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Synthesis, chiral resolution, and determination of novel furan lignan derivatives with potent anti-tumor activity

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ABSTRACT

A kind of racemic furan lignans were synthesized via a novel route, and two optical isomers were obtained through a selective hydrolization. The absolute configurations were determined by circular dichroism spectroscopy after separated through a preparative column. The different activities of isomers and the racemates were evaluated on QGY-7701 and HeLa cell lines, and compound **2c** showed the best activity on QGY-7701 and HeLa cell lines with IC_{50} 12 μM and 13 μM, respectively.

The development of efficient and mild methods for heterocyclic compound synthesis represents a broad area of organic chemistry because of the existence in numerous natural products.[1] Molecules containing such structure unit often play an essential role in their biological activity, particularly in cancer and virus researches.[2] This important class of heterocyclic products has therefore stimulated the development of popular synthetic five-membered oxygen heterocycles. Moreover, some derivatives of furan lignans which have been synthesized showed wide range of biological activities and could be used as versatile intermediates.[3–9] However, enough attention has not been paid to the chirality of this kind of heterocyclic compounds; especially the chiral center is locating on carbon atom of furan ring. Herein, we reported the synthesis, chiral separation and determination of two racemoid furan lignans, and the anti-cancer activity was evaluated on QGY-7701 and HeLa cell lines. The two pairs of chiral enantiomers are shown in Figure 1.

The preparation of target compound **2** was listed in Scheme 1. Compound **5** was synthesized through a conventional Knoevenagel Condensation between compound **3** and compound **4** refluxing in toluene with the catalytic piperidine and HOAc.[10] Compound **5** reacted with prop-2-yn-1-ol via a coupling reaction under nitrogen atmosphere to form compound **6**, which was catalyzed by $PdCl_2(Ph_3P)_2$ and *n*-BuLi at room temperature[11] in dry THF. Then a Heck reaction was employed to synthesize the key intermediate compound **7** from compound **6** and 1-iodo-4-methoxybenzene.[12] Compound **1** was prepared through a hydrolysis decarboxylation reaction in 20% of KOH aqueous solution in ethanol (v/v = 1:1) at room temperature with an excellent yield. In the end, compound **1** was reduced under $LiAlH_4$ to form compound **2** in THF with good yield.[14,23] Compounds **1**, **2**, and **6–8** were characterized by NMR spectra.[24]

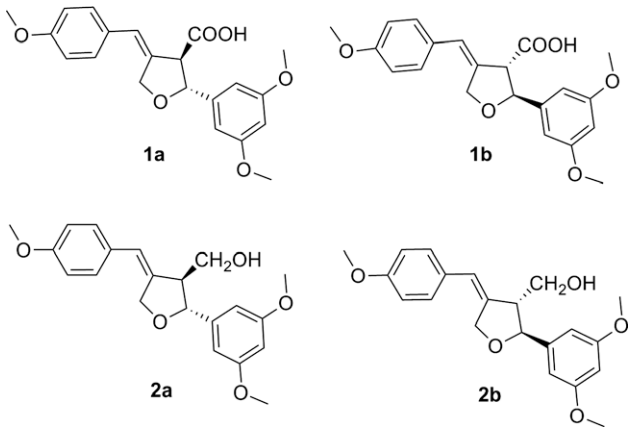

Figure 1. Four chiral target compounds.

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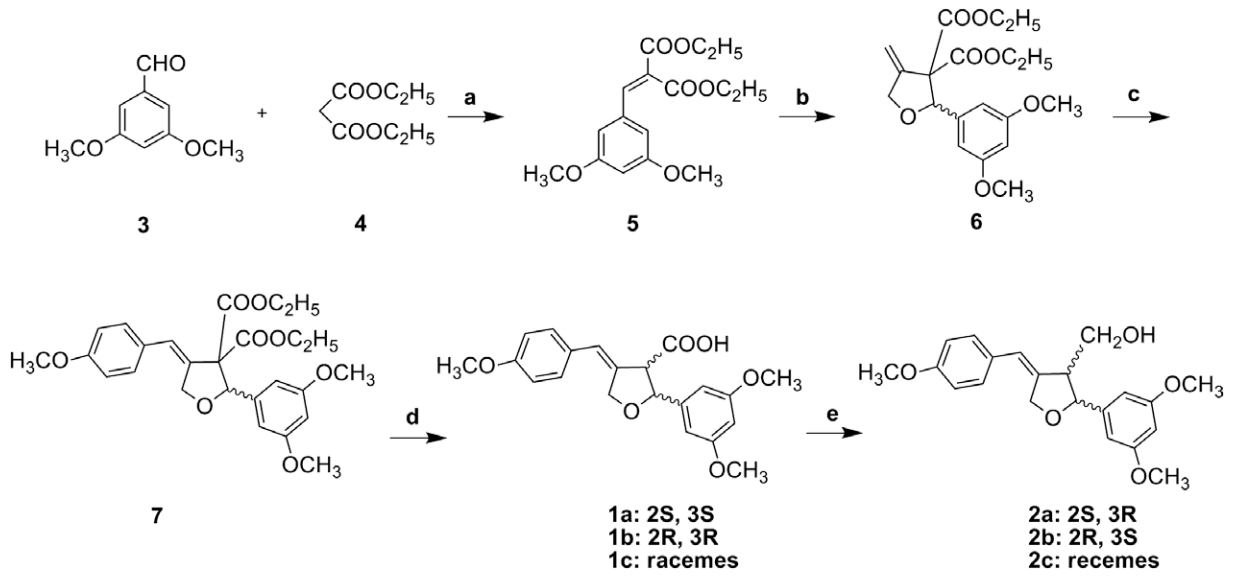

Scheme 1. Reagents and conditions: (a) piperidine, CH_3COOH, toluene, reflux, 6 h, 85%; (b) prop-2-yn-1-ol, NaH, *n*-BuLi, $Pd(Ph_3P)_2Cl_2$, THF, rt, 4 h, 90%; (c) 1-iodo-4-methoxybenzene, $Pd(OAc)_2$, PPh_3, TEA, DMF–DMSO, 8 h, 70%; (d) KOH 20%, EtOH, rt, 5 h, 90%; (e) $LiAlH_4$, THF, rt, 9 h, 80%.

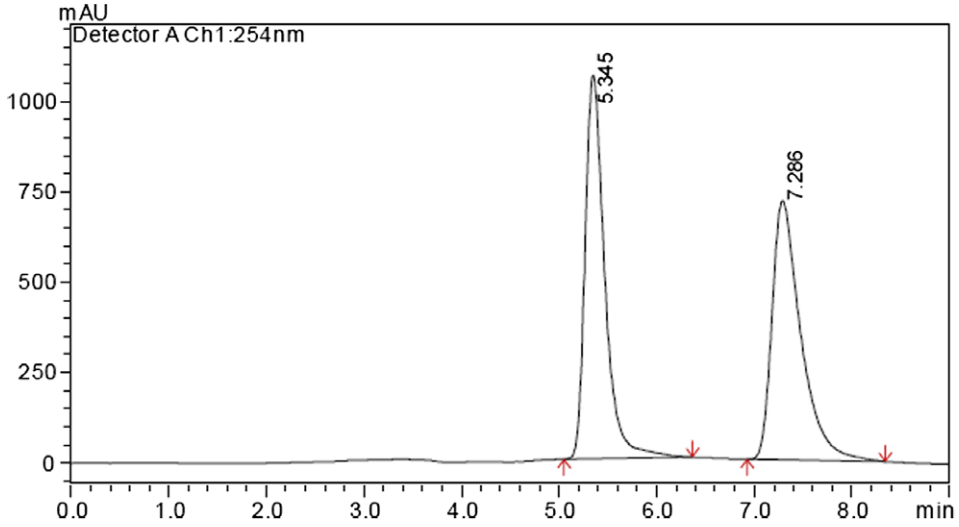

Figure 2. HPLC assay of compound **1c** (**1a**/**1b** = 50.3:49.7 m/m).

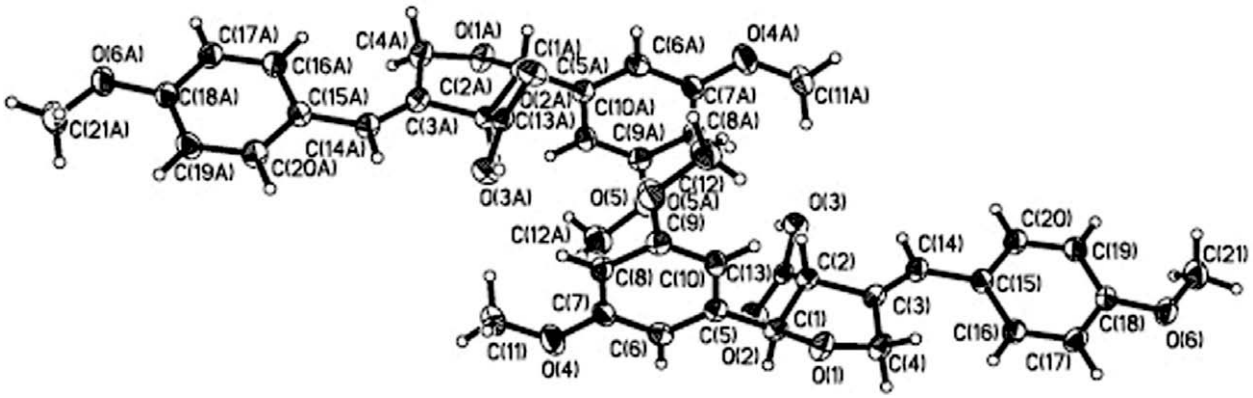

Figure 3. ORTEP drawing of compound **1c**.

As shown in Scheme 1, compound **6** was formed through a coupling reaction without orientation selectivity and two isomers should be obtained because of the existence of chiral center on carbon 2. Without changes, the chirality maintained after heck reaction, and *cis*-configuration was the only configuration due to the existence of stereospecific blockade. Then, compound **7** was hydrolyzed and one

carboxyl group was cut off during the hydrolysis. Therefore, four isomers should be generated theoretically. However, the substituted benzene ring locating on position 2 had a steric hindrance effect on the hydrolysis procedure; only two dominant configurations were generated. And the optical mixture was analyzed on high performance liquid chromatography (HPLC) equipped with a chiral column, only two isomer peaks (peak purity >99%) were found(Fig. 2). Besides, in the crystal cell[13] of single crystal of compound **1**, only two centrosymmetric enantiomers[15,16] could be observed (Fig. 3) and named as (2*S*,3*S*,*Z*)-2-(3,5-dimethoxyphenyl)-4-(4-methoxybenzylidene)tetra hydrofuran-3-carboxylic acid and (2*R*,3*R*,*Z*)-2-(3,5-dimethoxyphen yl)-4-(4-methoxybenzylidene)tetrahydrofuran-3-carboxylic acid, respectively. Consequently, the two isomers were isolated by a preparative Chiralpak IC column and specific rotations were determined. The specific rotation of compound **1a**, retention time was 5.345 min, was evaluated to be $[\alpha]_D^{20} = -30$ (*c*, methanol), while compound **1b**, retention time was 7.286 min, was evaluated to be $[\alpha]_D^{20} = +31.4$ (*c*, methanol). In order to determine the absolute configuration of **1a** and **1b**, circular dichroism (CD) spectra of the two compounds were detected (Fig. 4). The CD spectra were recorded in ethanol showed a quasi-mirror image pattern. In case of compound **1b**, the CD consists of a strong positive band at 258 nm, followed by another smaller negative band at 216 nm; for compound **1a**, the sequence of bands was similar but their signs were inverted. The CD of compound **1a** and **1b** were mainly allied to the transitions of carboxyl group and benzyl group perturbed by various chiral elements attached on the two chiral centers. In particular, the two bands in the CD spectra of **1a** and **1b** could be assigned, from right to left, to the n–π^* and the ^1L_b-type π–π^* transition of chromophore.[19] For the two major CD bands, the n–π^* transition at 258 nm showed a positive Cotton effect for the 3*S* configuration as in **1b**, and negative for the 3*R* configuration as in **1a**;[17–20] while the π–π^* transition at 216 nm showed a negative Cotton effect for the 2*S* configuration as in **1b**, and positive for the 2*R* configuration as in **1a**.[20] So, the absolute stereochemistry of compound **1a** and **1b** was established as (2*R*,3*R*,*Z*)-**1a** and (2*S*,3*S*,*Z*)-**1b**.[21]

The four optical monomers and two racemic compounds reported herein were evaluated, in vitro, cytotoxicity to HeLa (cervical adenocarcinoma) and QGY-7701 (human hepatoma cell) using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[22] 5-Fluorouracil (5-Fu) used in clinical trials was included as the positive control substance. Results of this biological activity study were summarized as Figure 5. IC_{50} (values concentration of the drug required to reduce cell viability by 50%) was

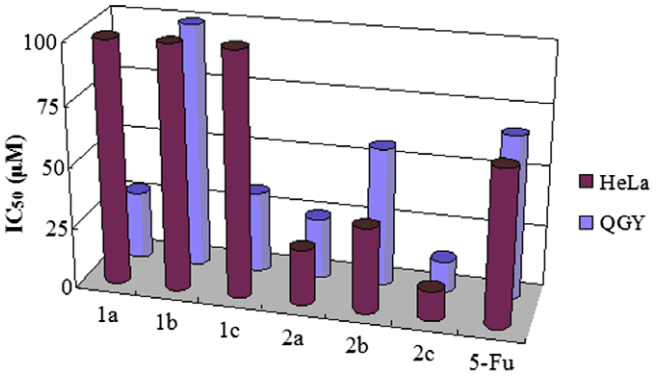

Figure 5. QGY and HeLa cell survival assessed by MTT.

used to evaluate the anti-tumor activity. For HeLa cell line, compound **1a**, **1b**, and **1c** (the racemate of **1a** and **1b**) showed no effectiveness (IC_{50} >100 μM), while compound **2c** (the racemate of **2a** and **2b**), **2a**, and **2b** displayed higher inhibition, 5.35, 2.79, and 1.83 folds, than 5-Fu (IC_{50} = 64.2 μM). For QGY-7701, compound **2c** displayed the best inhibition (IC_{50} = 13 μM), 5.17 folds more effective than 5-Fu (IC_{50} = 67.2 μM); followed by **2a**, **1a**, and **1c** (IC_{50} = 28, 28, and 33 μM). Though compounds **1a**, **1b**, and **1c** showed low cytotoxicity to HeLa, compound **1a** displayed good activity to QGY. For compounds **2a**, **2b**, and **2c**, moderate to good activity could be seen for both QGY and HeLa; especially **2c** performed the best activity among the six compounds.

We are satisfied with the different cytotoxicity between optical monomers and their racemate. Compound **1c**, the racemate of **1a** and **1b**, shows a little weaker active than compound **1a** because of the comparative lower concentration and silence of compound **1b** to QGY. Thanks to the moderate activity of **2a** and **2b**, compound **2c** emerged the highest cytotoxicity to both QGY-7701 and HeLa cancer cells.

In summary, we have found a novel route to synthesize racemic furan lignan from substituted benzaldehyde and diethyl malonate. The two ethyl ester was selectively hydrolyzed to form two optical isomers, which were separated by preparative Chiralpak IC column and their absolute configuration were established by the CD spectra. We have evaluated and compared the anti-cancer activity of optical monomer and racemate. The results revealed that enough attention should be paid to the isomers and their racemate of this

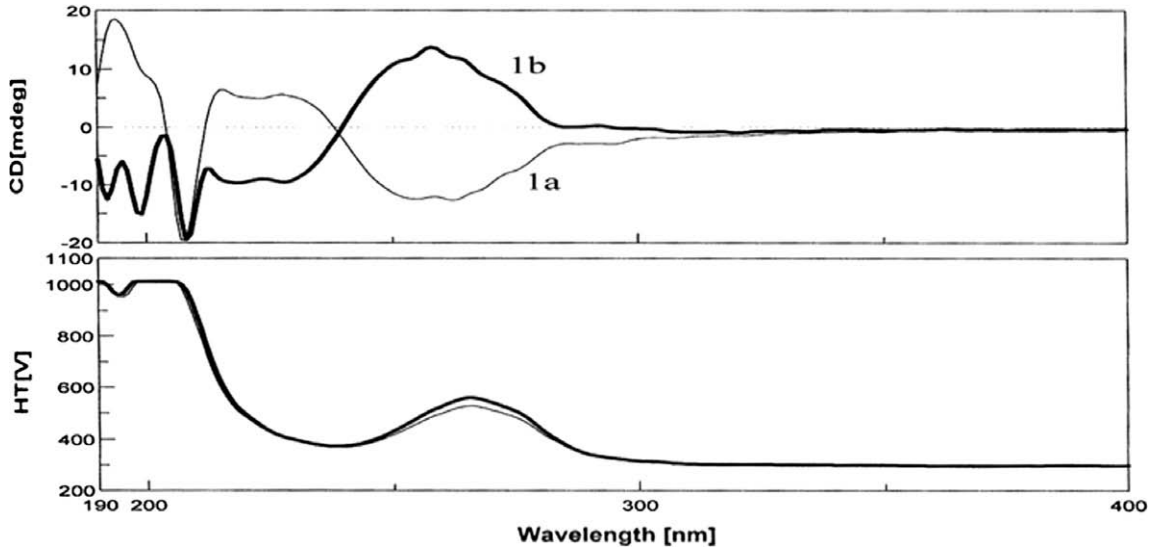

Figure 4. Circular dichroism spectra of compounds **1a** and **1b**.

kind of structures, especially when the different effects are generated due to the existence of chirality.

Acknowledgments

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23. *Typical procedure*: 370 mg of compound **1** (1 mmol) was dissolved in 50 mL of dry THF and 43 mg of $LiAlH_4$ (1.1 mmol) was added. The mixture was stirred at room temperature for 9 h, then 100 mL of 3 N HCl was added, and 100 mL of EtOAc was added to extract the crude product. Then the oil layer was washed with saturated NaCl solution and dried with $MgSO_4$. The crude product was purified with silica gel column. 289 mg of compound **2** was obtained, yield 81%.
24. *Spectral data*: Compound **6**: Oil; ^{1}H NMR (300 M, $CDCl_3$). δ 7.4–7.5 (M, 3H), 5.72 (s, 1H), 5.54 (s, 1H), 5.22 (S, 1H), 5.09 (dd, J = 13.7 and 2.2, 1H), 4.77 (dd, J = 13.7 and 2.7, 1H), 3.85–3.95 (m, 1H), 3.80 (S, 6H), 3.55–3.65 (m, 1H), 1.30 (t, J = 7.5 Hz, 3H), 0.75 (t, J = 7.0 Hz, 3H). Compound **7**: mp: 130 °C; IR (KBr, cm^{-1}): 1727 (C═O), 1614 (C═C), 1606 (Ar-H), 1486 (Ar-H); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.15 (d, J = 9, 2H), 6.95 (d, J = 9, 2H), 6.79 (S, 1H), 6.65 (S, 1H), 6.40 (S, 1H), 5.20 (s, 1H), 5.09 (dd, J = 13.7 and 2.2, 1H), 4.77 (dd, J = 13.7 and 2.7, 1H), 4.36 (q, J = 7.1, 2H), 3.89 (dq, J = 10.7 and 7.1, 1H), 3.86 (s, 3H), 3.78 (s, 6H), 3.61 (dd, J = 10.7 and 7.1, 1H), 1.30 (t, J = 7.0, 3H), 0.75 (t, J = 7.0, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 168.3, 168.0, 160.4, 159.0, 139.5, 135.6, 129.9, 129.2, 125.4, 114.0, 104.7, 100.7, 84.6, 70.3, 69.5, 61.9, 61.3, 55.3, 14.0, 13.4. EI-MS: 469 (M−H), 493 (M+Na). Compound **1**: $C_{21}H_{22}O_6$: mp: 167.3–168.2 °C; IR (KBr, cm^{-1}): 3450 (COOH), 1727 (C═O), 1614 (C═C), 1606 (Ar-H), 1464 (Ar -H); ^{1}H NMR (DMSO, 300 MHz): δ 12.86 (S,1H), 7.15 (d, J = 9 Hz, 2H), 6.94 (d, J = 9 Hz, 2H), δ 6.41–6.49 (m, 3H), 5.06 (d, J = 7.2 Hz, 1H), 4.83 (dd, J = 13.7 and 2.2 Hz, 1H), 4.75 (dd, J = 13.7 and 2.2 Hz, 1H), δ 3.76 (s, 3H), 3.72 (s, 6H), 3.61 (d, J = 7.2 Hz, 1H). ^{13}C NMR (DMSO, 300 MHz): δ 172.2, 160.4, 158.3, 142.7, 137.0, 129.4, 128.8, 121.3, 114.0, 103.7, 99.4, 81.6, 69.6, 58.3, 55.1. EI-MS: 369 (M−H), 393 (M+Na). Compound **2**: Oil, ^{1}H NMR (DMSO, 300 MHz), δ 7.10 (d, J = 9Hz, 2H), 6.90 (d, J = 9Hz, 2H), 6.57 (s, 2H), 6.30–6.41 (m, 2H), 5.67 (s, 1H), 4.88 (d, 1H), 4.75 (d, 1H), 3.78–3.96 (m, 11H), 2.97 (S, 1H, +D_2O disappeared).