Article

Concise total synthesis of (+)-Zeylenone with antitumor activity and structure-activity relationship of its derivatives

Zhonghao Sun [1],Shuxian Yang[1], Chengfang Xu[2], Guoxu Ma[1], Li Cao[1], Yu Tian[1], Jiahao Lin[2*], Xudong Xu [1*]

[1] Laboratory of Bioactive Substances and Resource Utilization of Chinese Herbal Medicine, Ministry of Education; Beijing Key Laboratory of Innovative Drug Discovery of Traditional Chinese Medicine (Natural Medicine) and Translational Medicine;
Beijing Key Laboratory of Innovative Drug Discovery of Traditional Chinese Medicine (Natural Medicine) and Translational Medicine;
Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, 100193, China
[2] China Agricultural University, Beijing, 100193, China

Abstract: (-)-Zeylenone is a promising cytotoxic agent，which is a natural product isolated from *Uvaria grandiflora Roxb*. Though substantial antitumor mechanism has been researched , little has focused on its enantiomer (+)-Zeylenone.This article will try to find a gram scale synthesis method of (+)-Zeylenone and explain the structure-activity relationship of this kind of compound. Total synthesis of (+)-zeylenone was completed in 13 steps with quinic acid as starting material in 8.8% overall yield. The highlight of the route was the control of the three carbon's chirality by clever use of single step dihydroxylation under the direction of the key C-3 chirality. In addition, zeylenone derivatives were designed and synthesized and their antitumor activity were evaluated against three human cancer cell lines using the CCK8 assay. Structure-activity relationship suggested compounds with both two absolute configurations exhibited good activity. Besides, hydroxyls at C-1/2 position were crucial for the activity and esterification of C-1 hydroxyl with large groups made the activity disappeared. Hydroxyl at C-3 position was also important as proper ester substituent could increase the potency.

Keywords: total synthesis; antitumor activity; structure-activity relationship.

1. Introduction

Nowadays despite the growth of different kind of tumor immunotherapy, small molecule inhibitors still possess significant role in cancer treatment. Natural products have long been recognized as the richest source of high chemical diversity and its novel scaffold structures can be used as anticancer agents. It is notable that of all the available anticancer drugs on the market, 70% have been sourced from natural products or have been derived from plants[1].Natural products with excellent anti-tumor activity is worthy of explore and research.

(-)-Zeylenone(figure 1) is a natural product isolated from *Uvaria grandiflora Roxb* with potent inhibition of different cell lins[2]. Its antitumor activity and underlying mechanism were researched by Cao's group[3-7].They have demonstrated that (-)-zeylenone strongly suppress chronic myelogenous leukemia[4], cervical carcinoma[5], prostate cancer[6], gastric cancer[7] both in vitro and in vivo, and undertook its drug discovery research. So far There existed two total synthesis methods for (-)-zeylenone. The first enantioselective synthesis was achieved by Ogasawara in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2019 doi:10.20944/preprints201904.0262.v1

1999[8, 9]. They utilized the chiral cyclohexanoid building block to form five naturally occurring polyoxygenated cyclohexanes, one of which was (–)-tonkinenin A proved to be identical with (-)-zeylenone. In 2006, Liu and co-workers [10, 11] reported a stereoselective synthesis of (-)-zeylenone and its enantiomer (+)-zeylenone from shikimic acid. This biosynthetic approach oritented starting material facilitated the construction of chiral center resulting in a shorter procedure. Besides, Palframan reported a synthesis route from a microbial arene oxidation product, and finally found a concise synthesis of representative examples of the (+)-zeylenol and (+)-zeylenone families of natural products[12]. The latest method was reported by using D-mannose as starting material to synthesize (-)-zeylenol based on mixed aldol condensation reaction[13].

So far little has been researched on (+)-zeylenone and its anti-tumor activity. Besides, structure-activity relationship of this kind of compounds were not clear. In this ariticle ,we will try to finish a concise synthesis method of (+)-zeylenone and explain the structure-activity relationship of its antitumor activity.

2. Results And Discussions

2.1. Total synthesis of (+)-zeylenone

2.1.1. Retrosynthesis of (+)-zeylenone

The structure of zeylenone contained three chiral center , α,β-unsaturated ketone and multiple oxygen substitutions. As we can see the reported methods, the starting material decided the efficiency and and Inspired by the shikimic acid started route, we found D-(−)-quinic acid possessed a more likely skeleton. Furthermore, compared the easily prepared synthetic agent QA-4 [14]and QA-4'[15] from D-(−)-quinic acid with zeylenone (Figure 1), we found three mainly different functional groups. And they are benzoylated methylol group at C-7 position, cis vicinal diols at C-1/2 position, olefinic bond between C-4/5 position.

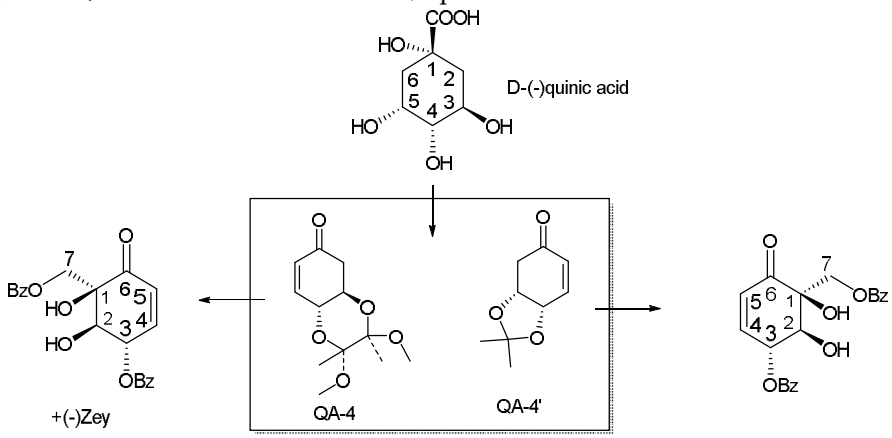
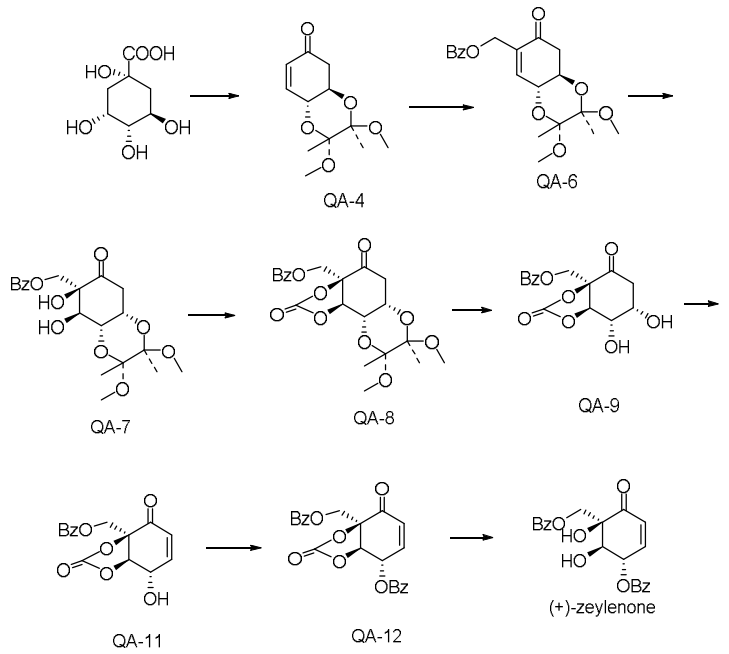


Figure 1. structures of zeylenone and synthetic agent QA-4 and QA-4' from D-(−)-quinic acid

So we designed a synthetic route from D-(−)-quinic acid via the synthetic agent **QA-4** (Scheme 1).

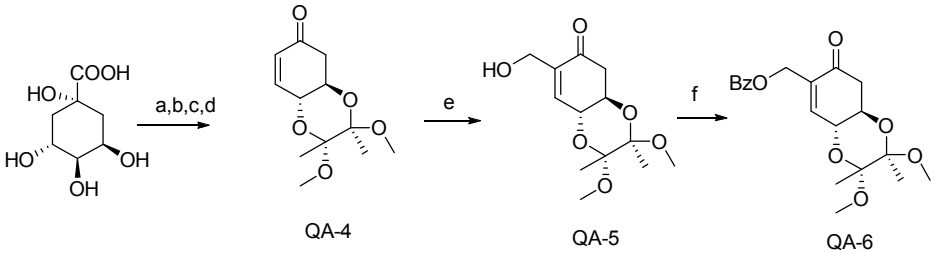
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2019 doi:10.20944/preprints201904.0262.v1



Scheme 1. Designed synthetic route from D-(−)-quinic acid to (+)-zeylenone.

2.1.2. Synthesis of (+)-zeylenone

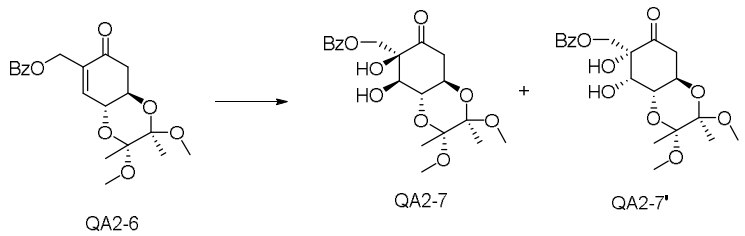
QA-6 was accomplished according to the literature in 44.3 % yield (Scheme 2)[14, 16].



(a)2,3-Butanedione,trimethylorthoformate,(±)csa(b): $NaBH_4$,MeOH,(c):$NaIO_4$,Phosphate buffer,(d):MsCl,NEt_3,CH_2Cl_2,(e):paraformaldehyde, imidazole,$NaHCO_3$(1M),THF,(f):BzCl, NEt_3, DMAP

Scheme 2. Synthesis of QA-6

The following sharpless dihydroxylation of **QA-6** was tried by potassium osmate without chiral ligands, but yielded the **QA-7** and **QA-7′** with a ratio of 1:0.6 calculated by ^{1}HNMR(Scheme 3, Table 1). When treated with the asymmetric catalyst AD-mix-α,single diol isomer **QA-7** was obtained in 65% yield(Table 1).



Scheme 3. Synthesis of QA-7

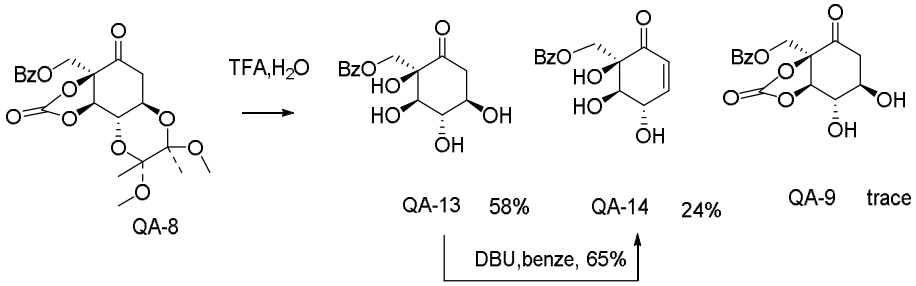
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2019 doi:10.20944/preprints201904.0262.v1

Table 1. Asymmetric dihydroxylation of QA-6

catalyst	Ratio
Potassium osmate, NMMO	QA-7:QA2-7′ (1:0.6)
AD-mix-α	QA-7 (65%), QA-7′ (trace)
AD-mix-β	QA-7′(48%), QA-7 (trace)

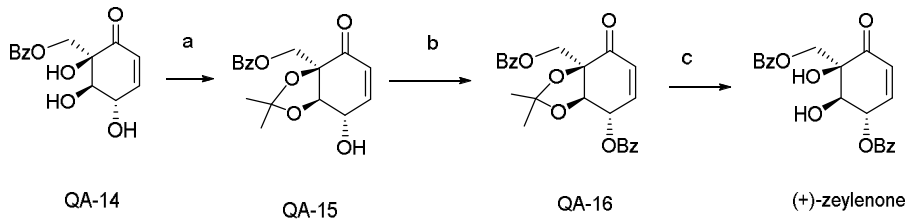
The *cis* vicinal diols **QA-7** were protected by acid-stable cyclic carbonate in consideration of following hydrolysis of six numbered 1,2-diacetal ring. **QA-7** was treated with triphosgene and pyridine in CH_2Cl_2 at -10 °C to give **QA-8** in 93% yield.

Hydrolysis of **QA-8** with TFA did not give desired **QA-9**, but produced the unexpected **QA-13** and **QA-14**(Scheme 4.We believed the product **QA-13** is prone to be eliminated. So we treated **QA-13** with catalytic amount DBU refluxed in benzene and obtained **QA-14** in 65% yield[17, 18].



Scheme 4. Synthesis of QA-14

The synthesized **QA-14** in advance made the route a little different from schedule(Scheme 5). The protection of *cis* vicinal diols of **QA-14** with propyl acetal by 2,2-dimethoxypropane gived **QA-15**. **QA-15** was benzoylated and hydrolyzed by benzoyl chloride and 1N hydrochloric acid successively to obtain the target compound (+)-zeylenone. The spectral data (including NMR, MS and IR) of (+)-zeylenone were identical with reference. Besides, the rotation value the value and sign of the optical rotation $[\alpha]^{20}$ D = +26.1(c = 0.2, MeOH)was in accordance with reference $[\alpha]^{20}$ D = +26(c = 0.2, MeOH).In addition, the Cotton effects in CD spectra (seen in supplementary information) of synthesized compound was opposite to (-)-zeylenone, and the same as (+)-zeylenone. All the data proved the synthesized compound was (+)-zeylenone.



(a):2,2-DMP,TsOH, 88%(b):BzCl,DMAP,95%(c):HCl, MeOH, 90%

Scheme 5. Synthesis of (+)-zeylenone

We have attempted to use this method to synthesize (-)-zeylenone via QA-4′, but failed at the Baylis-Hillman reaction to QA-5′.It was because of the less stable propylacetal protection of vicinal diols.

2.2. Design and synthesis of zeylenone Derivatives

Derivatives with the same absolute configuration of (-)-zeylenone was exhibited in figure 2. (-)-Zeylenone was isolated from the extraction of *Uvaria grandiflora Roxb* in Hainna province.The derivatives zey-1 was synthesized from (-)-zeylenone hydrolysis using ammonia water in methanol. zey-2 and zey-3 were synthesized with the same method as **QA-15** and **QA-16**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2019 doi:10.20944/preprints201904.0262.v1

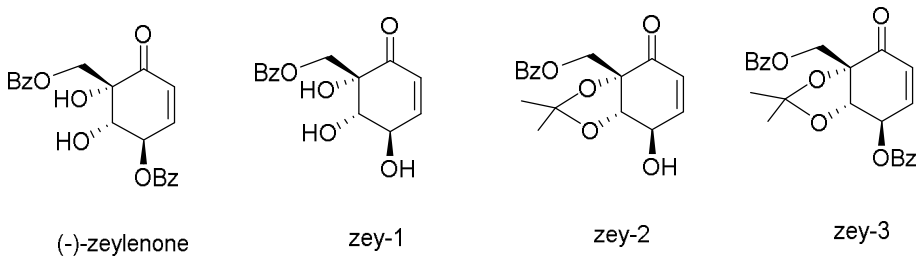


Figure 2. Derivatives of (-)-zeylenone

Besides, in order to investigate the influence of hydroxyls at position C-1/2/3 on the antitumor activity, we synthesized three series of analogues.**QA-15** was utilized as a starting material to synthesize Series A. The structure of Series A included acetalation of hydroxyls at C-1/2 positions and different acylation of hydroxyl at C-3 position. Series B was the acetal hydrolyzed products of corresponding Series A. Series C were acylated from (+)-zeylenone at C-2 position or both C-1/2 positions. The structure of these derivatives were outlined in figure 3.

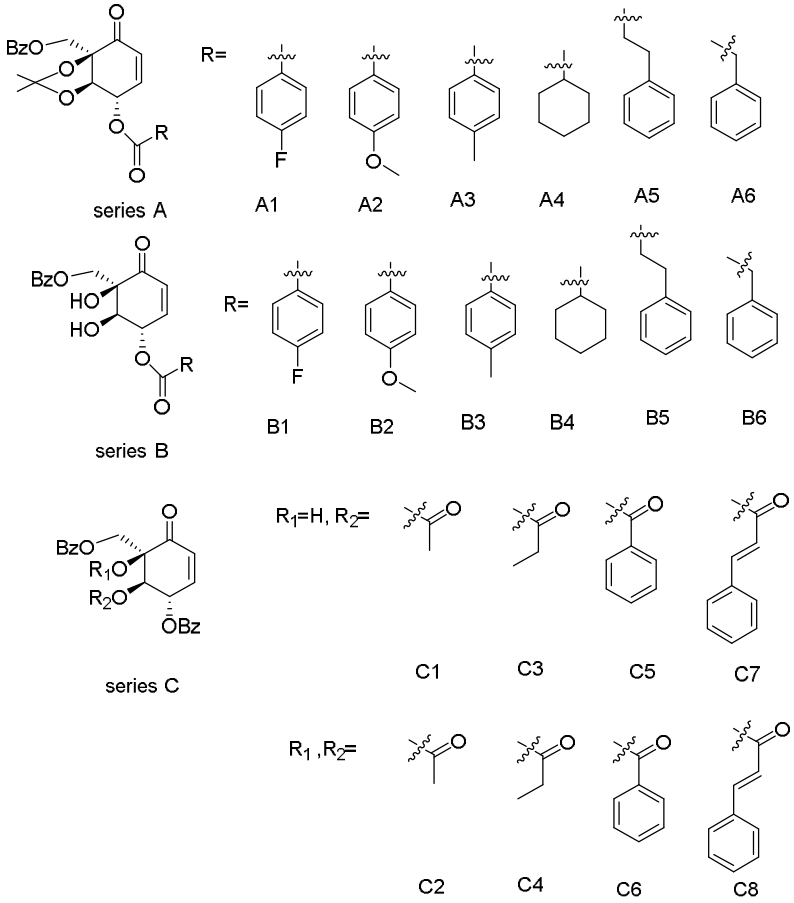


Figure 3. Derivatives of (+)-zeylenone

2.3. Antitumor activity

As shown in Table 2, the antitumor activity was measured by IC_{50} value against three human cancer cell lines including melanoma (B16), human hepatocellular carcinoma(HepG2), euroblastoma SH(SY5Y) using the CCK8 assay. Taxol was used as a standard for comparison.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2019 doi:10.20944/preprints201904.0262.v1

Table 2. Inhibitory effects of zey derivatives on proliferation of SY5Y, HepG2, B16 cells.

compound	IC50(μ M)		
	SY5Y	HepG2	B16
(+)-zey	3.411±0.08	4.001±0.18	5.326±0.3
QA-14	8.027±0.2	18.913±0.41	21.641±0.22
QA-15	10.365±0.15	55.759±2.9	88.256±1.55
QA-16	8.027±0.25	11.913±0.30	5.641±0.1
(-)-zey	2.916±0.05	4.267±0.17	3.873±0.26
zey-1	7.961±0.38	77.180±0.7	13.295±0.41
zey-2	8.018±0.34	40.311±0.55	12.131±0.24
zey-3	9.089±0.2	11.520±0.31	7.175±0.05
A1	1.964±0.19	2.899±0.04	2.563±0.11
A2	4.142±0.25	4.811±0.1	5.775±0.51
A3	3.165±0.08	4.385±0.09	2.908±0.22
A4	5.654±0.13	7.591±0.18	6.074±0.21
A5	8.512±0.02	17.340±0.5	13.950±0.5
A6	5.002±0.13	5.015±0.52	9.226±0.19
B1	2.845±0.92	4.614±0.48	3.242±0.24
B2	2.635±0.36	5.144±0.5	3.913±0.43
B3	3.291±0.31	14.690±0.22	3.589±0.74
B4	5.291±0.64	10.510±0.45	6.797±0.04
B5	3.251±0.52	3.226±0.15	3.722±0.1
B6	2.436±0.6	5.968±0.21	2.812±0.6
C1	4.018±0.50	10.300±0.74	2.131±0.58
C2	22.021±0.6	8.226±0.51	10.023±0.27
C3	6.225±0.2	8.824±0.11	16.253±0.06
C4	3.089±0.60	11.52±0.18	4.175±0.17
C5	9.275±0.19	5.664±0.08	9.478±0.04
C6	20.493±0.06	9.015±0.18	>100
C7	35.361±0.8	29.356±0.5	44.278±3.5
C8	>100	60.997±2.11	>100
taxol	2.88±0.24	5.42±0.38	3.56±0.35

2.4. Structure-activity relationship

Evaluation of (+)-zeylenone (3.411, 4.001, 53.26) and enantiomer (-)-zeylenone (2.916, 4.267, 3.873) showed similar IC_{50} value, which indicated both the two absolute configurations possessed potent activity. The relationship between absolute configuration and activity was proved by another pair of enantiomers **QA-16** and **zey-3,** which showed almost the same toxicity against three cell lines. Besides debenzoylated compounds at C-3 position (**QA-14**, **QA-15**, **zey-1**, **zey-2**) showed same declining trend of activity. But they were slightly more sensitive to SY5Y.The initial structure-activity relationship suggested both two absolute configurations exhibited good activity, and the debenzoylation at C-3 position could reduce the efficiency.

Generally, series A and B showed relatively potent activity. Compared series A with **QA-15** and series B with **QA-14**, we can conclude that acylation of hydroxyl at C-3 position is important to the activity. **A1** showed the highest IC_{50} value to three cell lines, and its acetalation counterpart **B1** also exhibited excellent activity, which suggested that the 4-fluorobenzoylation of hydroxyl at C-3 position could definitely improve the antitumor activity.The *para*-methyl and *para*-methoxyl substitutent of C-3 benzoylation could either improve or maintain the activity.But **B3** showed exceptionally low activity against hepg2. Cyclohexane esters **A4** and **B4** possessed a less than 10 micromole IC_{50} value. The activity of **A5** and **A6** were improved compared with **QA-16**, meanwhile **B5** and **B6** have a higher IC_{50} value than (-)-zeylenone,which indicated that increase of carbon chain

 doi:10.20944/preprints201904.0262.v1

between C-3 acyl and aromatic ring is beneficial to the increase of activity, but the correlation between carbon chain length and activity is not obvious.

Overall, esterification of hydroxyls at C-1/2 position decreased the activity, especially at C-1. Compared the mono esterification compounds (**C1**, **C3**, **C5**, **C7**) with the corresponding double esterification compounds (**C2**, **C4**, **C6**, **C8**) ,we could find an obvious decrease of activity. The cinnamic acid esterified **C8** almost showed no antitumor efficacy. All this information suggested hydroxyls at C-1/2 position were crucial for the activity and esterification of large groups at C-1 hydroxyl made the activity disappeared.

3. Experiment Section

3.1 General

The products were purified by column chromatography on silica gel (200～300 mesh, Qingdao Marine Chemical plant, Qingdao, People's Republic of China). Precoated silica gel GF254 plates (Zhi Fu Huang Wu Pilot Plant of Silica Gel Development, Yantai, China) were needed for TLC. High resolution mass spectra(HR-ESI-MS) was conducted with ThermoFisher Scientific LTQ-Orbitrap XL spectrometer. ^{1}HNMR and ^{13}CNMR spectra were acquired with a Bruker AV Ⅲ 600 NMR spectrometer (chemical shift values are shown as δ values with TMS as the internal standard). Abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublet) t (triplet), q (quartet), m (multiplet), bs (broad singlet). Chemical shifts (δ) are given in ppm relative to solvent residual peak ($CDCl_3$, δ = 7.26 ppm, CD_3OD, δ = 3.3 ppm) as external standard. All the in vitro tests were carried out in accordance with guidelines evaluated and approved by the Institute of Medicinal Plant Development(IMPLAD).

3.2 Synthesis and Characterization Data

3.2.1 QA-4

QA-4 was synthesis according to the ref[14] .

^{1}HNMR (600 MHz, $CDCl_3$) δ 6.87 (s, 1H), 6.00 (s, 1H), 4.51 (s, 1H), 4.06 (s, 1H), 3.32 (s, 3H), 3.27 (s, 3H), 2.74 (d, J = 14.8 Hz, 1H), 2.49– 2.47 (m, 1H), 1.34 (s, 3H), 1.33 (s, 3H).^{13}CNMR (150 MHz, $CDCl_3$) δ 196.90 (s), 148.59 (s), 130.10 (s), 100.79 (s), 99.71 (s), 69.23 (s), 68.08 (s), 48.24 (s), 48.16 (s), 42.02 (s), 17.73 (s), 17.68 (s). HRMS (ESI): m/z calcd. $C_{12}H_{18}NaO_5$ [M + Na]+: 265.1052, found 265.1175; data are in agreement with reported values.

3.2.2 QA-5

QA-4 (2.8 g, 12 mmol) was dissolved in 50 ml THF, 50 ml sodium bicarbonate solution (1M), imidazole (0.9 g, 13.2 mmol) and polyformaldehyde (720 mg, 24 mmol) were added successively.. After stirred for 2 h at RT, the mixture was extracted with ethyl acetate, washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 1:1) to give QA-5 (2.78g, 85%).White powder; ^{1}HNMR (600 MHz, $CDCl_3$) δ 6.83 (d, *J* = 1.4 Hz, 1H), 4.51 – 4.50 (m, 1H), 4.30 – 4.28 (m, 1H), 4.20 – 4.18 (m, 1H), 4.01 (ddd, *J* = 13.8, 9.0, 4.8, Hz, 1H), 3.30 (s, 3H), 3.24 (s, 3H), 2.74 (ddd, *J* = 16.4, 4.8, 2.0 Hz, 1H), 2.53 – 2.48 (m, 1H), 2.40 (bs, 1H, OH), 1.34 (s, 3H), 1.31 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 197.39 (s), 144.50 (s), 139.28 (s), 100.94 (s), 99.90 (s), 69.25 (s), 68.18 (s), 60.83 (s), 48.38 (s), 48.30 (s), 42.30 (s), 17.87 (s), 17.82 (s).HRMS (ESI): m/z calcd. $C_{13}H_{20}NaO_6$ [M + Na]$^{+}$: 295.1158, found 295.1175; data are in agreement with reported values[16].

3.2.3 QA-6

 doi:10.20944/preprints201904.0262.v1

QA-5 (2.8 g, 12 mmol) was dissolved in 30 ml DCM, then triethylamine (1.3 ml, 24 mmol), DMAP (150 mg, 1.2 mmol), benzoyl chloride (2.1 ml, 18 mmol) were added successively. After stirred for 2 h at RT, the reaction was quenched with saturated sodium bicarbonate solution, and extraction with DCM, washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 4:1) to give QA-6 (4.5g, 98%). White powder; ^{1}HNMR (600 MHz, $CDCl_3$) δ 8.09 (d, *J* = 6.3 Hz, 2H), 7.61 (s, 1H), 7.49 (d, *J* = 6.3 Hz, 2H), 6.93 (s, 1H), 5.09 (d, *J*=13.3 Hz, 1H), 5.05(d, *J*=13.3 Hz, 1H), 4.58 (d, *J* = 5.6 Hz, 1H), 4.09 (s, 1H), 3.34 (s, 3H), 3.32(s, 3H), 2.85 (dd, *J* = 15.7, 4.2 Hz, 1H), 2.59 (t, *J* = 14.5 Hz, 1H), 1.39 (s, 3H), 1.36 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 195.17 (s), 165.99 (s), 144.80 (s), 135.45 (s), 133.28 (s), 129.79 (s), 128.48 (s), 100.85 (s), 99.75 (s), 69.20 (s), 67.92 (s), 60.77 (s), 48.35 (s), 48.21 (s), 42.09 (s), 17.75 (s), 17.69 (s); HRMS (ESI): m/z calcd. $C_{20}H_{24}NaO_7$ [M + Na]$^+$: 399.1420, found 399.1440.

3.2.4 QA-7

QA-6 (3.76 g, 10 mmol) was dissolved in 50 ml THF and 10 ml of water.AD-mix-a (1g, equiv: 0.1g/mmol) was added while vigorously stirring. After stirred for 7 h at RT, the reaction was quenched with saturated sodium thiosulfate solution, and extraction with ethyl acetate, washed with saturated sodium chloride, dried (Na_2SO_4), and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate,1:1) to give QA-7 (2.66g, 65%). White powder; M p 190-192 ℃; $[\alpha]^{20}$ D = +32 (c = 0.6,$CHCl_3$); IR (KBr) cm^{-1} 3314, 2933, 1715, 1267, 978; ^{1}HNMR (600 MHz, $CDCl_3$) δ7.99 – 7.97(m, 2H), 7.62(m, 1H), 7.47 (m, 2H), 5.00 – 4.99 (m, 1H), 4.05 – 4.00(m, 1H), 3.94 – 3.90 (m, 1H), 3.85 (d, *J* = 10.4 Hz, 1H), 3.51 – 3.48 (m, 1H), 3.35 (s, 3H), 3.32 (s, 3H), 2.96 (dd, *J* = 18.7, 5.9 Hz, 1H), 2.74 (dd, *J* = 18.6, 11.9 Hz, 1H), 1.41 (s, 3H), 1.35 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 196.13 (s), 164.92(s), 133.35 (s), 129.78 (s), 129.42 (s), 128.48 (s), 99.61 (s), 99.43 (s), 82.02 (s), 79.40 (s), 71.97 (s), 60.54 (s), 48.49 (s), 48.43 (s), 41.44 (s) , 17.52 (s), 17.45 (s); HRMS (ESI): m/z calcd. $C_{20}H_{26}NaO_9$ [M + Na]$^+$: 433.1475, found 433.1499

3.2.5 QA-8

QA-7 (2.0 g, 5 mmol) was dissolved in 20 ml DCM at 0℃ under N_2.Triphosgene (2.2 g, 7.5mmol) dissolved in 10 ml DCM was added dropwise via syringe over 10 min, followed by pyridine (0.4 mL, 5mmol). After stirred for 1 h at RT, the reaction was quenched with saturated sodium bicarbonate solution, and extraction with DCM, washed with saturated sodium chloride, dried (Na_2SO4), and concentrated under reduced pressure. QA-8 (2.0g, 93%) was obtained by silica gel column chromatography (petroleum ether: ethyl acetate, 3:1). White powder; M p 188-189 ℃; $[\alpha]^{20}$ D = -18 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 2963, 1720, 1286, 978, 783; ^{1}HNMR (600 MHz, $CDCl_3$) δ 8.01 – 8.00 (m, 2H), 7.65(m, 1H), 7.49 (m, 2H), 4.91 (d, *J* = 8.4 Hz, 1H), 4.67 (d, *J* = 12.3 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.08 (td, *J* = 11.2, 6.6 Hz, 1H), 3.94 (dd, *J* = 11.1, 8.5 Hz, 1H), 3.34 (s, 3H), 3.28 (s, 3H), 3.02 (dd, *J* = 18.6, 6.5 Hz, 1H), 2.78 (dd, *J* = 18.6, 11.3 Hz, 1H), 1.38 (s, 3H), 1.34 (s, 3H);^{13}CNMR (150 MHz, $CDCl_3$) δ 196.85 (s), 168.61 (s), 167.52(s), 134.11 (s), 129.88 (s), 128.78 (s), 128.17 (s), 99.57 (s), 99.44 (s), 83.02 (s), 78.33 (s), 72.09 (s), 64.47 (s), 60.48 (s), 48.47 (s), 41.25 (s), 17.50 (s), 17.43 (s); HRMS (ESI): m/z calcd. $C_{21}H_{24}NaO_{10}$ [M + Na]+: 459.1267, found 459.1292.

3.2.6 QA-13 and QA-14

 doi:10.20944/preprints201904.0262.v1

QA-8 (2 g, 5 mmol) was dissolved in 20 ml DCM, and 3 ml TFA:H_2O (6:1) mixture was added. After stirred for 2 h at RT, the reaction was quenched with saturated sodium bicarbonate solution, and extraction with DCM, washed with saturated sodium chloride, dried (Na_2SO4), and concentrated under reduced pressure. QA-13 (858 mg, 58%) was obtained by silica gel column chromatography (DCM: MeOH, 20:1). QA-14 (450 mg, 24%) was obtained by silica gel column chromatography (DCM: MeOH , 10:1)

QA-13 (800 mg, 2.7 mmol) was dispersed in 10 ml benzene in a sealed tube. DBU (228 μL, 1.5 mmol) was added and the reaction was refluxed for 12 hours at 100 ℃. The reaction was concentrated under reduced pressure. QA-14 (480 mg, 65%) was obtained by silica gel column chromatography (DCM: MeOH , 10:1)

QA-13: Mp 178-179 ℃, white solid; $[\alpha]^{20}$ D = -33.8 (c = 0.2, MeOH); IR (KBr) cm^{-1} 3312, 2975, 1714, 1265, 981, 775; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.6 Hz, 2H), 7.54 (s, 1H), 7.40 (t, *J* = 7.6 Hz, 2H), 4.93 (s, 1H), 4.70 (d, *J* = 12.3 Hz, 1H), 4.64 (d, *J* = 12.6 Hz, 1H), 4.46 (s, 1H), 4.25 (s, 1H), 3.98 (s, 1H), 3.13 – 3.10 (m, 1H), 2.77 – 2.74 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.24 (s), 165.92 (s), 133.96 (s), 129.82 (s), 128.73 (s), 128.39 (s), 82.27 (s), 82.02 (s), 68.64 (s), 67.92 (s), 64.38 (s), 41.51 (s); HRMS (ESI): m/z calcd. $C_{14}H_{16}NaO_7$ [M + Na]+: 319.0794, found 319.0803

QA-14: Mp 145-146℃; white solid; $[\alpha]^{20}$ D = 21.9 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 3309, 2955, 1720, 1286, 978, 783; ^{1}HNMR (600 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.3 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 6.92 (dd, *J* = 10.2, 2.5 Hz, 1H), 6.17 (d, *J* = 10.2 Hz, 1H), 4.79 (d, *J* = 11.3 Hz, 1H), 4.70 (s, 1H), 4.59 (d, *J* = 11.3 Hz, 1H), 3.97 (d, *J* = 4.7 Hz, 1H), 3.63 (s, 1H), 3.48 – 3.42 (m, 1H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 195.33 (s), 166.94 (s), 147.88 (s), 133.68 (s), 129.91 (s), 129.01 (s), 128.56 (s), 126.96 (s), 74.08 (s), 68.00 (s), 64.12 (s); HRMS (ESI): *m/z* calcd. $C_{14}H_{14}NaO_6$ [M + Na]$^+$: 301.0688, found 301.0703

3.2.7. QA-15

QA-14 (900 mg, 3.2 mmol) was dissolved in 10 ml acetone, *p*-toluene sulfonic acid monohydrate (55 mg, 0.3 mmol), 2,2-dimethoxypropane (620 mL) was added successively. After stirred at room temperature for 2 hours, the reaction was concentrated. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 3:1) to give QA-15 (890 mg, 88%). white solid; M p 154-155℃; $[\alpha]^{20}$ D = -14.5 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 3311, 2975, 1698, 1640, 1186, 973, 778; ^{1}HNMR (600 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.7 Hz, 2H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.00 (dd, *J* = 10.0, 4.6 Hz, 1H), 6.26 (d, *J* = 10.2 Hz, 1H), 4.71 (d, *J* = 11.6 Hz, 2H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.54 (s, 1H), 2.68 (s, 1H), 1.47 (s, 4H), 1.39 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 195.95 (s), 166.11 (s), 145.71 (s), 133.69 (s), 129.73 (s), 128.69 (s), 128.63 (s), 109.61 (s), 80.36 (s), 79.87 (s), 64.67 (s), 64.54 (s), 27.42 (s), 26.34 (s); HRMS (ESI): *m/z* calcd. $C_{17}H_{18}NaO_6$ [M + Na]$^+$: 341.1001, found 341.1015.

3.2.8. QA-16

QA-15 (850 mg, 2.7 mmol) was dissolved in 5 ml DCM, then triethylamine (0.6 ml, 4 mmol), DMAP (25 mg, 0.2 mmol), benzoyl chloride (360 μ L, 3mmol)) were added successively. After stirred for 2 h at RT, the reaction was quenched with saturated sodium bicarbonate solution, and extraction with DCM, washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 4:1) to give QA-16 (1.0g, 95%). White powder; M p 147-148℃; $[\alpha]^{20}$ D = +20.4 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 2936, 1728, 1671, 1296, 972, 785; ^{1}HNMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.9 Hz, 2H),

7.88 (d, *J* = 7.9 Hz, 2H), 7.53 (ddd, *J* = 10.5, 9.9, 4.0 Hz, 2H), 7.39 (dd, *J* = 11.0, 4.3 Hz, 2H), 7.31 (d, *J* = 7.5 Hz, 2H), 7.09 (ddd, *J* = 10.0, 4.8, 1.7 Hz, 1H), 6.41 (d, *J* = 10.2 Hz, 1H), 6.00 (d, *J* = 4.8 Hz, 1H), 4.72 (m, 2H), 4.62 (d, *J* = 12.0 Hz, 1H), 1.51 (s, 3H), 1.44 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 195.61 (s), 165.67 (s), 165.25 (s), 141.49 (s), 133.68 (s), 133.41 (s), 130.68 (s), 129.77 (s), 129.72 (s), 129.14 (s), 128.50 (s), 128.48 (s), 110.07 (s), 80.02 (s), 77.70(s), 65.99 (s), 63.91 (s), 27.41 (s), 26.42 (s); HRMS (ESI): *m/z* calcd. $C_{24}H_{22}NaO_7$ $[M + Na]^+$: 445.1263, found 445.1291

3.2.8. (+)-zeylenone

To 5 ml methanol of QA-16 (1.0 g, 2.3 mmol) solution was added 2 ml 1N dilute hydrochloric acid. After stirred for 2 h at RT, the reaction was concentrated under reduced pressure as crude products. (+)-zeylenone (792 mg, 90%) was obtained by silica gel column chromatography purification (petroleum ether: ethyl acetate, 2:1). white powder; M p 147-148℃; $[\alpha]^{20}$ D = +26.1 (c = 0.2,MeOH); IR (KBr) cm^{-1} 3328, 2933, 1720, 1685, 978, 783; ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.03 – 8.01 (m, 2H), 7.94 – 7.93 (m, 2H), 7.56 (dt, *J* = 10.2, 7.5 Hz, 2H), 7.42 (dt, *J* = 16.0, 7.9 Hz, 4H), 6.97 (ddd, *J* = 10.2, 4.1, 1.2 Hz, 1H), 6.35 (d, *J* = 10.2 Hz, 1H), 5.96 (t, *J* = 3.7 Hz, 1H), 4.85 (d, *J* = 11.5 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.38 (s, 1H), 4.09 (s, OH), 3.20 (d, *J* = 2.4 Hz, OH); ^{13}CNMR (125 MHz, $CDCl_3$) δ 196.42 (s), 166.37 (s), 165.48 (s), 142.85 (s), 133.98 (s), 133.63 (s), 129.97 (s), 129.90 (s), 128.84 (s), 128.73 (s), 128.63 (s), 71.80 (s), 69.31 (s), 65.61 (s); HRMS (ESI): m/z calcd. $C_{21}H_{18}NaO_7$ $[M + Na]^+$: 405.0950, found 405.0972. CD spectrum seen in **Appendix** are in agreement with reported values[10].

3.2.9. (-)-zeylenone

Isolated from *Uvaria grandiflora Roxb* in Hainan Province. M p 147-148℃, white solid; $[\alpha]^{20}$ D = -26.1 (c = 0.2,MeOH); IR (KBr) cm^{-1} 3324, 2968, 1721, 1670, 975, 787; ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.08 – 8.00 (m, 2H), 8.00 – 7.88 (m, 2H), 7.65 – 7.52 (m, 2H), 7.42 (dt, *J* = 15.9, 7.9 Hz, 4H), 6.97 (ddd, *J* = 10.2, 4.1, 1.3 Hz, 1H), 6.34 (d, *J* = 10.2 Hz, 1H), 5.96 (t, *J* = 3.6 Hz, 1H), 4.85 (d, *J* = 11.5 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.38 (s, 1H), 4.10 (s, OH), 3.20 (d, *J* = 2.4 Hz, OH); ^{13}CNMR (125 MHz, $CDCl_3$) δ 196.42 (s), 166.37 (s), 165.49 (s), 142.85 (s), 133.97 (s), 133.63 (s), 129.97 (s), 129.90 (s), 129.24 (s), 128.88 (s), 128.84 (s), 128.73 (s), 128.63 (s), 71.80 (s), 69.32 (s), 65.60 (s); HRMS (ESI): m/z calcd. $C_{21}H_{18}NaO_7$ $[M + Na]^+$: 405.0950, found 405.0969.CD spectrum seen in **Appendix** are in agreement with reported values[11].

3.2.9. zey-1

The isolated (-)- zeylenone (1 g, 3.3 mmol) was dissolved in 20 ml methanol. 10 ml ammonia water was added, and the reaction was stirred for 4 hours at 80℃. The reaction was concentrated under reduced pressure, zey-1 (410 mg, 45%) was obtained by silica gel column chromatography (DCM: MeOH , 10:1); white solid; M p 145-146 ℃; $[\alpha]^{20}$ D = -22 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 3308, 2954, 1725, 977, 785; ^{1}HNMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.5 Hz, 1H), 7.57 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 6.92 (dd, *J* = 10.2, 2.5 Hz, 1H), 6.16 (d, *J* = 10.2 Hz, 1H), 4.79 (d, *J* = 10.8 Hz, 1H), 4.69 (s, 1H), 4.58 (d, *J* = 11.3 Hz, 1H), 3.96 (d, *J* = 4.7 Hz, 1H), 3.62 (s, 1H), 3.47 (s, 1H); ^{13}CNMR (150 HNz, $CDCl_3$) δ 195.47 (s), 167.08 (s), 148.02 (s), 133.82 (s), 130.05 (s), 129.15 (s), 128.70 (s), 127.10 (s), 76.43 (s), 74.22 (s), 68.14 (s), 64.26 (s); HRMS (ESI): m/z calcd. $C_{14}H_{14}NaO_6$ $[M + Na]^+$: 301.0688, found 301.0719.

3.2.10. zey-2

 doi:10.20944/preprints201904.0262.v1

Zey-2 was synthesized by the same method as QA-15. white solid; M p 154-155℃; $[\alpha]^{20}$ D = 14.5 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 3308, 2963, 1720, 1655, 1186, 978, 783; ^{1}HNMR (600 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 23.4, 7.7 Hz, 2H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 2H), 6.98 (dd, *J* = 10.0, 4.6 Hz, 1H), 6.23 (d, *J* = 10.2 Hz, 1H), 4.68 (d, *J* = 11.6 Hz, 2H), 4.56 (d, *J* = 11.2 Hz, 1H), 4.51 (s, 1H), 1.45 (s, 3H), 1.36 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 196.10 (s), 166.25 (s), 145.86 (s), 133.84 (s), 129.87 (s), 128.83 (s), 128.77 (s), 109.76 (s), 80.51 (s), 80.02 (s), 64.82 (s), 64.69 (s), 27.57 (s), 26.49 (s); HRMS (ESI): m/z calcd. $C_{17}H_{18}NaO_6$ $[M + Na]^+$: 301.1001, found 341.1007.

3.2.10. zey-3

Zey-3 was synthesized by the same method as QA-**16.** white solid; M p 147-148℃; $[\alpha]^{20}$ D = -20.4 (c = 0.2,$CHCl_3$); IR (KBr) cm^{-1} 2933, 1720, 1665, 1286, 978, 783; ^{1}HNMR (600 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.9 Hz, 2H), 7.84 (d, *J* = 7.9 Hz, 2H), 7.51 (dd, *J* = 6.7, 5.5 Hz, 2H), 7.35 (m, 2H), 7.28 (d, *J* = 7.5 Hz, 2H), 7.06 (m, 1H), 6.38(d, *J* = 10.2 Hz, 1H), 5.97(d, *J* = 4.7 Hz, 1H), 4.70 (d, *J* = 11.6 Hz, 1H), 4.68(s, 1H), 4.62 (d, *J* = 11.7 Hz, 1H), 1.48 (s, 3H), 1.40 (s, 3H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 195.61 (s), 165.67 (s), 165.25 (s), 141.49 (s), 133.68 (s), 133.41 (s), 130.68 (s), 129.77 (s), 129.72 (s), 129.14 (s), 128.50 (s), 128.48 (s), 110.07 (s), 80.02 (s), 77.70 (s), 65.99 (s), 63.91 (s), 27.41 (s), 26.42 (s); HRMS (ESI): m/z calcd. $C_{24}H_{22}NaO_7$ $[M + Na]^+$: 445.1263, found 445.1266.

3.2.10.synthesis of series A

Series A compounds were synthesized from Zey-2.

(1R, 2R, 3S)-1,2-propylacetal-3-*p*-fluorobenzoyl-zeylenone(A1): Zey-2 (50mg, 0.15mmol), DMAP (2.0mg, 0.015mmol) DCC (61mg, 0.3mmol) were dissolved in 1ml DCM, and *p*-fluorobenzoic acid (280mg, 0.2mmol) was added to the reaction. After the reaction was stirred for 12 h at room temperature, the solvent was dried and added 1 mL ether. The mixture was filtered and concentrated to get the crude product. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 4:1) to give A1 (60 mg, 90%).White powder; $[\alpha]^{20}$ D =13.1 (c = 0.3, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, CDCl3): δ (ppm)= 7.93 (dd, *J* = 8.2, 1.2 Hz, 2H, Ar-H), 7.88-7.82 (m, 2H, Ar-H), 7.56-7.50 (m, 1H, Ar-H), 7.37 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.04 (ddd, *J* = 10.2, 4.9, 1.8 Hz, 1H, H-4), 6.98-6.88 (m, 2H, Ar-H), 6.39 (d, *J* = 10.2 Hz, 1H, H-5), 5.94 (dd, *J* = 4.9, 1.2 Hz, 1H, H-3), 4.69-4.62 (m, 3H, H-2, H-7a, 7b), 1.48 (s, 3H, Me), 1.41 (s, 3H, Me). ^{13}CNMR (150 MHz, CDCl3): δ (ppm)= 195.7 (s, C-1), 166.8 (d, $J_{C\text{-}F}$ = 253.9.Hz, C-F), 166.4(s, C=O), 164.6 (s, C=O), 141.5 (s, C-4), 133.7 (s, Ar-C), 132.6 (s, Ar-C), 132.6 (s, Ar-C), 130.9 (s, C-5), 129.8 (s, 2×Ar-C)), 129.2 (s, Ar-C), 128.7 (s, 2×Ar-C), 124.9 (s, Ar-C), 115.9 (s, Ar-C), 115.8 (s, Ar-C), 110.2 (s, $CO_2(Me)_2$), 80.1 (s, C-2), 77.8 (s, C-1), 66.3 (s, C-3), 64.1 (s, C-7), 27.6 (s, Me), 26.6 (s, Me); HRMS (ESI): m/z calcd. $C_{24}H_{21}FNaO_7$ $[M + Na]^+$: 463.1169, found 463.1195.

(1R, 2R, 3S)-1,2-propylacetal-3-*p*-methoxybenzoyl-zeylenone(A2)(65 mg, 92%) was synthesized by the same method as A1, except changed *p*-fluorobenzoic acid to *p*-methoxybenzoic acid.White powder; $[\alpha]^{20}$ D =-16.8 (c = 0.3, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, $CDCl_3$): δ (ppm)= 7.95 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.80 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.53 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.37 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.05 (ddd, *J* = 10.2, 4.9, 1.8 Hz, 1H, H-4), 6.74 (d, *J* = 8.9 Hz, 2H, Ar-H), 6.37 (d, *J* = 10.2 Hz, 1H, H-5), 5.94 (dd, *J* = 4.9, 1.2 Hz, 1H, H-3), 4.69 (d, *J* = 11.8 Hz, 1H, H-7a), 4.67 (t, *J* = 1.7 Hz, 1H, H-7b), 4.62 (d, *J* = 11.8 Hz, 1H, H-2), 3.81 (s, 3H, OMe), 1.48 (s, 3H, Me), 1.41 (s, 3H, Me); ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm)= 195.8 (s, C-1), 165.9 (s, C=O), 165.1 (s, C=O), 164.0 (s, C=C(OMe)), 142.0 (s, C-4), 133.6 (s, Ar-C), 132.1 (s, 2 × Ar-C), 132.1 (s, 2 × Ar-C), 130.7 (s, C-5), 129.9(s, 2 × Ar-C), 129.3 (s, Ar-C), 128.6 (s, 2 × Ar-C), 120.9 (s, Ar-C), 113.9 (s, 2 × Ar-C), 110.1 (s, $CO_2(Me)_2$), 80.2 (s, C-2), 77.8 (s, C-1), 65.8 (s, C-3), 64.0 (s, C-7), 55.6 (s, OMe), 27.6 (s, Me), 26.6 (s, Me); HRMS (ESI): m/z calcd. $C_{25}H_{24}NaO_8$ $[M + Na]^+$: 475.1369, found 475.1390.

 doi:10.20944/preprints201904.0262.v1

(1R, 2R, 3S)-1,2-propylacetal-3-*p*-methylbenzoyl-zeylenone(A3)(55 mg, 85%) was synthesized by the same method as A1, except changed *p*-fluorobenzoic acid to *p*-methyl benzoic acid.White powder; $[\alpha]^{20}$ D =-25.9 (c = 0.3, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, CDCl3): δ (ppm)= 7.95 (dd, *J* = 8.3, 1.1 Hz, 2H, Ar-H), 7.73 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.52 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.36 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.07 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.05-7.03 (m, 1H, H-4), 6.37 (d, *J* = 10.2 Hz, 1H, H-5), 5.95 (dd, *J* = 4.8, 1.2 Hz, 1H, H-3), 4.70 (d, *J* = 11.8 Hz, 1H, H-7a), 4.68 (t, *J* = 1.7 Hz, 1H, H-7b), 4.61(d, *J* = 11.8 Hz, 1H, H-2), 2.35(s, 3H, Me-Ph), 1.48(s, 3H, Me), 1.41(s, 3H, Me); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 195.8(s, C-6), 165.8(s, C=O), 165.4(s, C=O), 144.7(s, C-4), 141.8(s, C=CMe), 133.5(s, Ar-C), 130.7(s, C-5), 130.0(s, 2×Ar-C), 129.9(s, 2×Ar-C), 129.4(s, 2×Ar-C), 129.3(s, Ar-C), 128.6(s, 2×Ar-C), 125.9(s, Ar-C), 110.2(s, $CO_2(Me)_2$), 80.2 (s, C-2), 77.8(s, C-1), 65.9(s, C-3), 64.0(s, C-7), 27.6 (s, Me), 26.6(s, Me), 21.9(s, Ar-Me); HRMS(ESI): m/z calcd.$C_{22}H_{20}NaO_8$[M + Na]$^+$: 435.1056,found 435.1080.

(1R, 2R, 3S)-1,2-propylacetal-3-(cyclohexanecarbonyl)-zeylenone(A4)(58 mg, 87%) was synthesized by the same method as A1, except changed *p*-fluorobenzoic acid to cyclohexanecarboxylic acid. White powder; $[\alpha]^{20}$ D =+22.5 (c = 0.2, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, $CDCl_3$) : δ (ppm)= 7.99 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-H), 7.58 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.44 (t, *J* = 7.8 Hz, 2H, Ar-H), 6.82 (ddd, *J* = 10.2, 4.2, 1.4 Hz, 1H, H-4), 6.28 (dd, *J* = 10.2, 0.8 Hz, 1H, H-5), 5.69 (dd, *J* = 5.3, 2.0 Hz, 1H, H-3), 4.71 (d, *J* = 11.6 Hz, 1H, H-7a), 4.52 (d, *J* = 11.6 Hz, 1H, H-7b), 4.18 (dd, *J* = 3.2, 1.4 Hz, 1H, H-2), 2.02-2.01 (m, 1H, $CHCO(CH_2)_2$), 1.70-1.67 (m, 2H, H of cyclohexyl), 1.67-1.60 (m, 2H, H of cyclohexyl), 1.54(s, 3H, Me), 1.45(s, 3H, Me), 1.28-1.24 (m, 3H, H of cyclohexyl), 1.07-1.05 (m, 3H, H of cyclohexyl); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 195.9 (s, C-1), 174.8 (s, C=O), 165.7 (s, C=O), 141.8 (s, C-4), 133.6 (s, Ar-C), 130.7 (s, C-5), 129.9 (s, 2×Ar-C), 129.4 (s, Ar-C), 128.7 (s, 2×Ar-C), 110.1(s, $CO_2(Me)_2$), 80.0 (s, C-2), 77.9 (s, C-1), 64.9 (s, C-3), 64.0 (s, C-7), 42.8 (s, C of cyclohexyl), 29.0 (s, C of cyclohexyl), 28.9 (s, C of cyclohexyl), 27.5(s, Me), 26.5(s, Me), 25.6 (s, C of cyclohexyl), 25.3 (s, C of cyclohexyl), 25.3 (s, C of cyclohexyl); HRMS (ESI): m/z calcd. $C_{24}H_{28}NaO_7$ [M + Na]$^+$: 451.1733, found 451.1731.

(1R, 2R, 3S)-1,2-propylacetal-3-(phenylpropanecarbonyl)-zeylenone(A5)(60 mg, 85%) was synthesized by the same method as A1, except changed *p*-fluorobenzoic acid to hydrocinnamic acid. White powder; $[\alpha]^{20}$ D =-13.1 (c = 0.3, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, $CDCl_3$): δ (ppm)= 7.97 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-H), 7.57-7.52 (m, 1H, Ar-H), 7.40 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.3 Hz, 2H, Ar-H), 7.21-7.18 (m, 1H, Ar-H), 7.02 (d, *J* = 7.1 Hz, 2H, Ar-H), 6.82 (ddd, *J* = 10.2, 5.0, 1.8 Hz, 1H, H-4), 6.30 (d, *J* = 10.3 Hz, 1H, H-5), 5.68 (dd, *J* = 5.0, 1.4 Hz, 1H, H-3), 4.60 (d, *J* = 11.5 Hz, 1H, H-7a), 4.51 (d, *J* = 11.5 Hz, 1H, H-7b), 4.34 (t, *J* = 1.8 Hz, 1H, H-2), 2.80 (t, *J* = 7.7 Hz, 2H, CH_2CH_2-Ph), 2.42 (dt, *J* = 15.7, 7.8 Hz, 1H, CH_2CH_2-Ph), 2.35-2.32 (m, 1H, CH_2CH_2-Ph), 1.44 (s, 3H, Me), 1.36 (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm)= 196.0 (s, C-1), 171.7 (s, C=O), 166.1 (s, C=O), 142.9 (s, C-4), 142.9 (s, Ar-C), 139.8 (s, Ar-C), 133.6(s, 2×Ar-C), 129.8 (s, Ar-C), 129.2 (s, 2×Ar-C)), 128.6 (s, 2×Ar-C), 128.6 (s, 2×Ar-C), 128.3 (s, C-5), 126.5 (s, Ar-C), 109.9(s, $CO_2(Me)_2$), 80.1 (s, C-2), 78.1 (s, C-1), 65.1 (s, C-3), 64.1 (s, C-7), 35.6 (s, CH_2), 30.9(s, CH_2), 27.5(s, Me), 26.5(s, Me); HRMS (ESI): m/z calcd. $C_{26}H_{26}NaO_7$ [M + Na]$^+$: 473.1576, found 473.1604.

(1R, 2R, 3S)-1,2-propylacetal-3-(phenethylcarbonyl)-zeylenone(A6)(55 mg, 85%) was synthesized by the same method as A1, except changed p-fluorobenzoic acid to phenylacetic acid.White powder; $[\alpha]^{20}$ D =-22.5 (c = 0.5, $CHCl_3$); ^{1}H-NMR (600 MHz, 298K, CDCl3): δ (ppm)= 8.01 (dd, *J* = 8.2, 1.1 Hz, 2H, Ar-H), 7.60 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.46 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.21-7.20 (m, 2H, Ar-H), 7.08 (dd, *J* = 7.4, 1.7 Hz, 2H, Ar-H), 6.90 (ddd, *J* = 10.2, 5.0, 1.8 Hz, 1H, H-4), 6.33 (d, *J* = 10.2 Hz, 1H, H-5), 5.70 (dd, *J* = 5.0, 1.6 Hz, 1H, H-3), 4.68 (d, *J* = 11.7 Hz, 1H, H-7a), 4.47 (t, *J* = 1.8

Hz, 1H, H-2), 4.44 (d, *J* = 11.7 Hz, 1H, H-7b), 3.41 (dd, *J* = 37.7, 15.4 Hz, 2H, CH_2-Ph), 1.43 (s, 3H, Me), 1.35 (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm)= 195.7 (s, C-6), 170.4 (s, C=O), 165.7 (s, C=O), 141.3 (s, C-4), 133.7 (s, Ar-C), 133.0 (s, Ar-C), 131.0 (s, C-5), 130.0 (s, 2 × Ar-C), 129.5 (s, Ar-C), 129.3(s, 2 × Ar-C), 128.8 (s, 2 × Ar-C), 128.8 (s, 2 × Ar-C), 127.6 (s, Ar-C), 110.2 (s, $CO_2(Me)_2$), 80.0 (s, C-2), 77.5 (s, C-1), 65.7 (s, C-3), 63.7 (s, C-7), 40.8 (s, CH_2(CO)Ar), 27.5 (s, Me), 26.5 (s, Me); HRMS (ESI): m/z calcd. $C_{22}H_{20}NaO_7$ $[M + Na]^+$: 459.1420, found 459.1446.

3.2.11.synthesis of series B

Series B compounds were synthesized from Series A using the same method. To a solution of Series A in 1 m THF, added 1N dilute hydrochloric acid 1ml, and stirred for 1h. The mixture was concentrated to get the crude product. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 2:1) to give Series B.

(1R, 2R, 3S)-3-*p*-fluorobenzoyl-zeylenone(B1):White solid; $[\alpha]^{20}$ D =18.0 (c = 0.3, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, $CDCl_3$): δ (ppm)= 8.03-8.01 (m, 2H, Ar-H), 7.93-7.92 (m, 2H, Ar-H), 7.56 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.41 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.08 (t, *J* = 8.6 Hz, 2H, Ar-H), 6.95 (ddd, *J* = 10.2, 4.1, 1.2 Hz, 1H, H-4), 6.34 (d, *J* = 10.2 Hz, 1H, H-5), 5.94 (m, 1H, H-3), 4.80 (d, *J* = 11.5 Hz, 1H, H-7a), 4.59 (d, *J* = 11.5 Hz, 1H, H-7b), 4.37 (s, 1H, H-2), 4.10 (s, OH), 3.24 (s, OH); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 196.3 (s, C-1), 166.8 (d, $J_{C\text{-}F}$ = 253.5.Hz, C-F), 166.4(s, C=O), 164.6 (s, C=O), 142.8 (s, C-4), 133.7 (s, Ar-C), 132.6 (s, Ar-C), 132.6 (s, Ar-C), 129.9 (s, 2×Ar-C)), 129.2 (s, Ar-C), 128.8 (s, C-5), 128.6 (s, 2 × Ar-C), 125.1 (s, Ar-C), 116.1 (s, Ar-C), 116.0 (s, Ar-C), 77.4 (s, C-2), 71.7 (s, C-1), 69.5 (s, C-3), 65.5 (s, C-7); HRMS m/z calcd. $C_{21}H_{17}FNaO_7$ $[M + Na]^+$: 423.0857, found 423.0856

(1R, 2R, 3S)-3-*p*-methoxybenzoyl-zeylenone(B2): White solid; $[\alpha]^{20}$ D =-22.9 (c = 0.5, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, $CDCl_3$): δ (ppm)= 7.96 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.94 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-H), 7.55 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.41 (dd, *J* = 8.1, 7.6 Hz, 2H, Ar-H), 6.96 (ddd, *J* = 10.2, 4.2, 1.5 Hz, 1H, H-4), 6.89 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.33 (dd, *J* = 10.2, 0.9 Hz, 1H, H-5), 5.95-5.90 (m, 1H, H-3), 4.84 (d, *J* = 11.5 Hz, 1H, H-7a), 4.59 (d, *J* = 11.5 Hz, 1H, H-7b), 4.36 (dd, *J* = 3.3, 1.5 Hz, 1H, H-2), 4.11 (s, OH), 3.85 (s, 3H, OMe), 3.18 (s, OH); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 196.6 (s, C-6), 166.3 (s, C=O), 165.2 (s, C=O), 164.1 (s, C=C(OMe)), 143.1 (s, C-4), 139.9 (s, Ar-C), 133.6 (s, Ar-C), 132.1 (s, 2×Ar-C), 129.9(s, 2×Ar-C), 129.3 (s, Ar-C), 128.6 (s, 2×Ar-C), 128.6 (s, C-5), 121.1 (s, Ar-C), 114.1 (s, 2×Ar-C), 77.4 (s, C-2), 71.8 (s, C-1), 69.0 (s, C-3), 65.7 (s, C-7), 55.7 (s, OMe); HRMS (ESI): m/z calcd. $C_{22}H_{20}NaO_8$ $[M + Na]^+$: 435.1056, found 435.1080

(1R, 2R, 3S)-3-*p*-methylbenzoyl-zeylenone(B3):White solid; $[\alpha]^{20}$ D =-14.9 (c = 0.2, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, CDCl3): δ (ppm)= 7.94 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.90 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.55 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.41 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.21 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.96 (dd, *J* = 10.1, 3.9 Hz, 1H, H-4), 6.33 (d, *J* = 10.2 Hz, 1H, H-5), 5.94 (t, *J* = 3.5 Hz, 1H, H-3), 4.84 (d, *J* = 11.5 Hz, 1H, H-7a), 4.59 (d, *J* = 11.5 Hz, 1H, H-7b), 4.37 (d, *J* = 2.2 Hz, 1H, H-2), 2.39 (s, 3H, Me-Ph); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 196.5 (s, C-6), 166.3 (s, C=O), 165.6 (s, C=O), 144.9 (s, C-4), 143.0 (s, C=CMe), 133.6 (s, Ar-C), 130.0 (s, 2×Ar-C), 129.9(s, 2×Ar-C), 129.5 (s, 2×Ar-C), 129.3 (s, Ar-C), 128.7 (s, C-5), 128.6 (s, 2×Ar-C), 126.1 (s, Ar-C), 77.4 (s, C-2), 71.8 (s, C-1), 69.2 (s, C-3), 65.6 (s, C-7), 21.9 (s, Ar-Me); HRMS (ESI): m/z calcd. $C_{22}H_{20}NaO_7$ $[M + Na]^+$: 419.1107, found 419.113.

(1R, 2R, 3S)-3-(cyclohexanecarbonyl)-zeylenone(B4):White solid; $[\alpha]^{20}$ D =+33.8 (c = 0.2, $CHCl_3$); ^{1}H-NMR (600 MHz, 298K, $CDCl_3$): δ (ppm)= 7.99 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-H), 7.58 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.44 (t, *J* = 7.8 Hz, 2H, Ar-H), 6.82 (ddd, *J* = 10.2, 4.2, 1.4 Hz, 1H, H-4), 6.28 (dd, *J* = 10.2, 0.8 Hz, 1H, H-5), 5.69 (dd, *J* = 5.3, 2.0 Hz, 1H, H-3), 4.71 (d, *J* = 11.6 Hz, 1H, H-7a), 4.52 (d, *J* = 11.6 Hz, 1H, H-7b), 4.18 (dd, *J* = 3.2, 1.4 Hz, 1H, H-2), 4.05 (br s, OH), 3.07 (br s, OH), 2.39-2.30 (m, 1H, $CHCO(CH_2)_2$), 1.98-1.84 (m, 2H, H of cyclohexyl), 1.77-1.63 (m, 3H, H of cyclohexyl), 1.44 (qd, *J* = 13.0, 3.5 Hz, 2H, H of cyclohexyl), 1.24 (ddd, *J* = 24.6, 12.2, 5.8 Hz, 3H, H of cyclohexyl); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 196.5 (s, C-1), 174.9 (s, C=O), 166.3 (s, C=O), 143.0 (s, C-4), 133.7 (s, Ar-C), 129.9 (s, C-5), 129.3 (s, 2×Ar-C)), 128.7 (s, Ar-C), 128.4 (s, 2×Ar-C), 77.3 (s, C-2), 71.8 (s, C-1), 68.4 (s, C-3), 65.7 (s, C-7), 42.1 (s, C of cyclohexyl), 29.1 (s, C of cyclohexyl), 29.1 (s, C of cyclohexyl), 25.7 (s, C of cyclohexyl), 25.4 (s, 2×C of cyclohexyl); HRMS (ESI): m/z calcd. $C_{21}H_{24}NaO_7$ $[M + Na]^+$: 411.1420, found 411.1441.

(1R, 2R, 3S)-3-(phenylpropanecarbonyl)-zeylenone(B5):White solid; $[\alpha]^{20}$ D =+20.6 (c = 0.3, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, $CDCl_3$): δ (ppm)=8.03-7.95 (m, 2H, Ar-H), 7.58 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.44 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.28 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.20 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.16 (d, *J* = 7.2 Hz, 2H, Ar-H), 6.71 (dd, *J* = 10.2, 3.9 Hz, 1H, H-4), 6.23 (d, *J* = 10.2 Hz, 1H, H-5), 5.68 (dd, *J* = 5.2, 2.4 Hz, 1H, H-3), 4.61 (d, *J* = 11.5 Hz, 1H, H-7a), 4.45 (d, *J* = 11.5 Hz, 1H, H-7b), 4.08 (d, *J* = 3.6 Hz, 1H, H-2), 4.08 (s, OH), 3.18 (s, OH), 2.95 (dd, *J* = 11.7, 4.5 Hz, 2H, CH_2CH_2-Ph), 2.69 (td, *J* = 7.7, 3.6 Hz, 2H, CH_2CH_2-Ph); ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 196.2 (s, C-1), 171.9 (s, C=O), 166.3 (s, C=O), 143.0 (s, C-4), 139.9 (s, Ar-C), 133.7 (s, Ar-C), 129.9(s, 2×Ar-C), 129.3 (s, Ar-C), 128.7 (s, 2×Ar-C)), 128.7 (s, 2×Ar-C), 128.4 (s, 2×Ar-C), 128.4 (s, C-5), 126.7 (s, Ar-C), 77.2 (s, C-2), 71.7 (s, C-1), 69.0 (s, C-3), 65.2 (s, C-7), 35.8 (s, CH_2), 31.0(s, CH_2); HRMS (ESI): m/z calcd. $C_{23}H_{22}NaO_7$ $[M + Na]^+$: 433.1263, found 433.1275.

(1R, 2R, 3S)-3-(phenethylcarbonyl)-zeylenone(B6):White solid; $[\alpha]^{20}$ D =+15.0 (c = 0.5, $CHCl_3$); ^{1}HNMR (600 MHz, 298K, CDCl3): δ (ppm)= 7.98 (d, J = 7.1 Hz, 1H, Ar-H), 7.59 (t, J = 7.5 Hz, 1H, Ar-H), 7.45 (dd, J = 8.1, 7.6 Hz, 1H, Ar-H), 7.35-7.28 (m, 1H, Ar-H), 7.28-7.22 (m, 3H, Ar-H), 6.79 (ddd, J = 10.2, 4.1, 1.4 Hz, 1H, H-4), 6.26 (dd, J = 10.2, 0.9 Hz, 1H, H-5), 5.72-5.67 (m, 1H, H-3), 4.61 (d, J = 11.6 Hz, 1H, H-7a), 4.35 (d, J = 11.6 Hz, 1H, H-7b), 4.14-4.09 (m, 1H, H-2), 4.03 (s, 1H, OH), 3.73-3.63 (m, 2H, CH_2-Ph), 3.10 (s, 1H, OH). ^{13}CNMR (150 MHz, $CDCl_3$): δ (ppm)= 196.3 (s, C-6), 170.5 (s, C=O), 166.2 (s, C=O), 142.5 (s, C-4), 133.7 (s, Ar-C), 133.1 (s, Ar-C), 129.9 (s, 2×Ar-C), 129.4(s, 2×Ar-C), 129.3 (s, Ar-C), 129.0 (s, 2×Ar-C), 128.7 (s, 2×Ar-C), 128.6 (s, C-5), 127.7 (s, Ar-C), 77.3 (s, C-2), 71.7 (s, C-1), 69.1 (s, C-3), 65.4 (s, C-7), 41.3 (s, CH_2(CO)Ar).

3.2.12. synthesis of series C

C1 and C2: (+)-Zeylenone (40 mg, 0.11 mmol) was dissolved in 2 ml DCM, then triethylamine (15 μl, 0.1 mmol), DMAP (1.5 mg, 0.1 mmol), acetyl chloride (15.5μL, 0.22mmol) were added successively. After stirred for 2 h at RT, the reaction was quenched with saturated sodium bicarbonate solution, and extraction with DCM, washed with saturated sodium chloride, dried (Na_2SO_4), and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (petroleum ether: ethyl acetate, 6:1) to give C1 (11 mg, 25%), and (petroleum ether: ethyl acetate, 4:1) to give C2 (27 mg, 71%).

(1R, 2R, 3S)-2-acetyl-zeylenone(C1):White solid; $[\alpha]^{20}$ D =+33.5 (c = 0.3, $CHCl_3$); ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.01 (ddd, *J* = 17.5, 8.2, 1.1 Hz, 4H), 7.59 – 7.55 (m, 2H), 7.44 (t, *J* = 7.6 Hz, 4H), 7.02

(ddd, *J* = 10.3, 3.6, 0.9 Hz, 1H), 6.38 (dd, *J* = 10.3, 1.1 Hz, 1H), 5.95 – 5.93 (m, 1H), 5.82 (dd, *J* = 4.4, 0.8 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.71 (d, *J* = 11.4 Hz, 1H), 3.76 (s, 1H, OH), 2.09 (s, 3H); ^{13}CNMR (125 MHz, $CDCl_3$) δ 195.00 (s), 169.52 (s), 166.19 (s), 165.39 (s), 143.27 (s), 134.09 (s), 133.62 (s), 130.05 (s), 129.95 (s), 129.33 (s), 129.08 (s), 128.91 (s), 128.69 (s), 76.27 (s), 71.71 (s), 68.77 (s), 65.20 (s), 20.80(s); HRMS (ESI): m/z calcd. $C_{243}H_{20}NaO_8$ $[M + Na]^+$: 447.1056, found 447.1056

(1R, 2R, 3S)-1,2-diacetyl-zeylenone(C2):White solid; $[\alpha]^{20}$ D =+12.05 (c = 0.1, $CHCl_3$); ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.00 (ddd, *J* = 13.5, 8.3, 1.1 Hz, 4H), 7.57 (ddd, *J* = 9.7, 6.8, 2.4 Hz, 2H), 7.45 (ddd, *J* = 7.7, 3.9, 2.3 Hz, 4H), 6.87 (dd, *J* = 10.5, 2.1 Hz, 1H), 6.32 (dd, *J* = 10.5, 2.3 Hz, 1H), 6.18 (d, *J* = 8.3 Hz, 1H), 5.92 (d, *J* = 8.3 Hz, 1H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.64 (d, *J* = 11.0 Hz, 1H), 2.18 (s, 3H), 2.01 (s, 3H); ^{13}CNMR (125 MHz, $CDCl_3$) δ 190.19 (s), 169.61 (s), 166.19 (s), 165.99 (s), 165.65 (s), 143.87 (s), 133.98 (s), 133.60 (s), 130.09 (s), 129.97 (s), 129.41 (s), 129.35 (s), 128.99 (s), 128.88 (s), 128.69 (s), 80.82 (s), 71.39 (s), 70.82 (s), 61.73 (s), 20.81 (s), 20.72 (s); HRMS (ESI): m/z calcd. $C_{25}H_{22}NaO_9$ $[M + Na]^+$: 489.1162, found 489.1158.

C3 and C4 were synthesized synthesized by the same method as C1 and C2, except changed acetyl chloride to propionyl chloride.

(1R, 2R, 3S)-2-propionyl-zeylenone(C3) (10 mg, 24%) White solid; $[\alpha]^{20}$ D =+16.3 (c = 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.3 Hz, 2H), 7.96 (d, *J* = 7.3 Hz, 2H), 7.56 (dd, *J* = 17.3, 7.5 Hz, 2H), 7.41 (td, *J* = 7.8, 3.7 Hz, 4H), 7.00 (dd, *J* = 10.2, 3.7 Hz, 1H), 6.37 (d, *J* = 10.2 Hz, 1H), 5.90 (t, *J* = 3.9 Hz, 1H), 5.81 (d, *J* = 4.2 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 3.59 (s, 1H, OH), 2.34 (dd, *J* = 7.4, 6.4 Hz, 2H), 1.08 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.12 (s), 172.93 (s), 166.14 (s), 165.30 (s), 143.08 (s), 134.04 (s), 133.57 (s), 130.01 (s), 129.89 (s), 129.06 (s), 128.85 (s), 128.65 (s), 76.31 (s), 71.45 (s), 68.66 (s), 65.40 (s), 27.52 (s), 9.14 (s); HRMS (ESI): m/z calcd. $C_{24}H_{22}NaO_8$ $[M + Na]^+$: 461.1212, found 461.1156.

(1R, 2R, 3S)-1,2-dipropionyl-zeylenone(C4) (30 mg, 71%): White solid; $[\alpha]^{20}$ D =-18.8 (c = 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (ddd, *J* = 15.8, 8.3, 1.2 Hz, 4H), 7.58 – 7.56 (m, 2H), 7.44 (ddd, *J* = 9.4, 5.9, 2.0 Hz, 4H), 6.88 (dd, *J* = 10.5, 2.0 Hz, 1H), 6.33 (dd, *J* = 10.5, 2.3 Hz, 1H), 6.19 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.95 (d, *J* = 8.4 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.60 (d, *J* = 10.9 Hz, 1H), 2.46 (q, *J* = 7.5 Hz, 2H), 2.30 – 2.25 (m, 2H), 1.17 (t, *J* = 7.5 Hz, 3H), 0.96 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.31 (s), 173.10 (s), 173.06 (s), 165.94 (s), 165.58 (s), 143.97 (s), 133.97 (s), 133.53 (s), 130.04 (s), 129.91 (s), 129.38 (s), 129.06 (s), 128.95 (s), 128.63 (s), 80.47 (s), 71.12 (s), 70.83 (s), 61.96 (s), 27.43 (s), 27.32 (s), 9.12 (s), 9.07 (s); HRMS (ESI): m/z calcd. $C_{27}H_{26}NaO_9$ $[M + Na]^+$: 517.1475, found 517.1472.

C5 and C6 were synthesized synthesized by the same method as C1 and C2, except changed acetyl chloride to benzoyl chloride.

(1R, 2R, 3S)-2-benzoyl-zeylenone(C5) (12 mg, 20%) White solid; $[\alpha]^{20}$ D =-24.5 (c = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.3 Hz, 2H), 7.98 (d, *J* = 7.5 Hz, 4H), 7.60 – 7.56 (m, 3H), 7.45 – 7.40 (m, 6H), 7.09 (ddd, *J* = 10.3, 3.4, 1.8 Hz, 1H), 6.49 (d, *J* = 10.3 Hz, 1H), 6.05 (d, *J* = 3.7 Hz, 2H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.76 (d, *J* = 11.5 Hz, 1H), 3.79 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.65 (s), 166.26 (s), 165.22 (s), 165.06 (s), 142.77 (s), 134.03 (s), 133.83 (s), 133.54 (s), 130.10 (s), 130.02 (s), 129.90 (s), 129.24 (s), 129.17 (s), 128.97 (s), 128.84 (s), 128.70 (s), 128.61 (s), 76.54 (s), 72.36 (s), 68.41 (s), 66.13 (s); HRMS (ESI): m/z calcd. $C_{28}H_{22}NaO_8$ $[M + Na]^+$: 509.1212, found 509.1208.

 doi:10.20944/preprints201904.0262.v1

(1R, 2R, 3S)-1,2-dibenzoyl-zeylenone(C6) (33 mg,64%) White solid; $[\alpha]^{20}$ D =10.9 (c = 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.97 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.89 (ddd, *J* = 15.8, 8.3, 1.2 Hz, 4H), 7.64 (dd, *J* = 10.6, 4.3 Hz, 1H), 7.53 – 7.52 (m, 5H), 7.37 (dt, *J* = 24.6, 7.9 Hz, 4H), 7.29 (d, *J* = 8.1 Hz, 2H), 6.99 (dd, *J* = 10.4, 2.3 Hz, 1H), 6.46 (dd, *J* = 10.2, 7.9 Hz, 2H), 6.32 (d, *J* = 8.1 Hz, 1H), 5.05 (d, *J* = 11.0 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.26 (s), 165.97 (s), 165.56 (s), 165.20 (s), 165.12 (s), 144.27 (s), 134.27 (s), 133.80 (s), 133.39 (s), 130.34 (s), 129.99 (d, *J* = 17.8 Hz), 129.55 (s), 128.96 (s), 128.92 (s), 128.71 (s), 128.65 (s), 128.62 (s), 128.52 (s), 128.44 (s), 80.62 (s), 72.10 (s), 70.94 (s), 62.98 (s); HRMS (ESI): m/z calcd. $C_{35}H_{26}NaO_9$ $[M + Na]^+$: 613.1475, found 613.1470.

C7 and C8 were synthesized synthesized by the same method as C1 and C2, except changed acetyl chloride to cinnamoyl chloride.

(1R, 2R, 3S)-2-cinnamoyl-zeylenone(C7) (15 mg, 18%) White solid; $[\alpha]^{20}$ D =-25.8 (c = 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 18.5, 7.3 Hz, 5H), 7.71 (d, *J* = 16.0 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.50 – 7.49 (m, 2H), 7.42 (m, 8H), 7.07 (dd, *J* = 10.3, 3.7 Hz, 1H), 6.45 – 6.42 (d, *J* = 10.2 Hz, 1H), 6.42 (d, *J* = 16.0 Hz, 1H), 6.04 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.96 (d, *J* = 4.4 Hz, 1H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.76 (d, *J* = 11.4 Hz, 1H), 3.78 (s, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.07 (s), 166.11 (s), 165.25 (d, *J* = 12.5 Hz), 146.88 (s), 143.08 (s), 133.90 (d, *J* = 11.6 Hz), 133.40 (s), 130.77 (s), 129.89 (s), 129.80 (s), 128.94 (s), 128.93 (s), 128.70 (s), 128.70 (s), 128.48 (s), 128.34 (s), 128.14 (s), 116.43 (s), 76.24 (s), 71.80 (s), 68.58 (s), 65.50 (s); HRMS (ESI): m/z calcd. $C_{30}H_{42}NaO_8$ $[M + Na]^+$: 535.1369, found 535.1367.

(1R, 2R, 3S)-1,2-dicinnamoyl-zeylenone(C8) (37 mg, 68%) White solid; $[\alpha]^{20}$ D =-37.9 (c = 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 – 8.01 (m, 4H), 7.83 (d, *J* = 16.0 Hz, 1H), 7.65 (d, *J* = 16.0 Hz, 1H), 7.60 – 7.58 (m, 3H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.46 – 7.41(m, 10H), 7.33 (t, *J* = 7.3 Hz, 2H), 6.97 (dd, *J* = 10.5, 2.1 Hz, 1H), 6.58 (d, *J* = 16.0 Hz, 1H), 6.44 – 6.40 (m, 2H), 6.39 (d, *J* = 15.9 Hz, 2H), 6.18 (d, *J* = 8.3 Hz, 1H), 5.03 (d, *J* = 11.0 Hz, 1H), 4.79 (d, *J* = 11.0 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.31 (s), 165.90 (s), 165.45 (s), 165.40 (s), 165.35 (s), 147.89 (s), 146.93 (s), 143.91 (s), 133.82 (s), 133.81 (s), 133.68 (s), 133.27 (s), 131.07 (s), 130.76 (s), 129.94 (s), 129.86 (s), 129.35 (s), 129.05 (s), 128.85 (s), 128.61 (s), 128.53 (s), 128.43 (s), 128.32 (s), 116.22 (s), 115.77 (s), 80.40 (s), 71.47 (s), 70.86 (s), 62.56 (s); HRMS (ESI): m/z calcd. $C_{39}H_{22}NaO_9$ $[M + Na]^+$: 665.1788, found 665.1795.

3.3. Antitumor activity assays

Cell Lines and Cell Culture

B16 melanoma cells, human hepatocellular carcinoma HepG2 cells, and human neuroblastoma SH-SY5Y were all obtained from the Chinese Academy of Medical Sciences Basic Medicine Cell Center (Beijing, China). B16 and SH-SY5Y cells were maintained in RPMI-1640 media containing 10% FBS and 1% penicillin/streptomycin in a 37 °C humidified incubator with 5% CO2. HepG2 cells were cultured in DMEM media under the same conditions.

Cell Viability Assay

Cell viability assay was determined using Cell Counting Kit-8 (CCK8) (Dojindo) according to manufacturer's protocol. Briefly, cells cultured in 96-well plates at a density of 4.5 × 10^3 cells/well were treated with various concentrations of test compound for 24 h. Then, 10μL CCK8 reagent was added to the media and allowed to incubate for another 2.5 h at 37 ℃ in an atmosphere of 5% CO_2.

Cell viability was quantified by reading the plates at an absorbance of 450 nm using a microplate reader.Three independent experiments were carried out.

3. Conclusion

The total synthesis of (+) - zeylenone was completed in 13 steps with quinic acid as starting material in 8.8% overall yield. The highlight of the route was the control of the three carbon's chirality by clever use of single step dihydroxylation under the direction of the key C-3 chirality. The yield of each step is excellent, and the gram scale preparation can be achieved.

In order to investigate the influence of absolute configuration and hydroxyls at position C-1/2/3 on the antitumor activity, zeylenone derivatives were designed and synthetized. Their antitumor activity was evaluated against three human cancer cell lines. Structure-activity relationship suggested that both two absolute configurations exhibited good activity, and (-)-zeylenone showed a little better efficacy.Besides, hydroxyls at C-1/2 position were crucial for the activity and esterification of large groups at C-1 hydroxyl made the activity disappeared. Proper ester group of hydroxyl at C-3 position could increase the efficient.**B1** showed the highest IC_{50} value to three cell lines, which was worthy to make further study. This article makes the structure-activity relationship clear and helps to find more potent antitumor agents.

Author Contributions:

Xu Xudong is the teacher who provided financial support for this project. Tian Yu helped to analysis and write the paper. Ma Guoxu helped to isolate the (-)-zeylenone from plants. Yang Shuxian, Xu Chengfang, Cao Li, Lin Jiahao helps to finish the biological activity assay.

Acknowledgments:

We thank analysis and testing center of The Institute of Medicinal Plant Development (IMPLAD) for NMR, LC-MS and IR analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kinghorn, A. D., Review of Anticancer Agents from Natural ProductsAnticancer Agents from Natural Products, 2nd Edition. . *Journal of Natural Products* **2015,** 78, (9), 2315-2315.
2. Yong-Hong, L.; Li-Zhen, X.; Shi-Lin, Y.; Jie, D.; Yong-Su, Z.; Min, Z.; Nan-Jun, S., Three cyclohexene oxides from Uvaria grandiflora. *Phytochemistry* **1997,** 45, (4), 729-732.
3. Zhang, L.; Jin, J.; Zhang, L.; Hu, R.; Gao, L.; Huo, X.; Liu, D.; Ma, X.; Wang, C.; Han, J.; Li, L.; Sun, X.; Cao, L., Quantitative analysis of differential protein expression in cervical carcinoma cells after zeylenone treatment by stable isotope labeling with amino acids in cell culture. *Journal of proteomics* **2015,** 126, 279-87.
4. Huo, X.; Liao, Y.; Tian, Y.; Gao, L.; Cao, L., Zeylenone promotes apoptosis in chronic myelogenous leukemia-derived K562 cells by a mechanism involving Jak2 and Src kinase. *RSC Advances* **2016,** 6, (115), 114096-114108.
5. Zhang, L.; Huo, X.; Liao, Y.; Yang, F.; Gao, L.; Cao, L., Zeylenone, a naturally occurring cyclohexene oxide, inhibits proliferation and induces apoptosis in cervical carcinoma cells via PI3K/AKT/mTOR and MAPK/ERK pathways. *Scientific reports* **2017,** 7, (1), 1669.

6. Zeng, S.; Zhu, B.; Zeng, J.; Wu, W.; Jiang, C., Zeylenone represses the progress of human prostate cancer by downregulating the Wnt/betacatenin pathway. *Molecular medicine reports* **2018,** 18, (6), 5572-5578.
7. Yang, S.; Liao, Y.; Li, L.; Xu, X.; Cao, L., Zeylenone Induces Mitochondrial Apoptosis and Inhibits Migration and Invasion in Gastric Cancer. *Molecules* **2018,** 23, (9).
8. Hiroya, K.; Kurihara, Y.; Ogasawara, K., Asymmetrization ofmeso 1,4-Enediol Ethers by Isomerization with a Chiral binap–RhI Catalyst. *Angewandte Chemie International Edition in English* **1995,** 34, (20), 2287-2289.
9. Hiroya, K.; Ogasawara, K., The first enantiocontrolled synthesis of naturally occurring polyoxygenated cyclohexenylmethanol dibenzoates (–)-zeylenol, (–)-uvarigranol G, (–)-tonkinenin A and (+)-pipoxide. *Chemical Communications* **1999**, (21), 2197-2198.
10. Liu, A.; Liu, Z. Z.; Zou, Z. M.; Chen, S. Z.; Xu, L. Z.; Yang, S. L., Synthesis of (+)-zeylenone from shikimic acid. *Tetrahedron* **2004,** 60, (16), 3689-3694.
11. Zhang, Y.; Liu, A.; Ye, Z. G.; Lin, J.; Xu, L. Z.; Yang, S. L., New Approach to the Total Synthesis of (−)-Zeylenone from Shikimic Acid. *Chemical & Pharmaceutical Bulletin* **2006,** 54, (10), 1459-1461.
12. Palframan, M. J.; Kociok-Kohn, G.; Lewis, S. E., Photooxygenation of a microbial arene oxidation product and regioselective Kornblum-DeLaMare rearrangement: total synthesis of zeylenols and zeylenones. *Chemistry* **2012,** 18, (15), 4766-74.
13. Vinaykumar, A.; Muniraju, C.; Rao, B. V., Stereoselective total synthesis of (−)-zeylenol, a key intermediate for the synthesis of (+)-pipoxide, (−)-uvarigranol G and (−)-tonkinenin A. *Tetrahedron Letters* **2017,** 58, (11), 1075-1077.
14. Arthurs, C. L.; Wind, N. S.; Whitehead, R. C.; Stratford, I. J., Analogues of 2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxycyclohex-2-enone (COTC) with anti-tumor properties. *Bioorganic & medicinal chemistry letters* **2007,** 17, (2), 553-7.
15. Arthurs, C. L.; Lingley, K. F.; Piacenti, M.; Stratford, I. J.; Tatic, T.; Whitehead, R. C.; Wind, N. S., (−)-Quinic acid: a versatile precursor for the synthesis of analogues of 2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxycyclohex-2-enone (COTC) which possess anti-tumour properties. *Tetrahedron Letters* **2008,** 49, (15), 2410-2413.
16. Schuster, H.; Martinez, R.; Bruss, H.; Antonchick, A. P.; Kaiser, M.; Schurmann, M.; Waldmann, H., Synthesis of the B-seco limonoid scaffold. *Chem Commun (Camb)* **2011,** 47, (23), 6545-7.
17. Barros, M. T.; Maycock, C. D.; Ventura, M. R., Approaches to the synthesis of (+)- and (−)-epibatidine†. *Journal of the Chemical Society, Perkin Transactions 1* **2001**, (2), 166-173.
18. Li, M.; Li, Y.; Ludwik, K. A.; Sandusky, Z. M.; Lannigan, D. A.; O'Doherty, G. A., Stereoselective Synthesis and Evaluation of C6''-Substituted 5a-Carbasugar Analogues of SL0101 as Inhibitors of RSK1/2. *Organic letters* **2017,** 19, (9), 2410-2413.

Sample Availability: Samples of the compounds (+)-Zeylenone, (-)-Zeylenone, A1-A6, B1-B6, C1-C8 are available from the authors.