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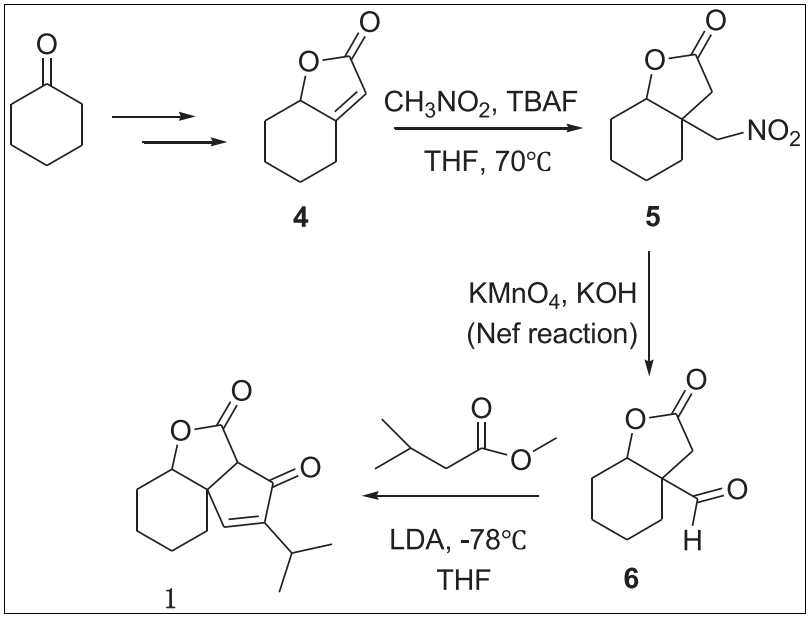

The synthesis of β-keto lactone was completed using cyclohexanone as starting material. The α,β-unsaturated lactone was obtained through Reformatsky reaction and iodolactonization. The aforementioned lactone was converted into target compound **1** (β-keto lactone) by Nef oxidation and Claisen condensation in six steps with 17.9% overall yield.

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INTRODUCTION

β-ketonic esters are important metabolites in plants and animals and are also important intermediates for the biosynthesis of a variety of useful organic compounds such as fats, ribose, β-amino acid, and β-hydroxyl acid [1,2]. They have found applications in treating tumors and hypertension as well as in the manufacture of protease inhibitors [3,4]. Most β-ketonic esters are nontoxic, aromatic, and are often used as food additives [5–7].

Przewalskin B isolated from *Salvia przewalskii* in Shangri-La, Yunnan Province, shows significant anti-HIV-1 activity and other physiological activities [8]. One salient feature of Przewalskin B is that it is a tetracyclic diterpene with the structure of β-ketonic esters (Fig. 1).

On the basis of the physiological activities of β-ketonic esters and relevant reports on the synthesis of Przewalskin B [9,10], we proposed a synthetic scheme of compound **1** (Fig. 2). Compound **1** contains the important active structural domains of the natural product Przewalskin B. The synthetic route of compound **1** is more simple and convenient than Przewalskin B. The synthesis study of compound **1** lays a foundation for the development of small-molecule drugs.

RESULTS AND DISCUSSION

By means of Reformatsky reaction, compound **2** can be easily synthesized using cyclohexanone. At the temperature of 0°C, compound **3** can be synthesized using $SOCl_2$/py./CH_2Cl_2 after 1 h of reaction. Compound **3** is hydrolyzed in 10% aqueous solution of KOH, and the resulting acids can be directly used in iodolactonization without purification. Using I_2/KI/sat. $NaHCO_3$ system and ethyl acetate as solvent, the reaction proceeded at room temperature for

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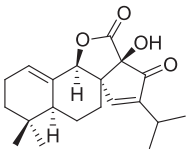
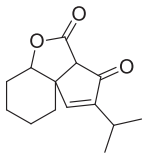


Przewalskin B

Figure 1. The structure of Przewalskin B.



compound 1.

Figure 2. The structure of compound **1**.

18 h. Then, the saturated aqueous solution of $Na_2S_2O_3$ was added. The extraction solution was dried and concentrated. Later, Et_3N was added for reaction at room temperature for 2 h. Finally, compound **4** was obtained by column chromatography (CC) (Scheme 1).

Because 1,4-addition is impossible between vinyl grignard reagent and compound **4**, fluorine anion was used as alkaline to facilitate the 1,4-addition of nitromethane to compound **5**. The oxidation of nitro group in compound **5** by $KMnO_4$ results in compound **6** (Scheme 2).

The condensation between compound **6** and methyl isovalerate leads to the target product, compound **1** (Scheme 3).

Compound **1** was tested for cytotoxicity against BEL-7402, A-549, HT-29, HL-60, MOLT-4 tumor cell lines, and anti-HIV-1 activity was evaluated by the inhibition for the cytopathic effects of HIV-1 (EC_{50}). Compound **1** exerted minimal cytotoxicity against the five cell lines ($IC_{50} > 200$ μg/mL) and showed anti-HIV activity with $EC_{50} = 28.45$ μg/mL and selectivity index = 3.51.

In summary, the synthesis of compound **1** has been achieved in six steps with 17.9% overall yield starting from the commercially available cyclohexenone, involving Reformatsky reaction, iodolactonization, Nef oxidation, and Claisen condensation as key steps to furnish the target compound.

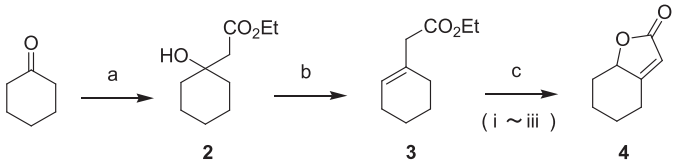
EXPERIMENTAL

General experimental. All regents were used as purchased from Acros, Aldrich, and Fluka without further purification. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2, and THF was dried by Na. For reactions carried out under Ar, the mixtures were degassed at high vacuum and purged with Ar at least three times. The course of reaction was monitored by TLC. 1H-NMR and 13C-NMR spectra were recorded at ambient temperature with a Bruker Avance 300 (300 MHz for 1H and 75.5 MHz for 13°C; Bruker, Germany) instrument in which TMS was used as internal standard for all measurements. MS data were recorded by using a VG Auto spec-3000 spectrometer or a Finnigan MAT 90 instrument. IR spectra were measured as KBr pellets by using a Bio-Rad FTS-135 spectrometer. CC was performed by using silica gel (200–300 mesh; Qingdao Marine Chemical, China).

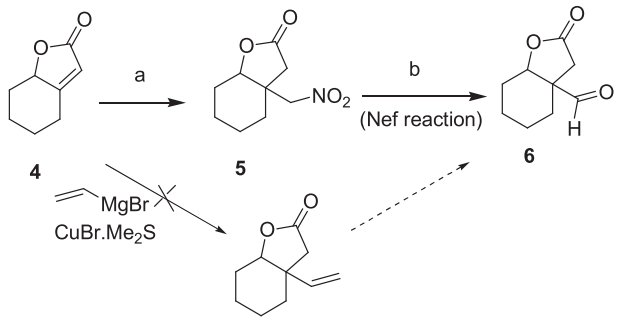
Preparation of Zn–Cu couple. Zinc dust (392 mg, 6 mmol) was added to a hot (80°C), well stirred solution of $Cu(OAc)_2 \cdot H_2O$ (22.4 mg, 0.1 mmol) in glacial acetic acid (1 mL). After about 30 s, the solution became colorless due to the deposition of the copper on the zinc. The silt-like couple was allowed to settle for about 1 min, followed by careful decantation of as much of the acetic acid as possible without loss of the deposits. The dark red-gray couple was then washed with a 1-mL portion of acetic acid and thrice with 1 mL of diethyl ether, followed by drying in a stream of nitrogen and subsequent suspension in dry THF (5 mL). The Zn/Cu couple resulting from this procedure was used for Reformatsky reaction. The procedure can readily be scaled up 10-fold.

Preparation of ethyl 2-(1-hydroxycyclohexyl)acetate (2). The Zn–Cu pair was suspended in dry THF (10 mL). A solution of ethyl 2-bromoacetate (1 g, 6 mmol) was added dropwise at 70°C and stirred for 1 h. Then, the solution of cyclohexanone (0.2 g, 2 mmol) in THF (1 mL) was added dropwise. The reaction mixture was stirred for 3 h at 70°C and cooled to room temperature, and then aqueous solution of NH_4Cl (10 mL) was added. The resulting mixture was extracted with ethyl acetate (3 × 5 mL), dried by Na_2SO_4, removed of the solvent *in vacuo*. The crude product was purified by flash chromatography giving compound **2** (87%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.97 (q, J = 7.10 Hz, 2H), 3.35

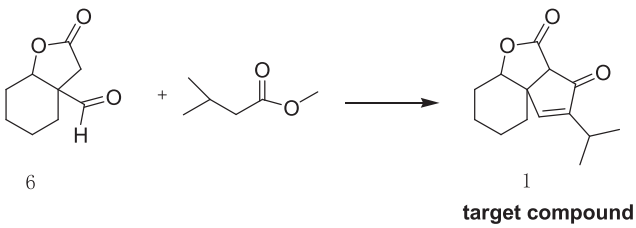
Scheme 1. Synthesis and reaction of 5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (4). Reagents and conditions: (a) $BrCH_2CO_2Et$, Zn-Cu, THF, reflux, 87%. (b) $SOCl_2$, pyridine, CH_2Cl_2, 0°C, 88%. (c) i: 10%KOH, MeOH; ii: I_2, KI, $NaHCO_3$; iii: Et_3N, three steps, 55%.



Scheme 2. Synthesis and reaction of octahydro-2-oxobenzofuran-3a-carbaldehyde (**6**). Reagents and conditions: (a) CH_3NO_2, TBAF, THF, 70°C, 85%. (b) $KMnO_4$, $MgSO_4$, KOH, CH_3OH, rt, 78%.



Scheme 3. Synthesis and reaction of target compound (**1**). Reagents and conditions: LDA, THF, −78°C, 64%.



(br.s, 1H), 2.23–2.28 (br.d, 2H), 1.21–1.48 (overlap, 10H), 1.10 (t, J = 7.10 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 172.46, 69.63, 60.18, 45.33, 37.22, 25.47, 22.22, 14.01 ppm; IR (KBr): υmax = 3516, 1718 cm^{-1}; MS (EI): *m/z*: 186.

Preparation of ethyl 2-cyclohexenylacetate (3). To an ice-water-cooled solution of compound **2** (3 g, 16.13 mmol) in dry pyridine (2.55 g, 32.26 mmol) and CH_2Cl_2 (10 mL) was added thionyl chloride (2.09 g, 17.74 mmol) dropwise under stirring over a 5-min period. After 1 h of stirring, the reaction mixture was poured into ice-water and extracted with ether. The ether layer was washed with water and dried over Na_2SO_4. Removal of solvent followed by CC gave compound **3** (88%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.56 (t, J = 7.88 Hz, 1H), 4.10 (q, J = 7.11 Hz, 2H), 2.76–2.92 (br.d, 2H), 1.96–2.08 (br.s, 3H), 1.51–1.62 (br.s, 5H), 1.24 (t, J = 7.11 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 171.96, 131.17, 125.49, 60.35, 43.64, 28.35, 25.25, 22.70, 21.97, 14.21 ppm; IR (KBr): υmax = 3413, 2938, 1738, 1459 cm^{-1}; MS (EI): *m/z*: 168.

Preparation of 5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (4). To a solution of 10% aqueous KOH (1.12 g, 20 mmol) was added compound **3** (1.68 g, 10 mmol) in CH_3OH (15 mL). After 24 h at room temperature, CH_3OH was removed on a rotary evaporator, and the aqueous mixture was diluted with 15 mL of water. The aqueous layer was acidified to pH 6 by the dropwise addition of concentrated HCl, which was extracted into ethyl acetate using three 15–mL portions. The organic phases were combined, dried over $MgSO_4$, and concentrated on a rotary evaporator without further purification.

A solution of KI (2.02 g, 12.15 mmol) and I_2 (0.93 g, 3.64 mmol) in 5 mL of water was added to a solution of crude carboxylic acid (0.34 g, 2.43 mmol) in ethyl acetate (5 mL) and sat. $NaHCO_3$ (1.02 g, 12.15 mmol), and the resulting mixture was allowed to stand for 18 h in the dark. Enough $Na_2S_2O_3$ was then added to decolorize the mixture, followed by 10 mL of ethyl acetate. The two layers were separated, and the aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated on a rotary evaporator. Et_3N (2.45 g, 24.3 mmol) was added to a solution of the crude iodolactone in 1 mL of dry benzene under N_2 at room temperature. After 24 h, the reaction mixture was concentrated to a brown residue, which was purified by CC to provide compound **4** (55%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.71 (s, 1H), 4.71 (dd, J = 6.41 Hz, 1H), 2.85–2.90 (br.d, 1H), 2.53 (br.s, 1H), 2.21–2.38 (m, 1H), 1.88–2.04 (m, 2H), 1.20–1.53 (m, 3H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 173.35, 172.49, 111.93, 81.33, 34.33, 27.97, 26.49, 22.31 ppm; IR (KBr): υmax = 2949, 1778, 1448 cm^{-1}; MS (EI): *m/z*: 138; HRMS: *m/z*: calcd for $C_8H_{10}O_2$: 138.0681; found: 138.0683.

Preparation of hexahydro-3a-(nitromethyl)benzofuran-2(3H)-one (5). Compound **4** (2.2 g, 16 mmol)was dissolved in THF (10 mL) and stirred at 70°C with nitromethane (1.95 g, 32 mmol) and tetrabutylammonium fluoride (1 M in tetrahydrofuran, 24 mL, 24.0 mmol). After 18 h, the mixture was cooled to room temperature, diluted with ethyl acetate (40 mL), and washed with 2 N HCl (20 mL) and then brine (2 × 30 mL). The organic phase was collected and dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue was purified by flash chromatography (silica, ethyl acetate/heptane, 1 : 9) to give compound **5** (85%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.58 (d, J = 11.78 Hz, 1H), 4.44 (d, J = 11.78 Hz, 1H), 2.76 (d, J = 17.10 Hz, 1H), 2.41 (d, J = 17.10 Hz, 1H), 1.37–1.63 (m, 8H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 174.73, 79.32, 78.44, 41.78, 28.87, 25.56, 20.23, 19.11 ppm; IR (KBr): υmax = 1771, 1549 cm^{-1}; MS (EI): *m/z*: 200 $[M+H]^+$.

Preparation of octahydro-2-oxobenzofuran-3a-carbaldehyde (6). A solution of compound **5** (0.3 g, 1.51 mmol) in MeOH (5 mL) at 0°C was mixed with a solution of KOH (85%) (0.09 g, 1.66 mmol) in MeOH (2 mL). The mixture was stirred at 0°C for 20 min and a freshly prepared solution of $KMnO_4$ (0.18 g, 1.13 mmol) and $MgSO_4$ (0.14 g, 1.15 mmol) in H_2O (10 mL) was added dropwise, over 40 min, to the aforementioned mixture. After the mixture was stirred at 0°C for an additional hour, the mixture was diluted with EtOAc (50 mL) and filtered through a pad of Celite. The Celite pad was washed with EtOAc (3 × 50 mL). The combined filtrates were washed with brine (1 × 20 mL) and H_2O (1 × 10 mL), dried ($MgSO_4$), filtered, and the

filtrate evaporated *in vacuo*. The residue was purified by flash chromatography (silica, ethyl acetate/heptane, 1 : 5) to give compound **6** (78%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.37 (s, 1H), 4.53 (t, J = 3.46 Hz, 1H), 2.47 (d, J = 17.12 Hz, 1H), 2.36 (d, J = 17.12 Hz, 1H), 1.18–1.63 (m, 8H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 198.88, 175.11, 72.83, 56.10, 38.77, 28.53, 25.96, 22.05, 21.28 ppm; IR (KBr): υmax = 1772, 1715 cm^{-1}; MS (EI): *m/z*: 168; MS (HR-ESI): *m/z*: calcd for $C_9H_{12}NaO_3^+[M+Na]^+$: 191.0679; found: 191.0681.

Preparation of compound 1. To a solution of methyl isovalerate (0.3 g, 2.62 mmol) in THF (20 mL) at −78°C under N_2 atmosphere was added LDA (1 M, 2.62 mmol, 2.62 mL) and stirred for 30 min. Then, the solution of compound **6** (0.4 g, 2.38 mmol) in THF (1 mL) was added dropwise. After 2 h of stirring, this mixture was added another LDA (1 M, 5.24 mmol, 5.24 mL) at the same temperature. After the addition was complete, the mixture was stirred for 18 h. Then, aqueous solution of NH_4Cl (10 mL) was added. The resulting mixture was extracted with ethyl acetate (3 × 5 mL), dried by Na_2SO_4, and removed of the solvent *in vacuo*. The crude product was purified by flash chromatography giving compound **1** (64%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.05 (s, 1H), 4.41 (t, J = 3.45 Hz, 1H), 3.30 (s, 1H), 2.57–2.61 (m, 1H), 2.21–2.26 (m, 2H), 1.65–1.79 (m, 5H), 1.24–1.39 (m, 7H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 197.87, 170.22, 158.71, 150.49, 80.11, 57.27, 50.31, 30.84, 29.66, 24.86, 22.34, 21.59, 21.00, 20.79 ppm; IR (KBr): υmax = 2954, 1771, 1707 cm^{-1}; MS (EI): *m/z*: 234; MS (HR-ESI): *m/z*: calcd for $C_{14}H_{18}NaO_3^+[M+Na]^+$: 257.1148; found: 257.1149.

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