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In vitro and in vivo antimalarial evaluation of semi-synthetic derivatives of gomphostenin

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ABSTRACT

A novel series of semi-synthetic gomphostenin derivatives (**1**–**9**) were prepared utilizing C-14 hydroxyl group for the first time and studied for their antimalarial properties. In vitro antiplasmodial activity was evaluated against both the chloroquine sensitive and resistant strains of *Plasmodium falciparum*. Most of the compounds exhibited superior or comparable antiplasmodial activity compared to parent compound, that is, gomphostenin (GN). Based upon in vitro antiplasmodial activity, compounds with IC_{50} values less than 10 μM were selected for in vivo antiplasmodial evaluation against *Plasmodium berghei* infection in mice model. GN derivatives **3** and **5** were found to have curative activity with moderate chemosuppression of 65% and 69%, respectively, at the dose level of 150 mg/kg/day.

Malaria is one of the most important parasitic infections[1] of humans due to its high morbidity and mortality, a threat to over 2 billion people living in areas of high incidence. *Plasmodium falciparum*, the causative agent of the malignant form of malaria, has high adaptability by mutation and is resistant to various types of antimalarial drugs, a serious setback to antimalarial programs, essentially because it precludes the use of cheap and previously effective drugs like chloroquine. Therefore, in order to decrease the risk of resistance,[2] new families of active compounds are needed with independent mechanism of action.[3] The success of the antimalarial drug quinine and the discovery of artemisinin, the most potent antimalarial drug both from plant source have lead to the study of plants to find out novel antimalarial agents.[4] In order to improve the efficacy and pharmaceutical profile, much attention has been given to the chemical modification[5] of bioactive compounds. Chemical modification of artemisinin[6] has resulted into new derivatives that combine higher antimalarial activity and bio-availability. For instance, derivatization of artemisinin at C10 has yielded compounds such as artemether and sodium artesunate that have already entered the clinics,[7] Another important derivative, in clinical use, is dihydroartemisinin.[8] All these drugs are characterized for important antiplasmocidal activities with IC_{50} values falling in the micro molar range.[9] As a result, it is always necessary to carry out extensive evaluations to determine the consequences of modifying a structure. As a part of our ongoing research activities on biologically active compounds, we have recently reported[10] the isolation and identification of Gomphostenins (Fig. 1) from the plant *Gomphostemma niveum* and their in vitro antimalarial evaluation against *P. falciparum*.

Gomphostenin (GN) is a clerodane class of diterpene with a α,β-unsaturated γ-lactone ring. From our initial in vitro studies, GN was found to have potential antiplasmodial properties; however, inhibition of malaria parasite was not comparable to that of standard antimalarial drug, that is, chloroquine and artemisinin. Consequently, in search of more effective derivative of GN, we synthesized several derivatives of GN through semi-synthetic route and herein, we report the synthesis and in vitro as well as in vivo antiplasmodial evaluation of semi-synthetic GN derivatives for the first time. The isolation of GN was achieved by the reported method.[10] Further by utilizing the C-14 hydroxyl group of GN, various derivatives were synthesized (Scheme 1) and characterized.[11]

The antiplasmodial efficacy of the GN derivatives (**1**–**9**) was evaluated by conventional[12]in vitro parasite culture method, using

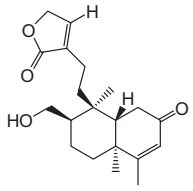

Figure 1. Gomphostenin (GN).

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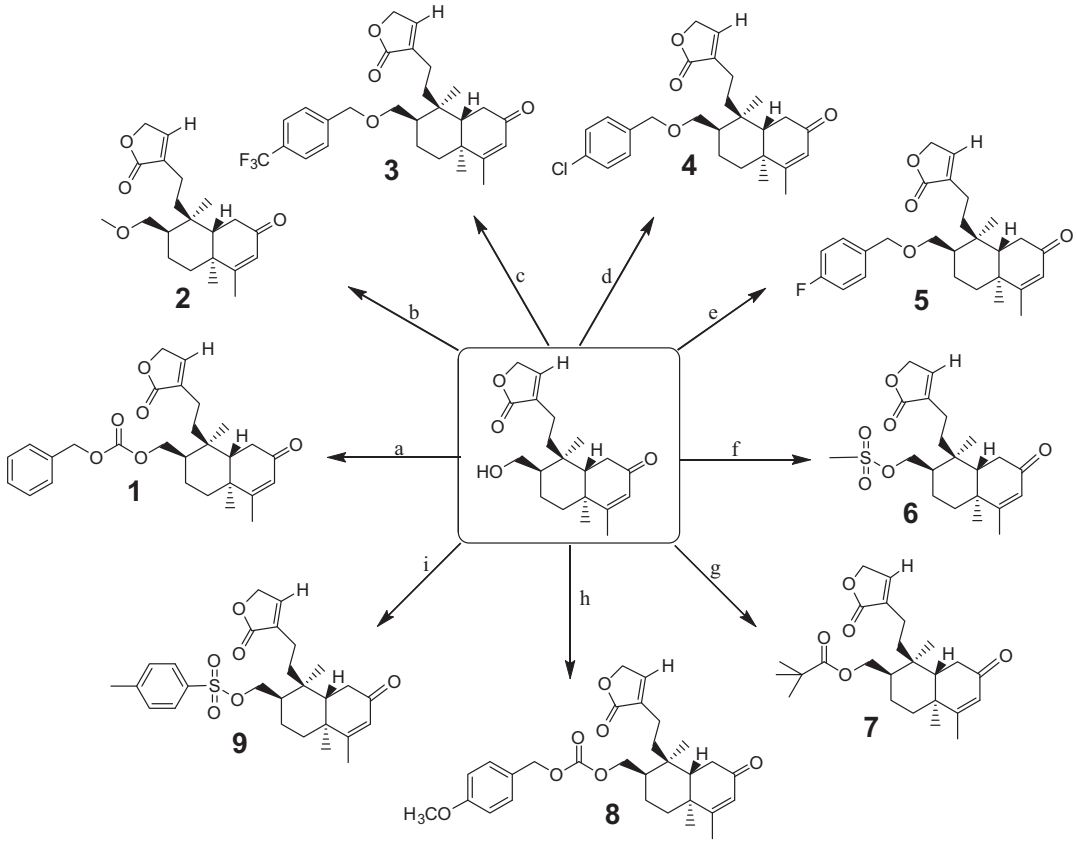

Scheme 1. Reagents and conditions: (a) Cbz-Cl, Et_3N, CH_2Cl_2; (b) CH_2N_2 (in Et_2O), dry DMSO; (c) *p*-$CF_3C_6H_4CH_2Br$, NaH, DMF; (d) *p*-$ClC_6H_4CH_2Br$, NaH, DMF; (e) *p*-$FC_6H_4CH_2Br$, NaH, DMF; (f) MsCl, Et_3N, CH_2Cl_2; (g) $(BOC)_2O$, Et_3N, CH_2Cl_2; (h) *p*-$OCH_3C_6H_4CH_2Br$, NaH, DMF; (i) TsCl, Et_3N, CH_2Cl_2.

chloroquine sensitive (MRC-2) and chloroquine resistant strain (RKL9) of *P. falciparum*. Parasites were cultured in human B (+) erythrocytes in Rosewell Park Memorial Institute (RPMI)-1640 media (GIBCO-BRL, Paisely, Scotland) supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) buffer, 10% human AB (+) serum, and 0.2% sodium bicarbonate (Sigma), and maintained at 5% CO_2. Cultures containing predominantly early ring stages were synchronized by addition of 5% D-sorbitol (Sigma) lysis, used for testing. Initial culture was maintained in small vials with 10% hematocrit, that is, 10 μL erythrocytes containing 1.0% ring-stage parasite in 100 μL complete media. The culture volume per well for the assay was 100 μL. The number of parasites for the assay was adjusted to 1–1.5% by diluting with fresh human B (+) red blood cells (RBC). Assays were carried out in 96-well microtitre flat-bottomed tissue culture plates incubated at 37 °C for 24 h in the presence of twofold serial dilutions of compounds and chloroquine diphosphate (CQ) for their effect on schizont maturation. All the GN derivatives **1**–**9** were dissolved in ethanol and further diluted with RPMI-1640 medium (the final ethanol concentration did not exceed 0.5%, which did not affect parasite growth). Chloroquine diphosphate was dissolved in aqueous medium. Tests were carried out in duplicate wells for each dose of the drugs. Solvent control cultures containing the same concentrations of the solvent present in the test wells were carried out with RPMI-1640 containing 10% AB (+) serum. Parasite growth was found to be unaffected by the solvent concentrations. Growth of the parasites from duplicate wells of each concentration was monitored in Giemsa-stained blood smears by counting the number of schizonts per 100 asexual parasites. Percentage schizont maturation inhibition was calculated by the formula: $(1 - Nt/Nc) \times 100$, where Nt and Nc represent the number of schizonts in the test and control. In vitro antimalarial activities of GN derivatives (**1**–**9**) as IC_{50} values for the inhibition of chloroquine sensitive and resistant strains are summarized in Table 1.

It is evident from the Table 1 that most of the derivatives were more potent than the parent compound. The introduction of phenyl ring at C-14 position had substantial effect on the inhibition of malarial parasite. Additionally when the phenyl group was substituted at 4 positions with Cl or F the activity increased with IC_{50} 14 μM and 8.3 μM, respectively. The activity was highest when tri-fluoro substituted methyl was used at 4 position of phenyl group. This emphasizes the fact that presence of fluorine group and phenyl ring favor the activity. Of the nine compounds, **3** and **5** demonstrated maximum in vitro antiplasmodial activity against

Table 1
IC_{50} values of GN derivatives (**1**–**9**) and chloroquine diphosphate (CQ) against *P. falciparum*

Compound	IC_{50} ± SD (μM) CQ-S	IC_{50} ± SD (μM) CQ-R
GN	115.0 ± 0.018	113.5 ± 0.011
1	42.2 ± 0.023	43.9 ± 0.017
2	94.4 ± 0.031	88.9 ± 0.025
3	4.8 ± 0.012	4.4 ± 0.022
4	14.0 ± 0.016	18.8 ± 0.028
5	8.3 ± 0.014	8.1 ± 0.017
6	69.9 ± 0.011	73.3 ± 0.011
7	78.5 ± 0.017	84.9 ± 0.021
8	37.6 ± 0.013	47.7 ± 0.017
9	26.1 ± 0.018	45.8 ± 0.012
CQ	0.024 ± 0.011	0.038 ± 0.017

IC_{50} values are expressed in micro molar concentrations (μM) ± standard deviations. All experiments were realized in triplicate.

Table 2
Blood schizonticidal activity of GN derivatives **3** and **5** against *P. berghei* in mice during 4-day test

GN derivative	Concentrations (mg/kg/day)	Average %* parasitemia	Average % suppression
3	150	11.5 ± 0.3	65.87 ± 0.6
	100	12.4 ± 1.8	63.20 ± 2.1
	50	17.5 ± 0.6	48.40 ± 1.1
5	150	10.3 ± 2.3	69.44 ± 1.3
	100	22.3 ± 2.3	33.21 ± 1.4
	50	26.4 ± 3.4	21.66 ± 3.2
CQ	5	1.07 ± 0.02	96.82 ± 0.3
Negative control		33.7 ± 3.7	0

* $P < 0.01$, values are expressed as mean ± S.D ($n = 3$); negative control = saline plus 0.5% Tween-80.

CQ sensitive and CQ resistant parasitic strain. In vitro studies revealed that all the semi-synthetic derivatives of GN are more active than the parent molecule however, less active than chloroquine. Based upon in vitro antiplasmodial activity, compounds with IC_{50} value less than 10 μM were selected for in vivo antiplasmodial activity evaluation against chloroquine sensitive *P. berghei* (ANKA strain) infection in mice model (Table 2) using the classical 4-day suppressive test.[13] Each swiss albino mice was inoculated on day 0, intraperitoneally with 0.2 ml of infected blood containing about 1×10^7 *P. berghei* parasitized red blood cells. Different dilutions of test compounds were prepared by suspending the compound in saline containing 0.5% Tween-80. The animal were divided into three groups of five mice each and orally administrated shortly after inoculation with **3** and **5**, respectively (50, 100, and 150 mg/kg/day), chloroquine (5 mg/kg/day), and an equal volume of saline plus 0.5% Tween-80 (negative control) for four consecutive days (day 0 to day 3), respectively. On the fifth day (day 4), thin films were made from the tail blood of each mouse and the parasitemia level was determined by counting the number of parasitized erythrocytes out of 600 erythrocytes in random fields of the microscope. Average percentage suppression was calculated as 100 (A − B/A), where A is the average percentage parasitemia in the negative control group and B is the average percentage parasitemia in the test group.

In case of in vivo experiment although chemosuppression of 69% and 65% was observed at the highest dose level of 150 mg/kg/day with GN derivative **3** and **5** however, both the compounds were found to possess curative activity for more then 28 days as none of the mice died before D +28. In summary, new semi-synthetic derivatives of GN were prepared and evaluated for antiplasmodial properties. Compounds with IC50 value less than 10 μM were selected for in vivo antiplasmodial evaluation against *P. berghei* infection in mice model. GN derivative **3** and **5** were found to have curative activity with moderate chemosuppression. It can be concluded that chemical modification of GN certainly holds great promise, and that further exploration in this field may lead to potent antimalarial agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.035.

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11. *Spectroscopic data for GN derivatives* **1**: viscous colorless liquid, IR (KBr): 1774, 1755, 1660, 1170, 1293 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (m, 3H), 1.23 (m, 1H), 1.39 (m, 3H), 1.41 (m, 2H), 1.47 (m, 2H), 1.66 (m, 2H), 1.8 (m, 3H), 1.85 (m, 1H), 1.89 (m, 2H), 2.23 (m, 2H), 3.80–3.86 (dd, $J = 7.5, 7.5$ 1H), 4.20–4.24 (dd, $J = 4.0, 4.0$ 1H), 5.17 (s, 2H), 5.73 (s, 1H), 4.7 (s, 2H), 7.2 (s, 1H), 7.32–7.47 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2, 18.4, 18.7, 18.8, 21.7, 34.2, 34.7, 34.8, 38.0, 39.5, 40.5, 45.3, 50.2, 65.4, 70.7, 71.2, 125.6, 127.1, 127.3, 127.6, 128.8, 128.9, 134.0, 136, 143.8, 171.2, 171.9, 199.4. ESI-MS 467 $(M+H)^+$; Anal. Calcd for $C_{28}H_{34}O_6$: C, 72.08; H, 7.35. Found: C, 72.14; H, 7.30. **2**: viscous colorless liquid, IR (KBr): 1750, 1735, 1663, 1170, 1333 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (m, 3H), 1.25 (m, 1H), 1.40 (m, 3H), 1.42 (m, 2H), 1.48 (m, 2H), 1.69 (m, 2H), 1.8 (m, 3H), 1.83 (s, 3H), 1.86 (m, 1H), 1.9 (m, 2H), 2.24 (m, 2H), 3.80–3.86 (dd, $J = 7.5, 7.5$ 1H), 4.20–4.25 (dd, $J = 4.0, 4.0$ 1H), 5.74 (s, 1H), 4.7 (s, 2H), 7.1 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.3, 18.6, 18.7, 18.9, 21.7, 34.3, 34.7, 34.8, 38.0, 39.5, 40.5, 45.3, 59.6, 65.3, 70.3, 125.6, 134.0, 143.8, 171.2, 171.9, 199.4. ESI-MS 347 $(M+H)^+$. Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.84; H, 8.76. For **3**: viscous colorless liquid, IR (KBr): 1750, 1735, 1663, 1400 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (m, 3H), 1.25 (m, 1H), 1.40 (m, 3H), 1.42 (m, 2H), 1.48 (m, 2H), 1.69 (m, 2H), 1.8 (m, 3H), 1.86 (m, 1H), 1.9 (m, 2H), 2.24 (m, 2H), 3.80–3.86 (dd, $J = 7.5, 7.5$ 1H), 4.20–4.25 (dd, $J = 4.0, 4.0$ 1H), 4.71 (s, 2H), 5.74 (s, 1H), 4.7 (s, 2H), 7.1 (s, 1H), 7.35 (t, $J = 7.69$ 1H), 7.54 (t, $J = 7.56$ 1H), 7.62 (d, $J = 8.1$ 1H), 7.73 (d, $J = 7.69$ 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.3, 18.5, 18.7, 18.9, 21.7, 34.3, 34.7, 34.8, 38.0, 39.6, 40.5, 45.3, 65.3, 70.3, 72.2, 122.1, 124.4, 125.2, 125.5, 125.6, 127.2, 127.4, 134.0, 140, 143.8, 171.2, 171.9, 199.4. ESI-MS 491 $(M+H)^+$. Anal. Calcd for $C_{28}H_{33}F_3O_4$: C, 68.56; H, 6.78. Found: C, 68.50; H, 6.72. **4**: viscous colorless liquid, IR (KBr): 1770, 1749, 1650, 1170, 980 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (m, 3H), 1.23 (m, 1H), 1.39 (m, 3H), 1.41 (m, 2H), 1.47 (m, 2H), 1.66 (m, 2H), 1.8 (m, 3H), 1.85 (m, 1H), 1.89 (m, 2H), 2.23 (m, 2H), 3.80–3.86 (dd, $J = 7.5, 7.5$ 1H), 4.20–4.24 (dd, $J = 4.0, 4.0$ 1H), 4.63 (s, 2H), 5.73 (s, 1H), 4.7 (s, 2H), 7.2 (s, 1H), 7.34–7.57 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2, 18.6, 18.7, 18.8, 21.7, 34.2, 34.7, 34.8, 38.0, 39.5, 40.5, 45.3, 50.1, 65.4, 70.7, 73.2, 125.6, 128.7, 128.8, 130, 134.2, 135, 136.6, 143.8, 171.2, 171.9, 199.2. ESI-MS 457 $(M+H)^+$. Anal. Calcd for $C_{27}H_{33}ClO_4$: C, 70.96; H, 7.28. Found: C, 70.92; H, 7.21. **5**: viscous colorless liquid, IR (KBr): 1778, 1749, 1640, 1165, 1100 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (m, 3H), 1.23 (m, 1H), 1.36 (m, 3H), 1.43 (m, 2H), 1.45 (m, 2H), 1.62 (m, 2H), 1.8 (m, 3H), 1.85 (m, 1H), 1.89 (m, 2H), 2.23 (m, 2H), 3.81–3.85 (dd, $J = 7.5, 7.5$ 1H), 4.20–4.24 (dd, $J = 4.0, 4.0$ 1H), 4.60 (s, 2H), 5.73 (s, 1H), 4.7 (s, 2H), 7.2 (s, 1H), 7.17–7.37 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2, 18.62, 18.7, 18.8, 21.7, 34.2, 34.7, 34.8, 38.0, 39.5, 40.5, 45.3, 52.2, 65.4, 70.7, 73.5, 115.3, 115.4, 129.2, 129.3, 133, 162.1 125.6, 143.6, 171.2, 171.7, 199.3. ESI-MS 441 $(M+H)^+$. Anal. Calcd for $C_{27}H_{33}FO_4$: C, 73.61; H, 7.55. Found: C, 73.64; H, 7.50. **6**: white solid, mp 62 °C, IR (KBr): 1758, 1733, 1667, 1330 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (m, 3H), 1.25 (m, 1H), 1.40 (m, 3H), 1.42 (m, 2H), 1.48 (m, 2H), 1.69 (m, 2H), 1.8 (m, 3H), 1.86 (m, 1H), 1.9 (m, 2H), 2.24 (m, 2H), 3.16 (s, 3H), 3.80–3.85 (dd, $J = 7.4, 7.4$ 1H), 4.20–4.25 (dd, $J = 4.2, 4.2$ 1H), 5.74 (s, 1H), 4.7 (s, 2H), 7.1 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.3, 18.6, 18.7, 18.9, 21.7, 34.3, 34.7, 34.8, 37.9, 38.0, 39.6, 40.5, 45.3, 65.3, 70.3, 125.2, 134.3, 143.1, 171.1, 171.7, 199.4. ESI-MS 411 $(M+H)^+$. Anal. Calcd for $C_{21}H_{30}O_6S$: C, 61.44; H, 7.37; S, 7.81. Found: C, 61.40; H, 7.33; S, 7.84. **7**: white solid, mp 85 °C, IR (KBr): 1756, 1743, 1735, 1664, 1050 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (m, 3H), 1.25 (m, 1H), 1.40 (m, 3H), 1.42 (m, 2H), 1.48 (m, 2H), 1.54 (s, 9H), 1.69 (m, 2H), 1.8 (m, 3H), 1.86 (m, 1H), 1.9 (m, 2H), 2.24 (m, 2H), 3.80–3.86 (dd, $J = 7.4, 7.4$ 1H), 4.20–4.25 (dd, $J = 4.5, 4.5$ 1H), 5.72 (s, 1H), 4.3 (s, 2H), 7.1 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.1, 18.4, 18.9, 19.1, 21.7, 28.7, 34.3, 35.1, 35.6, 38.6, 39.7, 45.9, 52, 63.2, 70.4, 77.3, 127.4, 128.2. 128.3, 133.9, 144.4, 151.2, 172.6, 174.6, 199.5. ESI-MS 417 $(M+H)^+$. Anal. Calcd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found C, 72.11; H, 8.60. **8**: viscous colorless liquid, IR (KBr): 1753, 1743, 1735, 1550, 1664, 1260, 1050 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (m, 3H), 1.22 (m, 1H), 1.40 (m, 3H), 1.38 (m, 2H), 1.46 (m, 2H), 1.67 (m, 2H), 1.8 (m, 3H), 1.86 (m, 1H), 1.9 (m, 2H), 2.23 (m, 2H), 3.78 (s, 3H), 3.82–3.86 (dd, $J = 7.5, 7.5$ 1H), 4.20–4.25 (dd, $J = 4.0, 4.0$ 1H), 4.73 (s, 2H), 5.74 (s, 1H), 4.7 (s, 2H), 7.1 (s, 1H), 7.3–7.6 (m, 4H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.1, 18.5, 18.6, 18.9, 21.7, 34.3, 34.7, 34.8, 38.0, 39.53, 40.5, 45.3, 55.8, 65.3, 70.3, 72.2, 124.1, 125.3, 125.5, 125.8, 127.2, 127.6, 134.4, 140.2, 143.9, 171.3, 171.8, 199.2. ESI-MS 453 $(M+H)^+$. Anal. Calcd for $C_{28}H_{36}O_5$: C, 74.31; H, 8.02. Found: C, 74.34; H, 8.02. **9**: brown solid, mp 123–125 °C. IR (KBr): 1772, 1741, 1646, 1400, 1380, 1170 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.93 (m, 3H), 1.22 (m, 1H), 1.31 (m, 3H), 1.42 (m, 2H), 1.43 (m, 2H), 1.60 (m, 2H), 1.81 (m, 3H), 1.83 (m, 1H), 1.89 (m, 2H), 2.21 (m, 2H), 2.34 (s, 3H), 3.82–3.85 (dd, J = 7.5, 7.5 1H), 4.20–4.24 (dd, J = 4.0, 4.0 1H), 5.73 (s, 1H), 4.7 (s, 2H), 7.2 (s, 1H), 7.47–7.75 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2, 18.6, 18.7, 18.8, 21.2, 21.7, 34.2, 34.7, 34.8, 38.0, 39.5, 40.5, 45.3, 50.2, 65.4, 70.7, 128.2, 128.3, 130.3, 131.5, 133, 140.2, 143.6, 144.4, 171.2, 171.7, 199.3; ESI-MS 487 (M+H); Anal. Calcd for $C_{27}H_{34}O_6S$: C, 66.64; H, 7.04. Found: C, 66.60; H, 7.09.

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