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SYNTHESIS AND ANTITUMOR ACTIVITY OF TANSHINONE ANALOGUES

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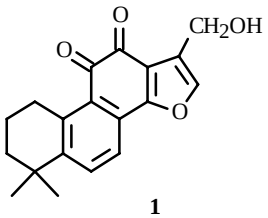
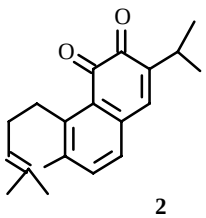
Diterpenoid tanshinones, which often bear an *o*-quinone moiety (such as compounds 1 and 2), have attracted particular attention of medicinal chemists and clinicians because many of them exhibit significant antibacterial [1], antidermatophytic [2], antioxidant [3], antiinflammatory [4], antineoplastic [5], and antiplatelet aggregation [6] activities. Przewaquinone A (**l**), a natural ortho-quinone isolated from *Salvia Przewalskii* Maxim. var. *Mandarinorum* Stib [7], showed antitumor activity in a variety of tumor models, such as Lewis lung carcinoma, melanoma B_{16}, sarcoma 180, and leukemia P-388, both in *vitro* and in *vivo* [8]. Sapr-ortho-quinone (**2**) was isolated from the roots of *Salvia prionitis Hance* [9], a plant used in Chinese folk medicine as an antiphlogistic, antibacterial, and antitubercular drug [10]. Sapr-ortho-quinone (**2**) was also obtained by acidic treatment of 15-deoxyfuerstione (**2a**), a component of the roots of *Salvia moororaftiana*, by Simoes et al [11]. The initial bioassay showed that sapr-ortho-quinone had activity in leukemia P-388 test [12]. Through the comparison between **1** and **2**, it seems that the furan ring in Przewaquinone might not be essential to its biological activity. Therefore, in order to seek more effective compounds that are easily synthesized, we designed and synthesized two analogues of tanshinone, namely **3** and **4**.



1



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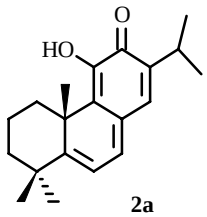
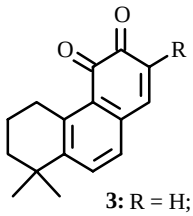


2a



3: R = H;
4: R = $CHOHCH_3$

Starting from anisole (**5**), compound **10** was prepared through a five-step procedure and then treated with methyllithium in ether to give alcohol **11**. After cyclization of compound **11** with boron trifluoride etherate in dichloromethane, the removal of the methyl group of the resulting cyclization product **12** was effected with boron tribromide to afford key intermediate **13**, which was oxidized with Fremy's salt to yield the title compound **3**.

The intermediate **12** was also able to undergo smooth reaction with acetyl chloride and aluminum chloride in dichloromethane to give **14**, which was then converted to "phenol" **15** by demethylation with boron tribromide. Compound **15** was easily reduced to give alcohol **16**, which was oxidized with Fremy's salt to give the target molecule **4**. The synthesized title compounds **13**, **14** were determined to possess the structure of **3** and **4**.

The data for **3**: mp 142–145°C. IR (KBr): 1686, 1657, 1578, 1248 cm^{-1}. EIMS *m/z*: 240 (M^+), 226, 212, 197. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz) 1.32 (6H, s, CH_3), 1.66 (2H, m, CH_2), 1.80 (2H, m, CH_2), 3.20 (2H, t, J = 6.4, CH_2), 6.38 (1H, d, J = 9.9, ArH), 7.16 (1H, d, J = 8.0, ArH), 7.38 (1H, d, J = 9.9, ArH), 7.63 (1H, d, J = 8.0, ArH). Anal. Calcd: C 79.97%; H 6.71%. Found: C 79.91%; H 6.61%.

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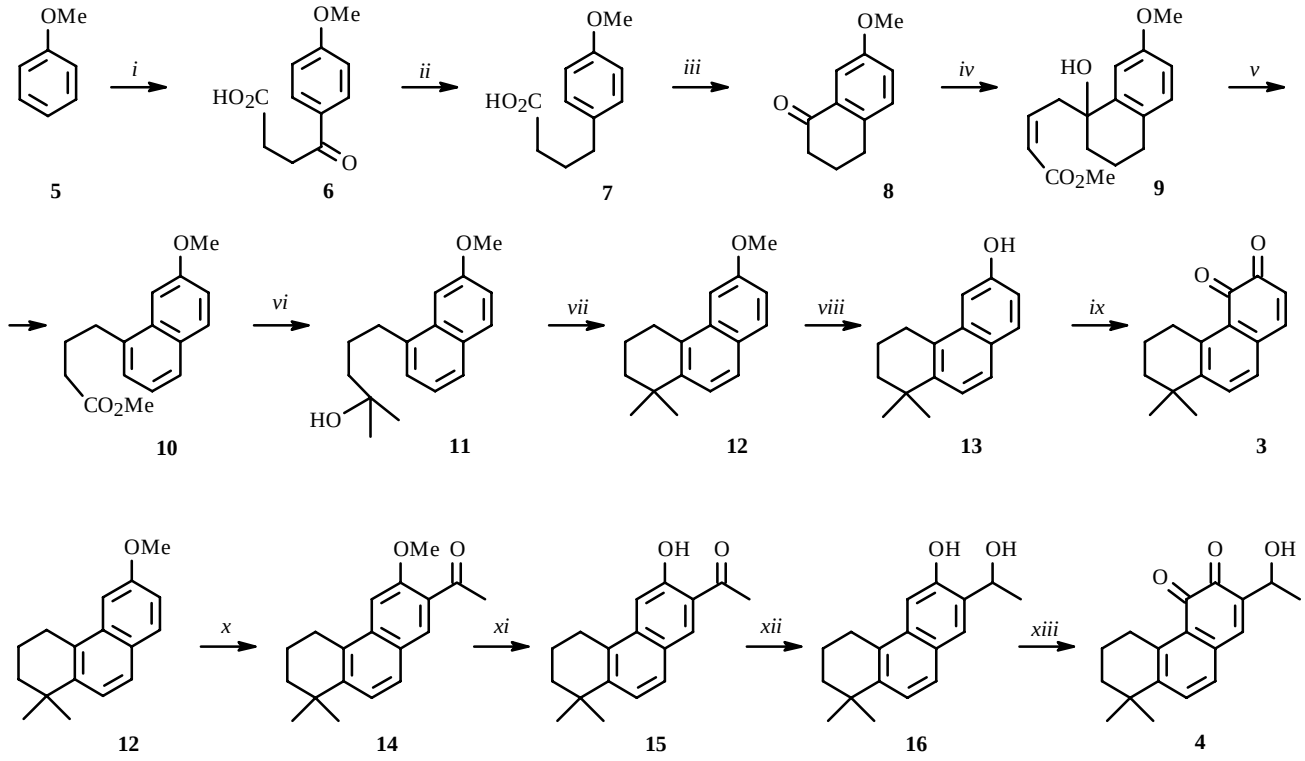
TABLE 1. Inhibition (%) of P-388 and A-549 Tumor Cell Line in *vitro*

Compound	Cell line	C (mol/L)				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
3	P-388[a]	97.0	97.0	98.5	6.1	3.0
	A-549[b]	89.3	83.9	32.1	30.4	30.0
4	P-388[a]	100.0	100.0	98.9	33.0	4.3
	A-549[b]	94.5	70.9	0	0	0

a. 48 h, MTT assay; b. 72 h, SRB assay.



i. 1.2 eq. succinic anhydride, 2.5 eq. $AlCl_3$, CH_3NO_2, 0°C→25°C, 12 h, 73.4%;
ii. 3.0 eq. $NH_2NH_2 \cdot H_2O$, 5.0 eq. KOH, DEG, 130 - 150°C 3 h, then 175 - 195°C 4 h, 95%;
iii. 25 eq. 85% H_3PO_4, 20 eq. P_2O_5 70 - 80°C 3 h, 87.7%;
iv. 4 eq. Zn, 3.5 eq. methyl-4-bromocrotonate, THF, 85.5%;
v. 0.3 eq. palladium black, 230 - 260°C 2h, 48.3%; *vi*. 7 eq. methyllithium, 0°C 4 h, 81.7%;
vii. 25 eq. $BF_3 \cdot Et_2O$, CH_2Cl_2, 20°C 1 h, 92.5%; *viii*. 3 eq. BBr_3 CH_2Cl_2, 0°C 3 h, 95.4%;
ix. 4 eq. Fremy's salt, KH_2PO_4 buffer, acetone, 30°C 10 h, 42.4%;
x. 5.8 eq. $AlCl_3$, 7.5 eq. acetyl chloride, CH_2Cl_2, 0°C - 25°C 12 h, 75%; $_{xi}$. 12 eq. BBr_3 CH_2Cl_2, 0°C 6 h, 78.9%;
xii. 4 eq. LiA1H_4; 0°C - 25°C 10 h, 70.9%; *xiii*. 4 eq. Fremy's salt, KH_2PO_4 buffer, acetone, 25°C, 40%
THF = tetrahydrofuran, DEG = Diethylene glycol.

The data for **4**: mp 47–50°C. IR (KBr): 1655, 1261, 1144 cm^{-1}. EIMS *m/z*: 284(M^+), 268, 254, 240, 225. HRMS: 284.1412 for $C_{18}H_{20}O_3$, Calcd. 284.1407. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz) 1.25 (6H, s, CH_3), 1.38 (3H, d,

J = 6.3, CH_3), 1.58 (2H, t, J = 5.6, CH_2), 1.70 (2H, m, CH_2,), 2.25 (1H, br, OH), 3.11 (2H, t, J = 6.3, CH_2-Ar), 4.76 (1H, m, CH), 7.05(1H, d, J = 8.0, ArH), 7.18 (1H, s, ArH), 7.53(1H, d, J = 7.7, ArH).

The cytotoxic effect of the target compounds on tumor cells was evaluated as assayed by the in *vitro* MTT method for the P-388 cell line and the SRB method for the A-549 cell line. As shown in Table 1, the synthesized compounds **3** and **4** exhibited relatively high activity against the P-388 cell line even at 10^{-6} M concentration.

In conclusion, using anisole as the starting material, we synthesized two analogues of tanshinone, namely 8,8-dimethyl-5,6,7,8-tetrahydrophenanthrene-3,4-dione **3** and 8,8-dimethyl-2-(1'-hydroxyethyl)-5,6,7,8-tetrahydrophenanthrene-3,4-dione **4**. Compounds **3** and **4** exhibited high activity against leukemia P-388 cell lines. Chemical synthesis and biological investigations of a series of structural analogues of the title compounds are in progress.

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