C=O), 160.6 (q), 158.3 (q), 128.9, 120.8 (q), 104.7, 98.8, 91.9 (C-5), 77.0 (C-12), 73.7 (q, C-6), 55.8 (C-1), 55.6 (OCH_3), 55.4 (OCH_3), 49.7 (C-7), 35.8 (C-9), 34.9 (C-10), 33.7 (C-11), 21.8 (C-8), 21.6 (CH_3 at C=O), 21.1 (CH_3 at C-10), 17.5 (CH_3 at C-2), 13.6 (CH_3 at C-11); IR (KBr): 1752, 1614, 1590, 1509, 1465, 1230, 1209, 1156, 1051, 832 cm^{-1}; MS (ESI): 179, 317, 427; EIMS: 405 (M^+ − 1), 317, 178, 151, 141, 121, 91, 77, 43; Anal. Calcd for $C_{23}H_{34}O_6$: C, 67.94; H, 8.44. Found: C, 68.21; H, 8.67%.

Compound 9. Mp: 163–166 °C (probably decomposed); ^{1}H NMR (600 MHz, CD_3COCD_3–D_2O): 7.49 (d, 1H, *J* = 7.2 Hz, Ar-H), 6.61 (d, 1H, *J* = 7.2 Hz, Ar-H), 6.55 (s, 1H, Ar-H), 6.24 (s, 1H, H-5), 5.01 (d, 1H, *J* = 7.2 Hz, H-12), 3.95 (m, 1H, H-2′), 3.83 (s, 6H, 2OCH_3), 3.27 (m, 1H, H-1′), 3.08 (m, 1H, H-1′), 2.78 (m, 1H, H-3), 2.68 (m, 1H, H-2), 2.62 (m, 1H, H-11), 2.50 (m, 1H, H-3), 2.15 (s, 3H, AcO), 1.87–1.70 (m, 4H, H-9β, H-8α, H-7, H-8β), 1.48 (m, 1H, H-10), 1.29 (m, 2H, H-1, H-2), 1.18 (m, 1H, H-9α), 0.99 (d, 3H, *J* = 6.0 Hz, CH_3 at C-10), 0.58 (d, 3H, *J* = 6.6 Hz, CH_3 at C-11); IR (KBr): 3432, 2932, 1758, 1615, 1590, 1510 cm^{-1}; MS (ESI): 549 (M + Na), 1052 (2(M + 1)), 1599 (3M + Na + 1); high resolution SIMS: Calcd for $C_{24}H_{35}O_7NSNa$ (M − Ac − H + Na): 504.2032. Found: 504.2032; Calcd for $C_{24}H_{35}O_6NSNa$ (M − AcOH + Na): 488.2083. Found: 488.2075; Calcd for $C_{24}H_{36}O_6NS$ (M − AcOH + H): 488.2263. Found: 488.2246.

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