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# Synthesis and structure–activity relationships of dehydroaltenusin derivatives as selective DNA polymerase α inhibitors

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ABSTRACT

Herein, we describe the synthesis and structure–activity relationships of dehydroaltenusin derivatives as inhibitors of a mammalian DNA polymerase α. We have newly synthesized nine dehydroaltenusin derivatives modified at the side chains or benzoquinone moiety. We also achieved the first synthesis of desmethylaltenusin and desmethyldehydroaltenusin, metabolites of *Alternaria* sp. or *Talaromyces flavus*, respectively. Among all synthesized derivatives, demethoxydehydroaltenusin was the most selective inhibitor of DNA polymerase α. The *o*-hydroxy-*p*-benzoquinone (2-hydroxycyclohexa-2,5-dienone) moiety is essential for the inhibition of DNA polymerases. Substitution at the 5-position of dehydroaltenusin is important for the inhibitory potency. Because dehydroaltenusin is conjugated with *N*-acetylcysteine methyl ester at the *o*-hydroxy-*p*-benzoquinone moiety, one or more cysteine residues of DNA polymerase α may act as a target for this compound.



## 1. Introduction

Development of highly potent and selective enzyme inhibitors is important for both drug development and chemical genetic studies.[1,2] Using this approach, several molecular target drugs have been developed that are effective and show relatively low toxicity in clinical use.[3] Furthermore, many of these enzyme inhibitors have been used as tools to characterize the biological function of the proteins of interest.[4]

Dehydroaltenusin (**1**) was first isolated from mycelium extracts of *Alternaria tennuis* and *Alternaria kikuchiana* by Rosett et al. in 1957[5] and its structure was subsequently determined by X-ray crystallography (Fig. 1).[6] Desmethyldehydroaltenusin (**2**), a related natural product, was isolated from the organic soluble metabolites of *Talaromyces flavus* by Ayer and Racok.[7] The structure of **2** was determined in the form of its acetate. After treatment of **2** with acetic anhydride in pyridine, the product was assigned as **3** (Fig. 2). The mass fragmentation pattern of desmethyldehydroaltenusin triacetate was similar to that of dehydroaltenusin diacetate except for the fragment ions having 14 mass units less. The $^1$H NMR of desmethyldehydroaltenusin triacetate was similar to that of dehydroaltenusin diacetate, which was prepared by acetylation of **1** with acetic anhydride in pyridine. The structure of dehydroaltenusin diacetate was originally suggested to be a δ-lactone (**4**) by Ayer and Racok,[7] but was later revised to be a γ-lactone (**5**) following our NMR analyses (COSY, HMQC, HMBC and INADEQUATE).[8] We found that compound **5** was a major product of acetylation of **1** with acetic anhydride in pyridine. Compound **4** was a minor product of the acetylation with acetyl chloride in pyridine and dichloromethane.

Nakanishi et al. reported that dehydroaltenusin (**1**) inhibits the calmodulin-dependent activity of myosin light chain kinase

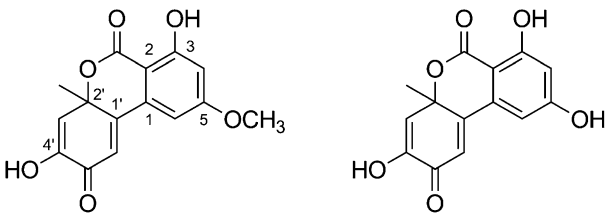


**Figure 1.** Structures of dehydroaltenusin (**1**) and desmethyldehydroaltenusin (**2**).

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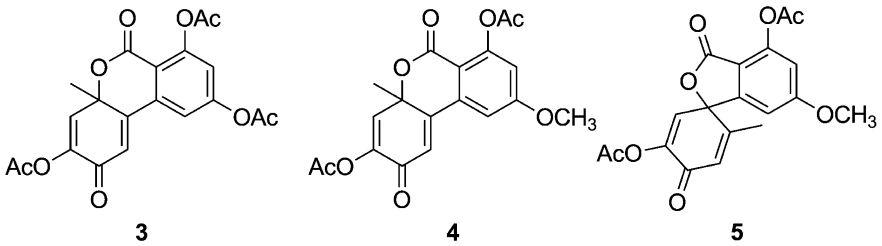



**Figure 2.** Proposed structure of desmethyldehydroaltenusin triacetate (**3**) prepared from **2** with acetic anhydride in pyridine, and structures of dehydroaltenusin diacetate (**4** and **5**, respectively).

(MLCK) with an $IC_{50}$ value of 0.69 μM.[9] We found that dehydroaltenusin (**1**), isolated from a fungus *A. tennuis*, selectively inhibited the activity of a mammalian DNA polymerase α. Compound **1** inhibited DNA polymerase α with an $IC_{50}$ value of 0.68 μM and had no effect on other replicative DNA polymerases such as DNA polymerase δ and ε.[10–16] Interestingly, related natural products, such as altenuene, alternariol and alternariol 9-methyl ether, did not inhibit the activity of DNA polymerases.[11]

Our group has reported the total synthesis of **1** with a palladium-catalyzed Suzuki coupling as a key reaction.[17,18] The structure–activity relationships of dehydroaltenusin acetates suggest that the *o*-hydroxy-*p*-benzoquinone (2-hydroxycyclohexa-2,5-dienone) moiety is essential for inhibition of DNA polymerase α.[8] We found that compound **1** existed in an equilibrium of two tautomers possessing γ-lactone and δ-lactone in polar solvents (Scheme 1). Thus, we proposed that the equilibrium between **1** and **1a** or plausible active *o*-quinone species (**1b**) might be important for the inhibition of DNA polymerase α.

In this paper, we prepared several dehydroaltenusin derivatives including desmethyldehydroaltenusin and investigated the structure–activity relationships as DNA polymerase α inhibitors in detail. We also examined the mechanism of inhibition of DNA polymerase α caused by **1**.

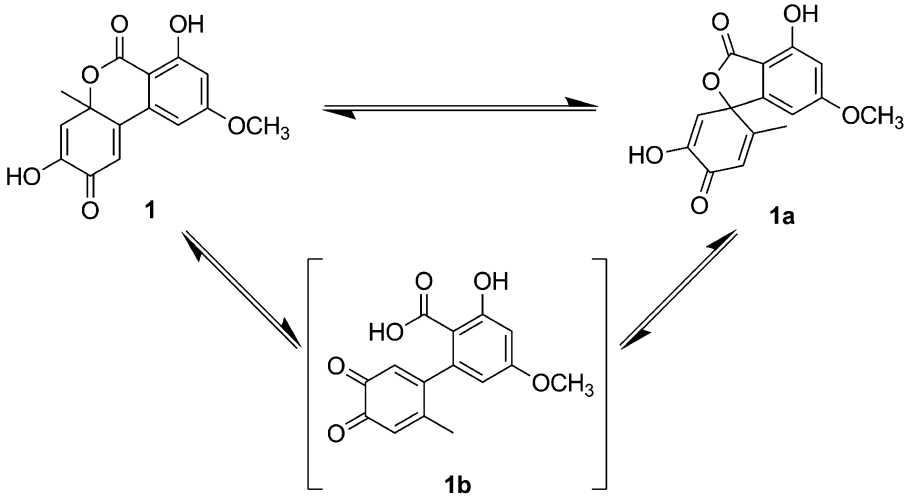
## 2. Results

### 2.1. Synthesis of dehydroaltenusin derivatives

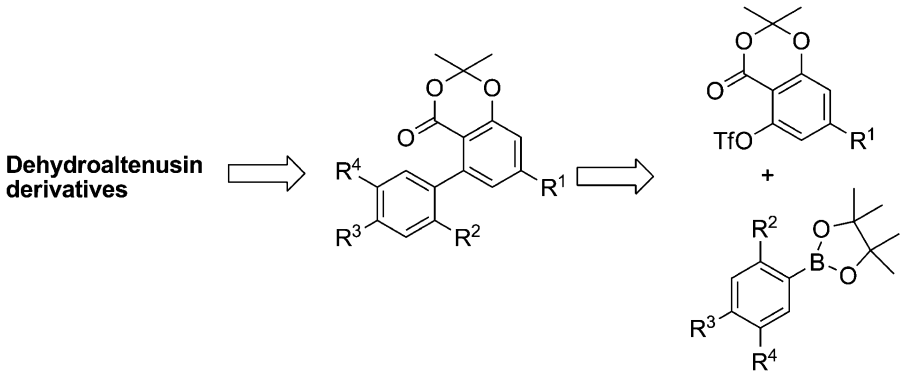
We planned to synthesize dehydroaltenusin derivatives by palladium-catalyzed Suzuki coupling of aryl triflates with aryl boronates (Scheme 2).[18–20] This convergent approach will be applicable to the synthesis of various dehydroaltenusin derivatives.

First, we decided to prepare desmethyldehydroaltenusin (**2**) (Scheme 3). Coupling of aryl triflate **6**[21] with aryl boronate **7**[18] in the presence of $PdCl_2$(dppf) (0.04 equiv) and $K_2CO_3$ (2.4 equiv) gave a biaryl **8** in 87% yield. Deprotection of all protecting groups in **8** by $BCl_3$ yielded desmethylaltenusin **9**.[22] Oxidation of **9** with $FeCl_3$ afforded desmethyldehydroaltenusin (**2**), which was converted into its triacetate by the procedure reported by Ayer and Racok.[7] The $^1H$ and IR spectra of desmethyldehydroaltenusin triacetate derived from synthetic **2** are identical to those reported. Ayer and Racok proposed the structure for desmethyldehydroaltenusin triacetate as **3**, which possessed the δ-lactone ring. However, the NMR and IR data of our desmethyldehydroaltenusin support the structure of γ-lactone **10**. In a previous paper we described the preparation and characterization of dehydroaltenusin diacetates (**4** and **5**) (Fig. 3).[8] The IR absorption at 1724 $cm^{-1}$ and three singlet peaks derived from H-3′, H-5′ and $CH_3$ in **4** are characteristic of a δ-lactone. On the other hand, the IR absorption at 1778 $cm^{-1}$ and the long-range coupling between H-3′ and $CH_3$ in **5** are characteristic of a γ-lactone. The IR absorption at 1778 $cm^{-1}$ and the long-range coupling between H-3′ and $CH_3$ in **10** indicates that the compound possesses a γ-lactone ring.

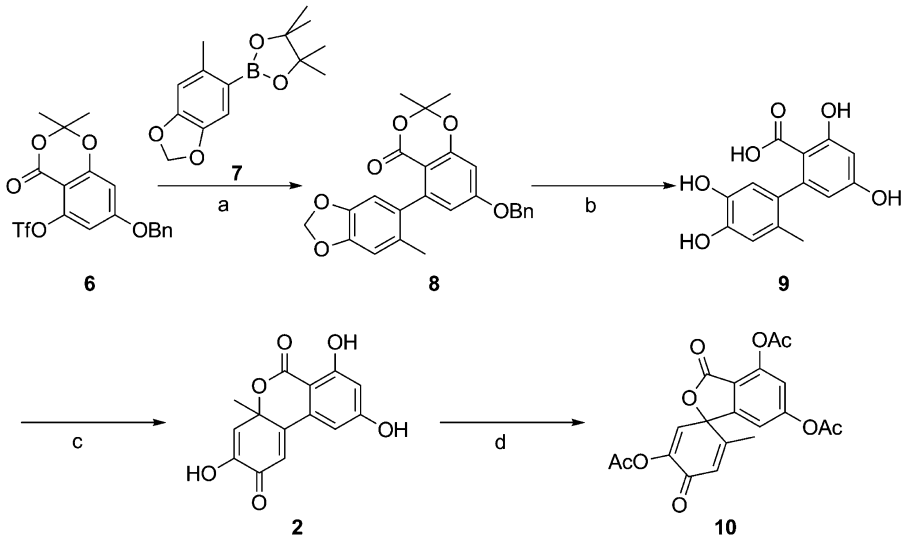
We next synthesized dehydroaltenusin derivatives by modifying the methoxy group at the C-5 position. The biaryl **8** serves as a potential starting material for the synthesis of various substituted dehydroaltenusin derivatives (Scheme 4). Deprotection of the benzyl group with $Pd(OH)_2$ on carbon under an atmosphere of $H_2$ gave a common intermediate **11**. Mitsunobu reaction of **11** with various alcohols gave ethers **12(a–d)**. Deprotection of the acetonide and methylene acetal in **12(a–d)**, followed by oxidation




**Scheme 1.** Tautomerization of dehydroaltenusin (**1**) in polar solvents.




**Scheme 2.** Synthetic approach for dehydroaltenusin derivatives.




**Scheme 3.** Synthesis of desmethyldehydroaltenusin (**2**) and its acetate (**10**). Reagents and conditions: (a) $PdCl_2$(dppf), $K_2CO_3$, DME, 85 °C (87%); (b) $BCl_3$, $CH_2Cl_2$–heptane, 0 °C (90%); (c) $FeCl_3$, EtOH–$H_2O$ (50%); (d) $Ac_2O$, pyridine (64%).

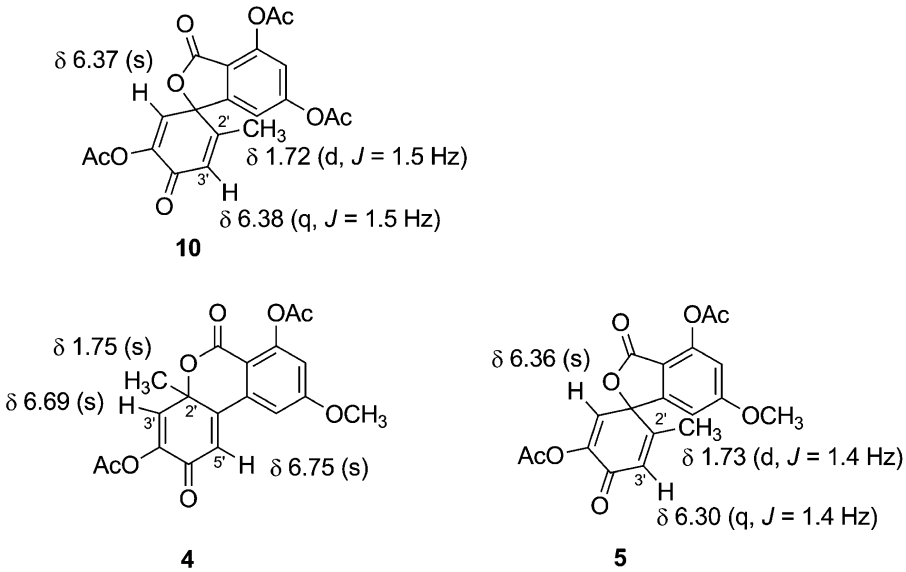
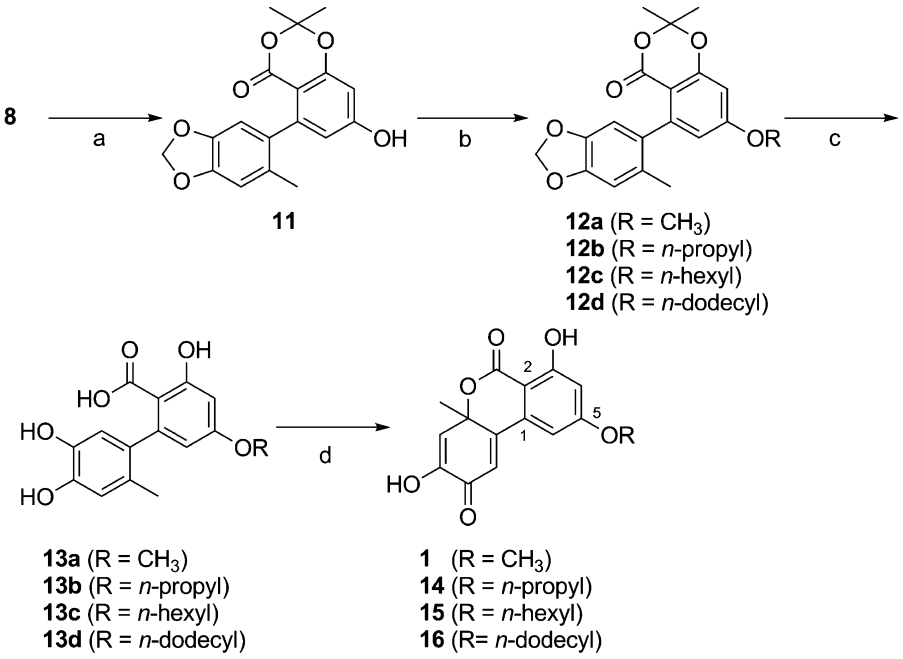



**Figure 3.** Assignment of desmethyldehydroaltenusin triacetate by comparison with that of dehydroaltenusin diacetates (**4** and **5**, respectively).



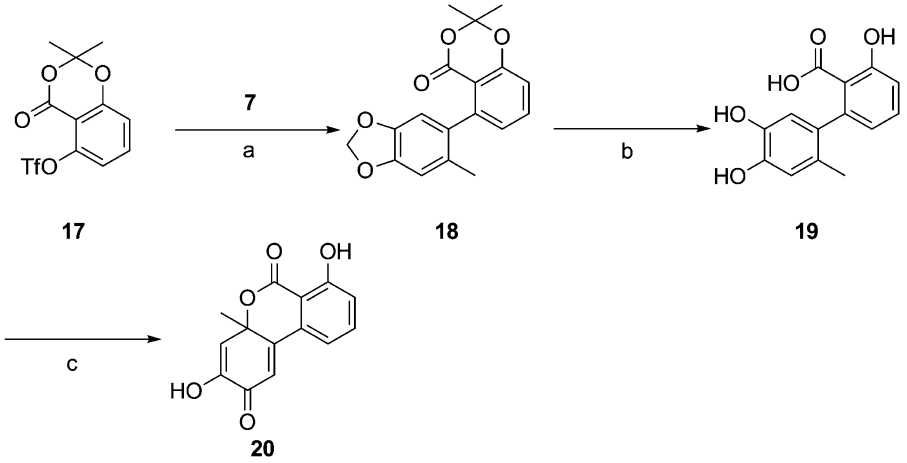

**Scheme 4.** Synthesis of dehydroaltenuisin (**1**) and its derivatives (**14**–**16**). Reagents and conditions: (a) $H_2$, $Pd(OH)_2$, THF–MeOH (87%); (b) alcohol, DIAD, $PPh_3$, THF (92% for **12a**, 79% for **12b**, 75% for **12c**, 73% for **12d**); (c) $BCl_3$, $CH_2Cl_2$–heptane, 0 °C (97% for **13a**, 77% for **13b**, 75% for **13c**, 82% for **13d**); (d) $FeCl_3$, EtOH–$H_2O$ (71% for **1**, 63% for **14**, 63% for **15**, 57% for **16**).

with $FeCl_3$ gave dehydroaltenusin (**1**) and its derivatives (**14**–**16**). We prepared demethoxydehydroaltenusin (**20**) by the same procedure in three steps starting from **17**[23] and **7** via the intermediates**18** and **19** (Scheme 5).

Although compounds **1**, **2**, **14**–**16** and **20** predominantly form the δ-lactone in $CDCl_3$, they exist in an equilibrium mixture of two tautomers, δ-lactone and γ-lactone, in polar solvents. The ratio of the tautomers in DMSO-$d_6$ is summarized in Table 1. The γ-lactone form of **1**, **2**, **14**–**16** and **20** is the major tautomer in DMSO-$d_6$.

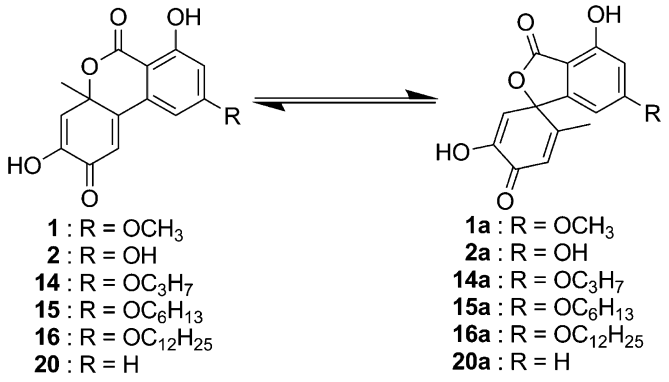
Next, we modified at the quinone moiety in dehydroaltenusin. An isocoumarin derivative was prepared according to a similar strategy (Scheme 6). Treatment of **21**[24] with bis(pinacolato)diboron in the presence of $PdCl_2$(dppf) and KOAc in DMF gave boronate **22** in 53% yield. Coupling of **22** with **23**[18] under Suzuki coupling conditions afforded **24**. Methanolysis of **24** followed by simultaneous lactone formation gave **25** in 77% yield. Deprotection of benzyl groups with $Pd(OH)_2$ on carbon under an atmosphere of $H_2$ afforded isocoumarin **26** in quantitative yield.

We also examined the importance of the hydroxyl group at C-4′ in dehydroaltenusin (**1**) in terms of its inhibitory activity against DNA polymerase α. Thus, we prepared dehydroaltenusin derivatives that lack the hydroxyl group at C-4′. 3-Iodo-4-methylphenol (**27**)[25] was converted into the corresponding boronate **28**. The palladium-catalyzed Suzuki coupling of **28** with **23** or **17** yielded **29** or **30**, respectively. Hydrolysis of **29** and **30** and oxidation of the resultant phenols with $PhI(OAc)_2$ gave **31** and **32** in 66% and 77% yield, respectively.[26] No reaction occurred when $FeCl_3$ was used as an oxidant in the oxidation of the phenol. Moreover, oxidation of the phenol with CAN resulted in decomposition of the phenol (Scheme 7).




**Scheme 5.** Synthesis of desmethyldehydroaltenusin **20**. Reagents and conditions: (a) $PdCl_2$(dppf), $K_2CO_3$, DME, 85 °C (89%); (b) $BCl_3$, $CH_2Cl_2$–heptane, 0 °C (77%); (c) $FeCl_3$, EtOH–$H_2O$ (80%).

**Table 1**
Ratio of γ-lactone and δ-lactone tautomers of dehydroaltenusin and its derivatives in DMSO-$d_6$

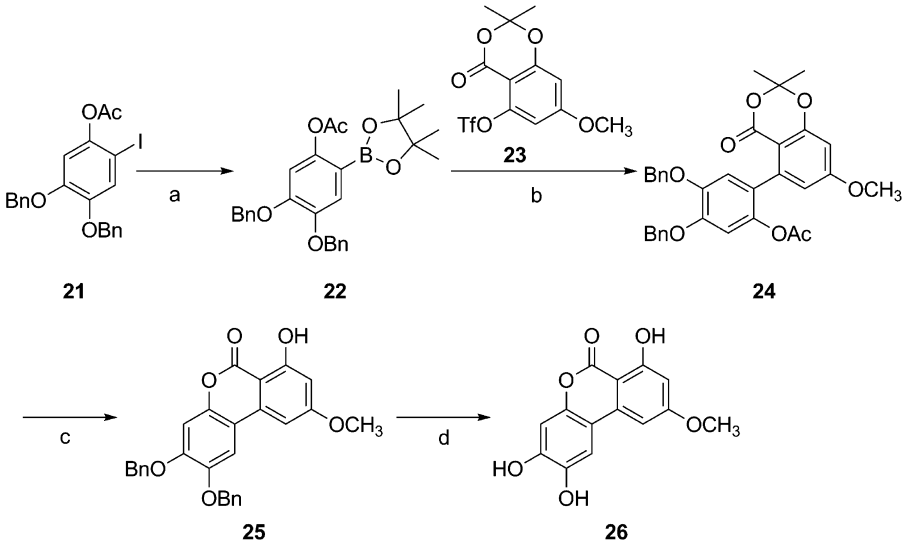



| Compound | Ratio of γ-lactone/δ-lactone |
|---|---|
| **1** | **1a**/**1** = 5 |
| **2** | **2a**/**2** = 7 |
| **14** | **14a**/**14** = 6 |
| **15** | **15a**/**15** = 6 |
| **16** | **16a**/**16** = 6 |
| **20** | **20a**/**20** = 13 |

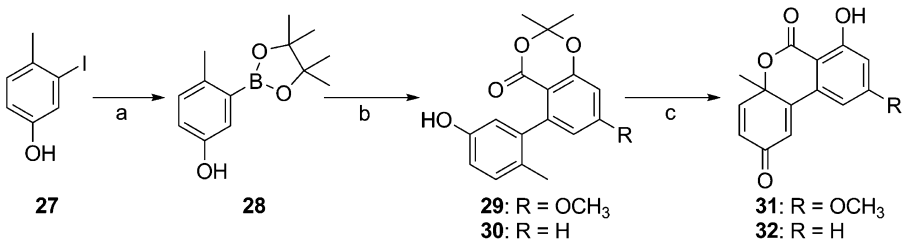
The ratio of γ-lactone and δ-lactone tautomers was determined on the basis of the $^1$H NMR integration.

## 2.2. Effect of synthetic dehydroaltenusin derivatives on mammalian DNA polymerases

The inhibitory effect of chemically synthesized dehydroaltenusin (**1**) and its derivatives (**2**, **14**–**16**, **20**, **25**, **26**, **31** and **32**) on two different DNA polymerases were investigated. Specifically, we used calf DNA polymerase α, which is a representative replicative DNA polymerase, and rat DNA polymerase β, which is a major repair-related DNA polymerase. Dehydroaltenusin (**1**) dose-dependently inhibited the activity of DNA polymerase α, with 50% inhibition observed at a concentration of 0.68 μM (Table 2) and almost complete inhibition at 4 μM.[17] Although we previously reported that the naturally purified dehydroaltenusin from a fungus (*Acremonium* sp.) was a specific inhibitor of mammalian DNA polymerase α,[10] this compound was also effective at inhibiting rat DNA polymerase β with an $IC_{50}$ value of 64 μM. From the prepared dehydroaltenusin derivatives, compounds **25**, **26**, **31** and **32** did not influence the activities of DNA polymerases α and β. The structural difference between dehydroaltenusin (**1**) and compound **2** is the group at 5-position of these compounds. Thus, five derivatives modified at this position were synthesized (**2**, **14**–**16** and **20**) and their inhibitory activities compared. All five compounds dose-dependently inhibited the activity of calf DNA polymerase α. Compound **16** had the strongest inhibitory effect among the compounds tested. The relative strength of the inhibitory effect of these derivatives could be ranked as follows: **16** > **15** > **14** >




**Scheme 6.** Synthesis of isocoumarin derivative **26**. Reagents and conditions: (a) $PdCl_2$(dppf), bis(pinacolato)diboron, KOAc, DMF, 80 °C (53%); (b) $PdCl_2$(dppf), $K_2CO_3$, DME, 80 °C (61% and 21% of recovered **23**); (c) $K_2CO_3$, MeOH (77%); (d) $H_2$, $Pd(OH)_2$, THF (quant.).




**Scheme 7.** Synthesis of dehydroaltenusin derivatives **31** and **32**. Reagents and conditions: (a) $PdCl_2$(dppf), bis(pinacolato)diboron, KOAc, DMF, 70 °C (86%); (b) $PdCl_2$(dppf), $K_2CO_3$, DME, 55 °C (76% for **29**, 67% for **30**); (c) LiOH, THF–$H_2O$, then $PhI(OAc)_2$, MeOH (77% for **31**, 64% for **32** in two steps).

**Table 2**
$IC_{50}$ values of dehydroaltenusin (**1**) and its derivatives on the activities of mammalian DNA polymerases α and β

| Compound | $IC_{50}$ value of DNA polymerase (μM) | | $IC_{50}$ value ratio pol β/pol α |
|---|---|---|---|
| | α | β | |
| Dehydroaltenusin (**1**) | 0.68 | 64 | 94.1 |
| **2** | 0.89 | 126 | 142 |
| **14** | 0.18 | 35 | 194 |
| **15** | 0.09 | 24 | 267 |
| **16** | 0.06 | 11 | 183 |
| **20** | 0.24 | 89 | 371 |
| **25** | >200 | >200 | — |
| **26** | >200 | >200 | — |
| **31** | >200 | >200 | — |
| **32** | >200 | >200 | — |

These compounds were incubated with calf DNA polymerase α or rat DNA polymerase β (0.05 units each). Enzyme activity in the absence of the compound was taken as 100%.

**20** > **1** > **2** (Table 2). However, in each case the effect of these compounds on rat DNA polymerase β activity was significantly weaker than on calf DNA polymerase α. The inhibitory effect of the dehydroaltenusin derivatives on rat DNA polymerase β varied markedly in the following order: **16** > **15** > **14** > **1** > **20** > **2** (Table 2). The $IC_{50}$ value ratio against DNA polymerase β/DNA polymerase α can be ranked as follows: **20** [371] > **15** [267] > **14** [194] > **16** [183] > **2** [142] > **1** [94.1] (Table 2). These results suggested that demethoxydehydroaltenusin (**20**) was the most specific mammalian DNA polymerase α inhibitor of the synthesized derivatives, and the specificity of this compound was approximately 4-fold stronger than that of dehydroaltenusin (**1**).

### 2.3. Model studies for determining the binding site of dehydroaltenusin in DNA polymerase α

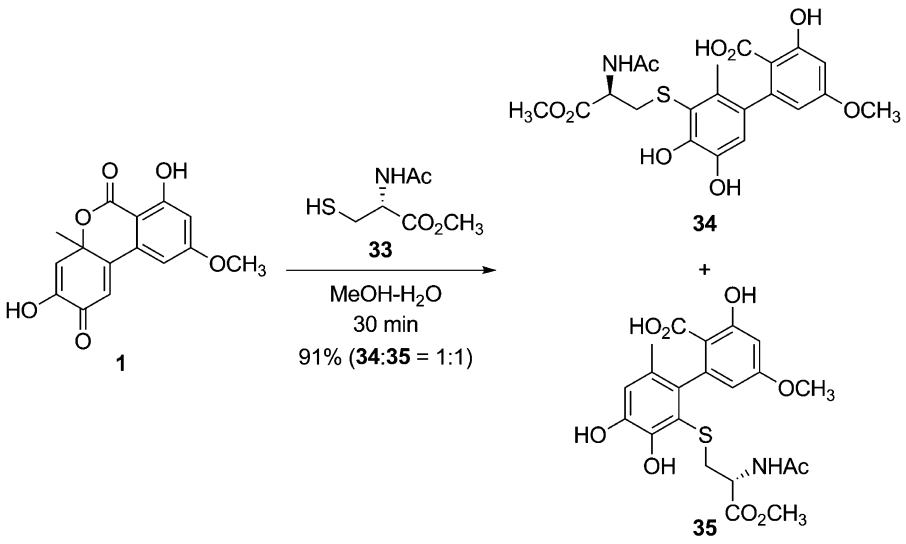
Dehydroaltenusin exists as an equilibrium of tautomers, **1** and **1a** via the plausible intermediate **1b** in polar solvents. Because *o*-quinone is a highly reactive species, which reacts with thiols or amines,[27–30] compound **1b** might conjugate to a sulfhydryl or amino group present in DNA polymerase α, and thereby inhibit the DNA polymerase activity. To clarify the type of residue that forms a dehydroaltenusin-adduct, **1** was reacted with various amino acids. Treatment of **1** with *N*-acetylcysteine methyl ester (**33**) in MeOH–$H_2O$ (1:1) for 30 min gave an inseparable 1:1 mixture of **34** and **35** in 91% yield (Scheme 8). Other amino acid derivatives, such as *N*-acetyllysine methyl ester, *N*-acetylarginine methyl ester, *N*-acetylhistidine methyl ester, *N*-acetyltyrosine ethyl ester and *N*-Boc-serine, did not react with **1**. These results suggested that **1** might bind to a cysteine residue of DNA polymerase α and inhibit the activity of the enzyme.

## 3. Discussion

In our previous report, we described the total synthesis of dehydroaltenusin using Suzuki coupling for construction of the biaryl moiety as a key transformation.[18] Here, we have successfully applied our synthetic method with slight modifications to the synthesis of dehydroaltenusin derivatives. We have accomplished a first synthesis of two natural products, desmethylaltenusin (**9**) and desmethyldehydroaltenusin (**2**). Our method is also applicable to the synthesis of related *Alternaria* toxins. Indeed, a similar strategy was used in the synthesis of alternariol, alternariol methyl ether, altenuene and isoaltenuene by Podlech et al.[19,20]

Using the newly synthesized derivatives of dehydroaltenusin (**2**, **14**–**16**, **20**, **25**, **26**, **31** and **32**) we investigated the structure–activity relationships for inhibition of DNA polymerase α and β. Compounds **2**, **14**–**16** and **20**, which have the *o*-hydroxy-*p*-benzoquinone unit, exhibited inhibitory activity against DNA polymerases α and β. The rank orders of the inhibitory effect against DNA polymerases α and β were **16** > **15** > **14** > **20** > **1** > **2** and **16** > **15** > **14** > **1** > **20** > **2**, respectively. These compounds were more selective DNA polymerase α inhibitors than dehydroaltenusin (**1**). Among the derivatives, demethoxydehydroaltenusin (**20**) was the most selective inhibitor of DNA polymerase α. On the other hand, isocoumarins (**25** and **26**) and *p*-benzoquinones (**31** and **32**) did not significantly influence on the activity of DNA polymerases. These results indicate that the *o*-hydroxy-*p*-benzoquinone moiety is essential for inhibiting DNA polymerases and that substitution of the alkoxy group at C-5 greatly influences the inhibitory potency. The long alkoxy side chains might interact with the hydrophobic region of DNA polymerases, resulting in the enhancement of the inhibitory activity.

Compounds **1**, **2**, **14**–**16** and **20**, which display inhibitory activity against DNA polymerase α, exist as an equilibrium mixture of two tautomers, δ-lactone and γ-lactone, in DMSO-$d_6$. The ratio of γ-lactone/δ-lactone does not correlate with either the strength or




**Scheme 8.** Reaction of dehydroaltenusin (**1**) with *N*-acetylcysteine methyl ester (**33**).

selectivity of the inhibitory activity. Our previous report shows that the inhibitory activities of dehydroaltenusin diacetates **4** and **5**, which form δ-lactone and γ-lactone, respectively, are much weaker than that of dehydroaltenusin.[18] These observations suggest that the *o*-quinone species of dehydroaltenusin derivatives such as compound **1b**, which possesses a plausible intermediate between δ-lactone and γ-lactone, are in fact the active species in the inhibition of DNA polymerase α. Actually, inactive compounds **25**, **26**, **31** and **32** did not form the *o*-quinone species, since these compounds do not tautomerize.

Generally, *o*-quinones are highly reactive species that react covalently with cellular nucleophiles.[27] Indeed, *o*-quinones can react with sulfhydryl, amine, amide, indole and imidazole groups of proteins or amino acids. The results obtained from the reaction of dehydroaltenusin (**1**) with various amino acid derivatives indicate that dehydroaltenusin might specifically conjugate with one or more cysteines of DNA polymerase α. We previously demonstrated that the inhibition of calf DNA polymerase α by dehydroaltenusin was competitive with the DNA template-primer and noncompetitive with the deoxyribonucleotide triphosphates (dNTPs).[10] We also showed that dehydroaltenusin binds to the core domain of the largest subunit of mouse DNA polymerase α, p180, but not to the smallest subunit or the DNA primase p46. The p180 domain contains many cysteine residues in the DNA binding domain of the enzyme.[31,32] These data suggest that dehydroaltenusin may directly conjugate with a cysteine residue of the template-primer DNA-binding site of DNA polymerase α, resulting in the inhibition of DNA polymerase α. Studies to identify the binding site of dehydroaltenusin in DNA polymerase α are currently underway.

## 4. Conclusion

We synthesized dehydroaltenusin derivatives and investigated their inhibitory activity against DNA polymerases α and β. Our results demonstrate that the *o*-hydroxy-*p*-benzoquinone (2-hydroxycyclohexa-2,5-dienone) moiety is essential for inhibition of DNA polymerases α and β. Substitution at the 5-position of dehydroaltenusin strongly influences the inhibitory potency. Of all the synthesized derivatives, demethoxydehydroaltenusin (**20**) was the most selective DNA polymerase α inhibitor. Results obtained from the reaction of dehydroaltenusin with *N*-acetylcysteine methyl ester indicates that dehydroaltenusin might specifically conjugate with a cysteine of DNA polymerase α.

## 5. Experimental

### 5.1. Analyses of structure determination

All non-aqueous reactions were carried out by using freshly distilled solvents under argon atmosphere. THF was distilled from sodium/benzophenone prior to use. Dichloromethane was distilled from $P_2O_5$ prior to use. *N*,*N*-Dimethylformamide was distilled from calcium hydride prior to use. All other reagents were commercially available and used without further purification.

All reactions were monitored by TLC, which was carried out on Silica Gel 60 $F_{254}$ plates (Merck, Germany). Flash chromatography separations were performed on PSQ 100B (Fuji Silysia Co., Ltd, Japan).

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker 600 MHz or 400 MHz spectrometer (Avance DRX-600, Avance DRX-400) or a JEOL 270 MHz spectrometer (EX-270W), using $CDCl_3$ (with TMS for $^1$H NMR and chloroform-*d* for $^{13}$C NMR as the internal reference) solution, unless otherwise noted. Chemical shifts were expressed in $\delta$ (ppm) relative to $Me_4Si$ or residual solvent resonance, and coupling constants ($J$) were expressed in hertz.

Melting points were determined with Yanaco MP-3S and were uncorrected. Infrared spectra (IR) were recorded on a Jasco FT/IR-410 spectrometer using NaCl (neat) or KBr pellets (solid), and were reported on wavenumbers ($cm^{-1}$). Mass spectra (MS) were obtained on a Hitachi M-80 spectrometer or an Applied Biosystems mass spectrometer (APIQSTAR pulsar i) under conditions as High resolution, using poly (ethylene glycol) as internal standard.

#### 5.1.1. 7-Benzyloxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (8)

To a degassed stirred solution of **6** (445.5 mg, 1.30 mmol), **7** (355.7 mg, 1.36 mmol) and $K_2CO_3$ (429.3 mg, 3.11 mmol) in DME (10.0 mL) was added $PdCl_2$ (dppf) (37.7 mg, 0.052 mmol), and the mixture was stirred at 85 °C for 17 h. After addition of Celite, the mixture was stirred for 30 min and filtered through a pad of Celite. The filtrate was diluted with $CHCl_3$, and this organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (5:1–1:1) yielded **8** (376.2 mg, 87%) as white solids. Mp = 200.5–201.5 °C. IR $\nu_{max}$ (KBr): 2994, 2917, 1730, 1604, 1575, 1486, 1425, 1381, 1342, 1281, 1231, 1200, 1166, 1129, 1034, 929, 856 $cm^{-1}$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.40 (4H, m), 7.36 (1H, m), 6.70 (1H, s), 6.58 (1H, s), 6.52 (1H, d, $J$ = 2.5 Hz), 6.51 (1H, d, $J$ = 2.5 Hz), 5.96 (1H, d, $J$ = 1.5 Hz), 5.94 (1H, d, $J$ = 1.5 Hz), 5.09 (2H, s), 2.00 (3H, s), 1.73 (3H, s), 1.73 (3H, s). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 163.7, 158.8, 158.5, 147.0, 146.9, 145.3, 135.6, 133.0, 128.7, 128.7, 128.4, 128.2, 127.5, 127.5, 113.7, 109.9, 108.6, 106.0, 105.1, 101.5, 100.9, 70.4, 26.0, 25.3, 19.7. HRMS (ESI) calcd for $C_{25}H_{22}O_6Na$ ($[M+Na]^+$) 441.1308, found 441.1304. Anal. Calcd for $C_{25}H_{22}O_6$: C, 71.76; H, 5.30. Found: C, 71.52; H, 5.02.

#### 5.1.2. Desmethylaltenusin (3,4′,5,5′-tetrahydroxy-2′-methylbiphenyl-2-carboxylic acid) (9)

To a stirred solution of **8** (313.8 mg, 0.75 mmol) in $CH_2Cl_2$ (8.0 mL) was added $BCl_3$ (1.0 M solution in heptane, 4.50 mL, 4.5 mmol) at 0 °C and the mixture was stirred at room temperature for 19.5 h. Then the mixture was diluted with EtOAc. The organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc–AcOH (4000:1000:1–2000:1000:1) yielded **9** (186.4 mg, 90%) as yellow foam. IR $\nu_{max}$ (KBr): 3381, 2928, 2850, 1650, 1614, 1516, 1454, 1171, 1066, 1011, 852, 790 $cm^{-1}$. $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ 6.58 (1H, s), 6.49 (1H, s), 6.29 (1H, d, $J$ = 2.5 Hz), 6.08 (1H, d, $J$ = 2.5 Hz), 1.91 (3H, s); $^{13}$C NMR (150 MHz, $CD_3OD$): $\delta$ 174.3, 165.7, 163.2, 148.3, 144.7, 143.1, 135.6, 127.4, 117.3, 116.6, 112.3, 105.9, 102.4, 19.2; HRMS (ESI) calcd for $C_{14}H_{12}O_6Na$ ($[M+Na]^+$) 299.0526, found 299.0519.

#### 5.1.3. Desmethyldehydroaltenusin (2)

To a stirred solution of **9** (186.4 mg, 0.675 mmol) in EtOH–$H_2O$ (1:1, 4.0 mL) was added a 0.2 M solution of $FeCl_3$ in $H_2O$ (10.1 mL) and the mixture was stirred at room temperature for 15 min and diluted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (1:1) and recrystallization from EtOAc–hexane gave **2** (92.3 mg, 50%) as yellow crystals. Mp = 185.0–185.5 °C (decomp.). IR $\nu_{max}$ (KBr): 3368, 3053, 2980, 2928, 1678, 1640, 1490, 1395, 1263, 1173, 1107, 1073, 1025, 856, 802 $cm^{-1}$. $^1$H NMR (600 MHz, $CDCl_3$–$CD_3OD$): $\delta$ 6.69 (1H, d, $J$ = 2.0 Hz), 6.66 (1H, s), 6.55 (1H, d, $J$ = 2.0 Hz), 6.26 (1H, s), 1.72 (3H, s). $^1$H NMR (600 MHz, DMSO-$d_6$): **2a**: $\delta$ 10.90 (1H, s), 10.59 (1H, s), 9.54 (1H, s), 6.35 (1H, d, $J$ = 1.4 Hz), 6.28 (1H, br q, $J$ = 1.1 Hz), 5.98 (1H, d, $J$ = 1.4 Hz), 1.58 (3H, d, $J$ = 1.4 Hz). Compound **2**: $\delta$ 11.10 (1H, s), 11.10 (1H, s), 9.58 (1H, s), 6.84 (1H, d, $J$ = 2.0 Hz), 6.75 (1H, s), 6.50 (1H, d, $J$ = 2.0 Hz), 6.16 (1H, s), 1.64 (3H, s). $^{13}$C NMR (150 MHz, $CDCl_3$–$CD_3OD$): $\delta$ 181.1, 167.4, 164.9, 163.8, 151.6,

146.9, 135.1, 121.2, 116.4, 105.2, 104.2, 98.3, 78.8, 28.6. HRMS (ESI) calcd for $C_{14}H_{11}O_6Na$ ([M+H]$^+$) 275.0550, found 275.0543. Anal. Calcd for $C_{14}H_{11}O_6$: C, 61.32; H, 3.68. Found: C, 61.06; H, 3.58.

#### 5.1.4. Desmethyldehydroaltenusin triacetate (10)

A solution of **2** (5.4 mg, 19.5 μmol) in $Ac_2O$ and pyridine (1:1, 1.0 mL) was stirred at room temperature for 25 h and then concentrated. Chromatography on silica gel with hexane–EtOAc (3:2) and yielded **10** (5.0 mg, 64%) as amorphous solids. IR (KBr) 3537, 3026, 2928, 2850, 1778, 1687, 1665, 1613, 1472, 1431, 1369, 1302, 1188, 1152, 1123, 1090, 1055, 1013, 979, 913, 756 cm$^{-1}$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.10 (1H, d, $J$ = 1.7 Hz), 6.97 (1H, d, $J$ = 1.7 Hz), 6.37 (1H, s), 6.31 (1H, d, $J$ = 1.4 Hz), 2.43 (3H, s), 2.32 (3H, s), 2.29 (3H, s), 1.72 (3H, d, $J$ = 1.4 Hz). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 178.0, 168.2, 168.2, 167.8, 165.3, 157.1, 154.4, 149.7, 149.6, 145.6, 129.9, 127.8, 118.4, 115.2, 113.0, 82.8, 21.1, 20.6, 20.4, 17.0. HRMS calcd for $C_{20}H_{16}O_9Na$ ([M+Na]$^+$) 423.0686, found 423.0702.

#### 5.1.5. 7-Hydroxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (11)

To a stirred solution of **8** (747.4 mg, 1.81 mmol) in THF–MeOH (1:1, 400 mL) was added 10% $Pd(OH)_2$ on carbon (74.7 mg, 0.53 mmol), and the mixture was stirred under an atmosphere of $H_2$ at room temperature for 3 h. After addition of Celite, the mixture was stirred for 30 min and filtered through a pad of Celite. The filtrate was concentrated, and the crude product was recrystallized from EtOAc to afford **11** (530.0 mg, 87%) as crystals. Mp = 247.0–247.5 °C (decomp.). IR $\nu_{max}$ (KBr): 3755, 3382, 3002, 2892, 1703, 1610, 1479, 1392, 1282, 1189, 1035, 921, 859, 727 cm$^{-1}$. $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 10.9 (1H, s), 6.76 (1H, s), 6.61 (1H, s), 6.39 (1H, d, $J$ = 2.3 Hz), 6.26 (1H, d, $J$ = 2.3 Hz), 5.99 (1H, s), 5.97 (1H, s), 1.90 (3H, s), 1.66 (3H, s), 1.65 (3H, s). $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 163.7, 158.3, 158.3, 146.4, 146.3, 144.9, 133.4, 127.7, 114.1, 109.6, 108.6, 104.9, 104.0, 102.2, 100.8, 25.6, 24.9, 19.4. HRMS (ESI) calcd for $C_{18}H_{16}O_6Na$ ([M+Na]$^+$) 351.0839, found 351.0842. Anal. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.78; H, 4.67.

### 5.2. General method for the preparation of 12

To a stirred solution of **11** (1.0 equiv), alcohol (1.5 equiv) and $PPh_3$ (1.5 equiv) in THF was added DIAD (1.5 equiv) at 0 °C, and then the mixture was stirred at room temperature for 20 min. After concentration of the mixture, chromatography on silica gel with hexane–EtOAc yielded **12**.

#### 5.2.1. 7-Methoxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (12a)

The title compound was prepared by the coupling of **11** and methanol in 92% yield as white acicular crystals. The spectral data of **12a** were identical to those of our authentic sample.[18]

#### 5.2.2. 7-Propoxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (12b)

The title compound was prepared by the coupling of **11** and 1-pronanol in 79% yield as white acicular crystals. Mp = 133.5–134.0 °C. IR $\nu_{max}$ (KBr): 2979, 2939, 2883, 1733, 1606, 1576, 1481, 1428, 1382, 1342, 1282, 1233, 1186, 1130, 1034, 970, 932, 914, 854 cm$^{-1}$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 6.70 (1H, s), 6.59 (1H, s), 6.43 (1H, d, $J$ = 2.5 Hz), 6.42 (1H, d, $J$ = 2.5 Hz), 5.96 (1H, d, $J$ = 1.3 Hz), 5.94 (1H, d, $J$ = 1.3 Hz), 3.96 (2H, m), 2.02 (3H, s), 1.82 (2H, sextet, $J$ = 7.6 Hz), 1.73 (3H, s), 1.72 (3H, s), 1.04 (3H, t, $J$ = 7.6 Hz). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 164.3, 158.9, 158.6, 146.9, 146.9, 145.3, 133.1, 128.3, 113.5, 109.8, 108.7, 105.6, 105.0, 101.0, 100.9, 70.1, 26.1, 25.3, 22.3, 19.7, 10.4. HRMS (ESI) calcd for $C_{21}H_{22}O_6Na$ ([M+Na]$^+$) 393.1314, found 393.1299. Anal. Calcd for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99. Found: C, 68.04; H, 5.84.

#### 5.2.3. 7-Hexyloxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (12c)

The title compound was prepared by the coupling of **11** and 1-hexanol in 75% yield as white acicular crystals. Mp = 43.0–43.7 °C. IR $\nu_{max}$ (KBr): 2995, 2956, 2928, 2860, 1732, 1608, 1576, 1481, 1430, 1384, 1344, 1283, 1241, 1189, 1132, 1058, 1033, 969, 933, 912, 868 cm$^{-1}$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 6.70 (1H, s), 6.59 (1H, s), 6.42 (1H, d, $J$ = 2.4 Hz), 6.41 (1H, d, $J$ = 2.4 Hz), 5.96 (1H, d, $J$ = 1.4 Hz), 5.93 (1H, d, $J$ = 1.4 Hz), 3.98 (2H, t, $J$ = 6.5 Hz), 2.02 (3H, s), 1.79 (2H, quin, $J$ = 6.5 Hz), 1.73 (3H, s), 1.72 (3H, s), 1.45 (2H, quin, $J$ = 7.3 Hz), 1.34 (4H, m), 0.90 (3H, t, $J$ = 6.8 Hz). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 164.3, 158.9, 158.6, 146.9, 146.9, 145.3, 133.2, 128.3, 113.5, 109.9, 108.7, 105.6, 105.0, 101.0, 100.9, 68.6, 31.5, 28.9, 26.1, 25.6, 25.3, 22.5, 19.7, 13.9. HRMS (ESI) calcd for $C_{24}H_{28}O_6Na$ ([M+Na]$^+$) 435.1778, found 435.1796. Anal. Calcd for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84. Found: C, 69.69; H, 7.12.

#### 5.2.4. 7-Dodecyloxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (12d)

The title compound was prepared by the coupling of **11** and 1-dodecanol in 73% yield as white solids. Mp = 111.9–112.2 °C. IR $\nu_{max}$ (neat): 2919, 2851, 1738, 1604, 1580, 1476, 1430, 1389, 1343, 1279, 1237, 1182, 1132, 1039, 937, 912, 869 cm$^{-1}$. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 6.70 (1H, s), 6.59 (1H, s), 6.42 (1H, d, $J$ = 2.5 Hz), 6.41 (1H, d, $J$ = 2.5 Hz), 5.96 (1H, d, $J$ = 1.3 Hz), 5.94 (1H, d, $J$ = 1.3 Hz), 3.98 (2H, t, $J$ = 6.5 Hz), 2.02 (3H, s), 1.81–1.76 (2H, m), 1.73 (3H, s), 1.72 (3H, s), 1.46–1.41 (2H, m), 1.21–1.38 (16H, br m), 0.88 (3H, t, $J$ = 7.0 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.3, 158.9, 158.5, 146.8, 146.8, 145.3, 133.1, 128.2, 113.5, 109.9, 108.6, 105.5, 105.0, 101.0, 100.9, 68.6, 31.9, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 28.9, 26.0, 25.9, 25.3, 22.7, 19.7, 14.1. HRMS (ESI) calcd for $C_{30}H_{40}O_6Na$ ([M+Na]$^+$) 519.2717, found 519.2750. Anal. Calcd for $C_{30}H_{40}O_6$: C, 72.55; H, 8.12. Found: C, 72.31; H, 8.42.

### 5.3. General method for the preparation of 13

To a stirred solution of **12** (1.0 equiv) in $CH_2Cl_2$ was added $BCl_3$ (5.0 equiv) at 0 °C and the mixture was stirred at room temperature for 18–20 h. Then the mixture was diluted with EtOAc. The organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc–AcOH yielded **13**.

#### 5.3.1. Altenusin (13a)

The title compound was prepared from **12a** in 97% yield as yellow amorphous solids. The spectral data of **13a** were identical to those of our authentic sample.[18]

#### 5.3.2. 3,4′,5′-Trihydroxy-2′-methyl-5-propoxybiphenyl-2-carboxylic acid (13b)

The title compound was prepared from **12b** in 77% yield as yellow amorphous solids. IR $\nu_{max}$ (KBr): 3536, 3397, 2972, 2873, 2556, 1640, 1613, 1519, 1456, 1367, 1340, 1284, 1255, 1219, 1184, 1065, 955, 868 cm$^{-1}$. $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ 6.49 (1H, s), 6.40 (1H, s), 6.30 (1H, d, $J$ = 3.0 Hz), 6.06 (1H, d, $J$ = 3.0 Hz), 3.81 (2H, t, $J$ = 7.0 Hz), 1.80 (3H, s), 1.65 (2H, quin, $J$ = 7.0 Hz), 0.90 (3H, t, $J$ = 7.0 Hz). $^{13}$C NMR (150 MHz, $CD_3OD$): $\delta$ 174.3, 165.7, 164.5, 148.0, 145.0, 143.3, 135.5, 127.5, 117.5, 116.8, 112.0, 107.0, 101.2, 70.8, 23.5, 19.3, 10.7. HRMS (ESI) calcd for $C_{17}H_{18}O_6Na$ ([M+Na]$^+$) 341.0995, found 341.0996. Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.16; H, 5.93.

#### 5.3.3. 5-Hexyloxy-3,4′,5′-trihydroxy-2′-methylbiphenyl-2-carboxylic acid (13c)

The title compound was prepared from **12c** in 75% yield as yellow foam. IR $\nu_{max}$ (KBr): 3400, 2928, 1645, 1611, 1516, 1457, 1364, 1255, 1177, 1068, 1028, 854 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 6.71 (1H, s), 6.61 (1H, s), 6.45 (1H, d, $J$ = 2.5 Hz), 6.21 (1H, d, $J$ = 2.5 Hz), 3.96 (2H, t, $J$ = 6.9 Hz), 1.93 (3H, s), 1.76 (2H, quin, $J$ = 6.9 Hz), 1.42 (2H, quin, $J$ = 6.9 Hz), 1.32 (4H, m), 0.88 (3H, t, $J$ = 6.6 Hz). $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 173.4, 165.5, 145.5, 164.2, 143.5, 141.3, 132.6, 128.4, 117.1, 115.9, 111.8, 103.6, 100.9, 68.5, 31.5, 28.9, 25.6, 22.5, 19.0, 13.9. HRMS (ESI) calcd for $C_{20}H_{24}O_6Na$ ([M+Na]$^+$) 383.1465, found 383.1491.

#### 5.3.4. 5-Dodecyloxy-3,4′,5′-trihydroxy-2′-methylbiphenyl-2-carboxylic acid (13d)

The title compound was prepared from **12d** in 82% yield as yellow foam. IR $\nu_{max}$ (neat): 3334, 3021, 2926, 2855, 1650, 1609, 1517, 1461, 1217, 1071, 1038, 853 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 6.70 (1H, s), 6.61 (1H, s), 6.44 (1H, d, $J$ = 2.3 Hz), 6.20 (1H, d, $J$ = 2.3 Hz), 3.95 (2H, t, $J$ = 6.5 Hz), 1.93 (3H, s), 1.76 (2H, m), 1.42 (2H, m), 1.38–1.20 (16H, br m), 0.87 (3H, t, $J$ = 7.0 Hz). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 173.2, 165.4, 164.1, 145.4, 143.5, 141.3, 132.6, 128.4, 117.1, 115.9, 111.8, 103.5, 100.8, 68.5, 31.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.3, 28.9, 25.9, 22.7, 19.0, 14.1. HRMS (ESI) calcd for $C_{26}H_{36}O_6Na$ ([M+Na]$^+$) 467.2404, found 467.2412.

### 5.4. General method for the preparation of 1, 14, 15 and 16

To a solution of **13** (1.0 equiv) in EtOH–$H_2O$ (1:1, 25 mL) was added a 0.2 M solution of $FeCl_3$ (3.0 equiv) in $H_2O$, and the mixture was stirred at room temperature for 10–35 min. The mixture was diluted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (3:2) yielded **1** (0.84 g, 71%).

#### 5.4.1. Dehydroaltenusin (1)

The title compound was prepared from **13a** in 71% yield as yellow acicular crystals. The spectral data of **1** were identical to those of our authentic sample.[18]

#### 5.4.2. 3,7-Dihidroxy-4a-metyl-9-propoxy-4a*H*-benzo[*c*]chromene-2,6-dione (14)

The title compound was prepared from **13b** in 63% yield as yellow acicular crystals. Mp = 124.5–125.0 °C. IR $\nu_{max}$ (KBr): 3284, 3048, 2968, 2938, 2878, 1684, 1644, 1573, 1496, 1455, 1387, 1357, 1108, 1077, 1027, 952, 891 851 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 11.27 (1H, s), 6.73 (1H, d, $J$ = 2.4 Hz), 6.70 (1H, s), 6.61 (1H, d, $J$ = 2.4 Hz), 6.40 (1H, s), 6.28 (1H, s), 4.05 (2H, m), 1.86 (2H, quin, $J$ = 7.3 Hz), 1.73 (3H, s), 1.07 (3H, t, $J$ = 7.3 Hz). $^1H$ NMR (600 MHz, DMSO-$d_6$): **14a**: $\delta$ 11.03 (1H, s), 9.53 (1H, s), 6.42 (1H, d, $J$ = 1.8 Hz), 6.31 (1H, d, $J$ = 1.8 Hz), 6.29 (1H, br q, $J$ = 1.3 Hz), 5.71 (1H, s), 3.92 (2H, m), 1.68 (2H, sextet, $J$ = 7.2 Hz), 1.57 (3H, d, $J$ = 1.3 Hz), 0.94 (3H, t, $J$ = 7.2 Hz). Compound **14**: $\delta$ 11.15 (1H, s), 9.59 (1H, s), 7.10 (1H, d, $J$ = 2.2 Hz), 6.96 (1H, s), 6.70 (1H, d, $J$ = 2.2 Hz), 6.18 (1H, s), 4.11 (2H, m), 1.76 (2H, sextet, $J$ = 7.4 Hz), 1.64 (3H, s), 0.99 (3H, t, $J$ = 7.4 Hz).$^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 180.8, 167.3, 165.9, 164.7, 153.2, 146.1, 134.9, 120.7, 116.1, 104.7, 104.1, 99.6, 79.1, 70.4, 29.5, 22.3, 10.4. HRMS (ESI) calcd for $C_{17}H_{16}O_6Na$ [M+Na]$^+$ 339.0839, found 339.0844. Anal. Calcd for $C_{17}H_{16}O_6$: C, 64.55; H, 5.10. Found: C, 64.68; H, 5.01.

#### 5.4.3. 9-Hexyloxy-3,7-dihidroxy-4a-metyl-4a*H*-benzo[*c*]chromene-2,6-dione (15)

The title compound was prepared from **13c** in 63% yield as yellow solids. Mp = 136.3–136.7 °C. IR $\nu_{max}$ (KBr): 3277, 3053, 2940, 2866, 1681, 1644, 1576, 1514, 1463, 1412, 1386, 1356, 1307, 1259, 1212, 1184, 1110, 1080, 1028, 972, 891, 857 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 11.26 (1H, s), 6.73 (1H, d, $J$ = 2.1 Hz), 6.68 (1H, s), 6.61 (1H, d, $J$ = 2.1 Hz), 6.39 (1H, s), 6.26 (1H, s), 4.05 (2H, t, $J$ = 6.6 Hz), 1.82 (2H, quin, $J$ = 6.6 Hz), 1.73 (3H, s), 1.48 (2H, quin, $J$ = 6.6 Hz), 1.35 (4H, m), 0.92 (3H, t, $J$ = 6.5 Hz). $^1H$ NMR (600 MHz, DMSO-$d_6$): **15a**: $\delta$ 11.02 (1H, s), 9.53 (1H, s), 6.42 (1H, d, $J$ = 1.9 Hz), 6.31 (1H, d, $J$ = 1.9 Hz), 6.29 (1H, br q, $J$ = 1.4 Hz), 5.71 (1H, s), 3.95 (2H, m), 1.66 (2H, m), 1.57 (3H, d, $J$ = 1.4 Hz), 1.37 (2H, m), 1.31–1.26 (4H, m), 0.86 (3H, t, $J$ = 6.9 Hz). Compound **15**: $\delta$ 11.14 (1H, s), 9.59 (1H, s), 7.09 (1H, d, $J$ = 2.2 Hz), 6.96 (1H, s), 6.70 (1H, d, $J$ = 2.2 Hz), 6.18 (1H, s), 4.14 (2H, m), 1.72 (2H, m), 1.64 (3H, s), 1.42 (2H, m), 1.31–1.26 (4H, m), 0.89 (3H, t, $J$ = 6.9 Hz).$^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 180.8, 167.3, 166.0, 164.7, 153.3, 146.1, 134.9, 120.7, 116.1, 104.7, 104.1, 99.6, 79.0, 69.0, 31.4, 29.5, 28.8, 25.5, 22.5, 13.9. HRMS (ESI) calcd for $C_{20}H_{22}O_6Na$ ([M+Na]$^+$) 381.1308, found 381.1316. Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19. Found: C, 67.03; H, 6.19.

#### 5.4.4. 9-Dodecyloxy-3,7-dihidroxy-4a-metyl-4a*H*-benzo[*c*]chromene-2,6-dione (16)

The title compound was prepared from **13d** in 57% yield as yellow acicular crystal. Mp = 119.2–119.8 °C. IR (KBr): 3281, 3054, 2921, 2851, 1681, 1645, 1572, 1508, 1462, 1384, 1356, 1304, 1257, 1180, 1111, 1080, 1029, 893, 855 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 11.30 (1H, s), 6.73 (1H, d, $J$ = 2.2 Hz), 6.69 (1H, s), 6.61 (1H, d, $J$ = 2.2 Hz), 6.40 (1H, s), 6.28 (1H, s), 4.04 (2H, m), 1.82 (2H, m), 1.73 (3H, s), 1.47 (2H, m), 1.39–1.22 (16H, br m), 0.88 (3H, t, $J$ = 7.0 Hz). $^1H$ NMR (600 MHz, DMSO-$d_6$): **16a**: $\delta$ 11.02 (1H, s), 9.53 (1H, s), 6.41 (1H, d, $J$ = 1.7 Hz), 6.30 (1H, d, $J$ = 1.7 Hz), 6.28 (1H, br q, $J$ = 1.2 Hz), 5.70 (1H, s), 3.95 (2H, m), 1.65 (2H, m), 1.57 (3H, d, $J$ = 1.2 Hz), 1.36 (2H, m), 1.23 (16H, br m), 0.85 (3H, t, $J$ = 7.1 Hz). Compound **16**: $\delta$ 11.14 (1H, s), 9.59 (1H, s), 7.09 (1H, d, $J$ = 2.1 Hz), 6.96 (1H, s), 6.70 (1H, d, $J$ = 2.1 Hz), 6.18 (1H, s), 4.13 (2H, m), 1.73 (2H, m), 1.65 (3H, s), 1.41 (2H, m), 1.23 (16H, br m), 0.85 (3H, t, $J$ = 7.1 Hz). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 180.8, 167.4, 166.0, 164.7, 153.3, 146.1, 134.9, 120.7, 116.2, 104.7, 104.1, 99.6, 79.1, 69.0, 31.9, 29.6, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 28.9, 25.9, 22.7, 14.1. HRMS (ESI) calcd for $C_{26}H_{34}O_6Na$ ([M+Na]$^+$) 465.2247, found 465.2249. Anal. Calcd for $C_{26}H_{34}O_6$: C, 70.56; H, 7.74. Found: C, 70.77; H, 8.00.

#### 5.4.5. 2,2-Dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (18)

To a stirred solution of **17** (365.2 mg, 0.95 mmol), **7** (352.4. mg, 1.08 mmol) and $K_2CO_3$ (448.0 mg, 3.24 mmol) in DME (10 mL) was added $PdCl_2$ (dppf) (39.2 mg, 0.054 mmol), and the mixture was stirred at 85 °C for 17 h. After addition of Celite, the mixture was stirred for 30 min and filtered through a pad of Celite. The filtrate was diluted with chloroform, and the organic layer was washed successively with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (8:1) yielded **18** (299.0 mg, 89%) as a white solid. Mp = 187.8–188.4 °C. IR $\nu_{max}$ (KBr): 3003, 2895, 1738, 1582, 1474, 1385, 1311, 1277, 1225, 1119, 1074, 1035, 930, 871, 829 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 7.52 (1H, t, $J$ = 7.8 Hz), 6.97 (1H, dd, $J$ = 1.07 Hz, 7.8 Hz), 6.88 (1H, dd, $J$ = 1.07), 6.71 (1H, s), 6.58 (1H, s), 5.97 (1H, d, $J$ = 1.5 Hz), 5.95 (1H, d, $J$ = 1.5 Hz), 3.96 (2H, m), 2.00 (3H, s), 1.75 (3H, s), 1.74 (3H, s). $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 159.0, 156.7, 146.9, 145.3, 145.1, 135.0, 132.9, 128.2, 125.9, 116.4, 112.9, 109.9, 108.7, 105.2, 100.8, 26.0, 25.3, 19.7. HRMS (ESI) calcd for $C_{18}H_{16}O_5Na$ ([M+Na]$^+$) 335.0889, found 335.0909. Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.42; H, 5.06.

#### 5.4.6. 3,4′,5′-Trihydroxy-2′-methyl-biphenyl-2-carboxylic acid (19)

To a stirred solution of **18** (268.5 mg, 0.86 mmol) in $CH_2Cl_2$ (9.0 mL) was added $BCl_3$ (1.0 M solution in heptane, 4.3 mL,

4.3 mmol) at 0 °C and the mixture was stirred at room temperature for 18 h. Then the mixture was diluted with EtOAc. The organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc–AcOH (1333:667:1) yielded **19** (172.8 mg, 77%) as a yellow foam. IR $\nu_{max}$ (KBr): 3462, 3366, 3044, 1662, 1601, 1514, 1442, 1340, 1281, 1249, 1224, 1171, 1045, 951, 871, 813 $cm^{-1}$. $^1H$ NMR (600 MHz, MeOD): $\delta$ 7.32 (1H, t, $J$ = 7.8 Hz), 6.87 (1H, d, $J$ = 7.8 Hz), 6.61 (1H, d, $J$ = 7.8 Hz), 6.59 (1H, s), 6.50 (1H, s), 1.73 (3H, s). $^{13}C$ NMR (150 MHz, MeOD): $\delta$ 173.7, 161.2, 145.3, 144.9, 143.1, 134.7, 133.7, 127.7, 123.5, 117.4, 117.0, 116.4, 19.4. HRMS (ESI) calcd for $C_{14}H_{12}O_5Na$ $[M+Na]^+$ 283.0576, found 283.572. Anal. Calcd for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65. Found: C, 64.52; H, 4.64.

### 5.4.7. 3,7-Dihidroxy-4a-methyl-4a*H*-benzo[*c*]chromene-2,6-dione (20)

To a solution of **19** (134.7 mg, 0.66 mmol) in EtOH–$H_2O$ (1:1, 3.0 mL) was added a 0.2 M solution of $FeCl_3$ in $H_2O$ (6.0 mL) and the mixture was stirred at room temperature for 10 min. The mixture was diluted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (2:3) yielded **20** (134.8 mg, 80%) as a yellow acicular crystal. Mp = 180.0–181.2 °C. IR $\nu_{max}$ (KBr): 3388, 3103, 3053, 2980, 2927, 1682, 1647, 1578, 1494, 1458, 1419, 1379, 1341, 1318, 1271, 1233, 1207, 1170, 1102, 1051, 1024, 943, 887, 843, 815 $cm^{-1}$. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 11.13 (1H, s), 7.64 (1H, t, $J$ = 8.01 Hz), 7.22 (1H, dd, $J$ = 0.89, 8.02 Hz), 7.19 (1H, dd, $J$ = 0.89, 8.02 Hz), 6.74 (1H, s), 6.73 (1H, s) 6.70 (1H, s), 6.47 (1H, s), 6.29 (1H, s), 1.75 (3H, s). $^1H$ NMR (600 MHz, DMSO-$d_6$): **20a**: $\delta$ 11.10 (1H, s), 9.58 (1H, s), 7.54 (1H, t, $J$ = 7.9 Hz), 6.96 (1H, d, $J$ = 7.9 Hz), 6.71 (1H, d, $J$ = 7.9 Hz), 6.32 (1H, br s), 5.77 (1H, s), 1.55 (3H, br s). Compound **20**: $\delta$ 10.99 (1H, s), 9.61 (1H, s), 7.37 (1H, t, $J$ = 8.4 Hz), 7.51 (1H, d, $J$ = 8.4 Hz), 7.20 (1H, d, $J$ = 8.4 Hz), 6.88 (1H, s), 6.19 (1H, s), 1.67 (3H, s). $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 180.7, 167.5, 162.3, 152.9, 146.3, 137.0, 133.8, 121.1, 120.9, 115.9, 115.7, 106.5, 79.6, 29.7. HRMS (ESI) calcd for $C_{14}H_{10}O_5Na$ ($[M+Na]^+$) 281.0420, found 281.0425. Anal. Calcd for $C_{14}H_{10}O_5$: C, 65.12; H, 3.90. Found: C, 65.42; H, 3.76.

### 5.4.8. 4,4,5,5,-Tetramethyl-2-(2-acetoxyl-4,5-dibenzyloxyphenyl)-1,3,2-dioxaborolane (22)

To a degassed solution of **21** (1.33 g, 2.81 mmol), bis(pinacolato)diboron (1.02 g, 4.02 mmol) and KOAc (0.83 g, 8.46 mmol) in DMF (20 mL) was added $PdCl_2$(dppf) (204 mg, 0.28 mmol). The mixture was stirred at 80 °C for 6 h, then cooled to room temperature and filtrated through a pad of Celite and washed with EtOAc. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed over silica gel. Elution with hexane–ethyl acetate (6:1) gave **22** (711 mg, 53%) as white solids. Mp = 113–115 °C. IR $\nu_{max}$ (KBr): 2983, 1757, 1622, 1514, 1001, 912, 849, 744, 696, 584, 499 $cm^{-1}$. $^1H$ NMR (270 MHz, $CDCl_3$): $\delta$ 7.27–7.45 (11H, m), 6.63 (1H, d, $J$ = 1.6 Hz), 5.10 (4H, s), 2.27 (3H, s), 1.29 (12H, s). $^{13}C$ NMR (68 MHz, $CDCl_3$): $\delta$ 170.4, 152.2, 150.6, 150.6, 146.1, 137.0, 136.3, 128.3 (×2), 128.1 (×2), 127.6, 127.5, 127.3 (×2), 126.9 (×2), 121.4, 108.2, 83.4 (×2), 71.7, 70.7, 24.8 (×4), 21.1. HRMS (ESI) calcd for $C_{28}H_{31}BO_6Na$ ($[M+Na]^+$) 497.2105, found 497.2103.

### 5.4.9. 7-Methoxy-2,2-dimethyl-5-(2-acetoxy-4,4-dibenzyloxyphenyl)-4*H*-1,3-benzodioxin-4-one (24)

To a degassed solution of **22** (126 mg, 0.265 mmol), **23** (76.3 mg, 0.214 mmol) and $K_2CO_3$ (92.3 mg, 0.668 mmol) in DME (3 mL) was added $PdCl_2$(dppf) (8.2 mg, 11.2 μmol). The mixture was stirred at 80 °C for 6.5 h, cooled to room temperature and then filtrated through a pad of Celite and washed with EtOAc. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed over silica gel. Elution with hexane–ethyl acetate (6:1) gave **24** (73.2 mg, 61%) amorphous solids and recovered **23** (15.7 mg, 21%). IR $\nu_{max}$ (KBr): 3001, 2937, 1739, 1610, 1516, 1458, 1383, 1277, 1203, 1093, 1016, 914, 850, 741, 698, 636 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$): $\delta$ 7.27–7.47 (10H, m), 6.83 (1H, s), 6.77 (1H, s), 6.44 (1H, d, $J$ = 2.7 Hz), 6.42 (1H, d, $J$ = 2.7 Hz), 5.13 (4H, s), 3.80 (3H, s), 2.01 (3H, s), 1.74 (6H, s). $^{13}C$ NMR (68 MHz, $CDCl_3$): $\delta$ 169.0, 164.4, 158.6, 158.1, 149.2, 146.4, 141.9, 141.3, 137.1, 136.7, 128.4 (×2), 128.3 (×2), 127.8, 127.6, 127.4 (×2), 127.3 (×2), 125.3, 116.5, 113.0, 108.7, 105.5, 104.9, 101.1, 72.0, 71.3, 55.7, 26.5, 24.9, 20.9. HRMS (ESI) calcd for $C_{33}H_{30}O_8Na$ ($[M+Na]^+$) 577.1832, found 577.1822.

### 5.4.10. 2,3-Dibenzyloxy-7-trihydroxy-9-methoxy-6*H*-benzo[*c*]-chromen-6-one (25)

To a solution of **24** (73.2 mg, 0.132 mmol) and $K_2CO_3$ (92.3 mg, 0.139 mmol) in MeOH (5 mL) was stirred at room temperature for 5 h. The mixture was diluted with $CHCl_3$, and the reaction was quenched by the addition of 1 M HCl solution. The layers were separated. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed over silica gel. Elution with $CHCl_3$ gave **25** (46.3 mg, 77%) as white solids. Mp = 199–200 °C. IR $\nu_{max}$ (KBr): 3444, 3068, 2941, 2875, 1668, 1626, 1568, 1512, 1456, 1410, 1371, 1315, 1275, 1188, 1157, 1095, 1034, 993, 924, 868, 833, 750, 696 $cm^{-1}$. $^1H$ NMR (270 MHz, $CDCl_3$): $\delta$ 11.52 (1H, s), 7.50–7.32 (11H, m), 6.50 (1H, d, $J$ = 2.4 Hz), 5.22 (4H, s), 3.91 (3H, s). $^{13}C$ NMR (68 MHz, $CDCl_3$): $\delta$ 166.7, 165.2, 164.6, 151.6, 146.0, 146.0, 136.7, 136.6, 135.8, 128.6 (×2), 128.5 (×2), 128.1, 128.0, 127.4 (×2), 127.1 (×2), 110.5, 108.9, 102.9, 99.8, 99.2, 98.7, 72.3, 71.1, 55.8. HRMS (ESI) calcd for $C_{28}H_{22}O_6Na$ ($[M+Na]^+$) 477.1308, found 477.1327. Anal. Calcd for $C_{28}H_{22}O_6$: C, 74.00; H, 4.88. Found: C, 74.13; H, 4.77.

### 5.4.11. 2,3,7-Trihydroxy-9-methoxy-6*H*-benzo[*c*]chromen-6-one (26)

A solution of **25** (34.3 mg, 75.5 μmol) and $Pd(OH)_2$ on carbon powder (20% Pd, 5.8 mg) in THF (6 mL) was stirred under an atmosphere of $H_2$ at room temperature for 9 h. The mixture was filtrated through a pad of Celite and washed with $CHCl_3$–MeOH (10:1). The filtrate was concentrated. The residue was chromatographed over silica gel. Elution with $CHCl_3$–MeOH (20:1→10:1) gave **26** (21.1 mg, quant.) as white solids. Mp = 272 °C (decomp.). IR $\nu_{max}$ (KBr): 3531, 1645, 1516, 1454, 1277, 1161, 1032, 976, 914, 837, 785 $cm^{-1}$. $^1H$ NMR (270 MHz, $CD_3OD$): $\delta$ 7.36 (1H, s), 6.88 (1H, d, $J$ = 2.2 Hz), 6.82 (1H, s), 6.49 (1H, d, $J$ = 2.2 Hz), 3.94 (3H, s). $^{13}C$ NMR (68 MHz, DMSO-$d_6$): $\delta$ 166.6 (×2), 164.9, 163.6, 149.3, 144.2, 143.7, 137.5, 108.9, 103.4, 100.0, 98.6, 97.9, 56.1. HRMS (ESI) calcd for $C_{14}H_{10}O_6Na$ ($[M+Na]^+$) 297.0369, found 297.0359.

### 5.4.12. 4,4,5,5-Tetramethyl-2-(2-methyl-4-hydroxyphenyl)-1,3,2-dioxaborolane (28)

To a degassed solution of **27** (180 mg, 0.770 mmol), bis(pinacolato)diboron (256 mg, 1.00 mmol) and KOAc (246 mg, 2.51 mmol) in DMF (5 mL) was added $PdCl_2$(dppf) (56.3 mg, 76.4 μmol). The mixture was stirred at 70 °C for 11 h, cooled to room temperature and then filtrated through a pad of Celite and washed with EtOAc. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed over silica gel. Elution with hexane–ethyl acetate (7:1) gave **28** (155 mg, 86%) as white solids. Mp = 113–114 °C. IR $\nu_{max}$ (KBr): 3394, 2976, 2929, 1599, 1506, 1433, 1356, 1290, 1217, 1142, 1063, 962, 914, 856, 820, 752, 727 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$): $\delta$ 11.52 (1H, s), 7.50–7.32 (11H, m), 6.50 (1H, d, $J$ = 2.4 Hz), 5.22 (4H, s), 3.91 (3H, s). $^{13}C$ NMR (68 MHz, $CDCl_3$): $\delta$ 152.6, 136.5, 131.0, 121.9,

117.8, 117.8, 83.6 (×2), 24.9 (×4), 21.9. HRMS (EI) calcd for $C_{13}H_{19}BO_3$ ([M]$^+$) 234.1427, found 234.1426.

#### 5.4.13. 5-(5-Hydroxy-2-methylphenyl)-7-methoxy-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (29)

To a degassed solution of **23** (90.6 mg, 0.254 mmol), **28** (49.6 mg, 0.212 mmol) and $K_2CO_3$ (89.2 mg, 0.645 mmol) in DME (4 mL) was added $PdCl_2$(dppf) (19.2 mg, 26.2 μmol). The mixture was stirred at 55 °C for 9 h, cooled to room temperature and then filtrated through a pad of Celite and washed with EtOAc. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed over silica gel. Elution with hexane–ethyl acetate (3:1) gave **29** (44.1 mg, 76%) as amorphous solids. IR $\nu_{max}$ (KBr): 3437, 3074, 2925, 2854, 1738, 1616, 1579, 1423, 1281, 1209, 1144, 1049, 939, 868, 768, 704, 671 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ 7.03 (1H, d, $J$ = 8.4 Hz), 6.68 (1H, dd, $J$ = 8.4 Hz, 2.7 Hz), 6.56 (1H, d, $J$ =2.7 Hz), 6.45 (1H, d, $J$ = 2.7 Hz), 6.43 (1H, d, $J$ = 2.7 Hz), 5.45 (1H, br s), 3.84 (3H, s), 2.00 (3H, s), 1.73 (6H, s). $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ 164.6, 159.2, 158.4, 153.4, 146.8, 141.0, 130.4, 126.5, 115.2, 114.6, 112.5, 105.3, 105.2, 100.7, 55.8, 26.2, 25.2, 19.0. HRMS (ESI) calcd for $C_{18}H_{18}O_5Na$ ([M+Na]$^+$) 337.1046, found 337.1040.

#### 5.4.14. 5-(5-Hydroxy-2-methylphenyl)-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (30)

To a degassed solution of **17** (258 mg, 0.792 mmol), **28** (144 mg, 0.614 mmol) and potassium carbonate (256 mg, 1.85 mmol) in DME (7 mL) was added $PdCl_2$(dppf) (86.4 mg, 185 μmol). The mixture was stirred at 55 °C for 23 h, cooled to room temperature and then filtrated through a pad of Celite and washed with EtOAc. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed over silica gel. Elution with hexane–ethyl acetate (3:1) gave **30** (118 mg, 67%) as amorphous solids. IR $\nu_{max}$ (KBr): 3377, 2997, 2924, 2860, 1718, 1581, 1477, 1385, 1319, 1205, 1045, 926, 845, 814, 754, 698 cm$^{-1}$. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ 7.51 (1H, br t, $J$ = 7.6 Hz), 7.05–6.94 (2H, m), 6.86 (1H, br d, $J$ = 7.6 Hz), 6.54 (1H, d, $J$ = 2.7 Hz), 6.63 (1H, m), 5.93 (1H, br s), 1.97 (3H, s), 1.73 (6H, s). $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ 159.6, 156.4, 153.5, 145.1, 140.1, 135.2, 130.3, 126.2, 125.5, 116.3, 115.3, 114.7, 112.5, 105.2, 26.1, 25.2, 19.0. HRMS (EI) calcd for $C_{17}H_{16}O_4$ ([M]$^+$) 284.1049, found 284.1049.

#### 5.4.15. 7-Hydroxy-9-methoxy-4a-methyl-4a*H*-benzo[*c*]chromene-2,6-dione (31)

A solution of **29** (44.1 mg, 0.140 mmol) and LiOH·$H_2O$ (130 mg, 3.09 mmol) was stirred at 50 °C for 18 h. The mixture was diluted with EtOAc. The reaction was quenched by the addition of 1 M HCl solution. The layers were separated. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated.

To a solution of the crude carboxylic acid (39.7 mg) in MeOH (3 mL) was added PhI(OAc)$_2$ (67.6 mg, 0.209 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated. The residue was chromatographed over silica gel. Elution with $CHCl_3$ gave **31** (33.7 mg, 77% in two steps) as pale yellow solids. Mp = 225–230 °C. IR $\nu_{max}$ (KBr): 3450, 3209, 3060, 2925, 2852, 1676, 1614, 1570, 1487, 1433, 1389, 1358, 1290, 1196, 1167, 1113, 1070, 1036, 978, 839, 644 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ 11.3 (1H, s), 7.10 (1H, d, $J$ = 10.0 Hz), 6.74 (1H, d, $J$ = 2.4 Hz), 6.62 (1H, d, $J$ = 2.4 Hz), 6.62 (1H, d, $J$ = 1.9 Hz), 6.33 (1H, dd, $J$ = 10.0 Hz, 1.9 Hz), 3.91 (3H, s), 1.70 (3H, s). $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ 184.4, 166.9, 166.3, 164.6, 149.3, 147.0, 135.1, 127.8, 123.3, 104.0, 103.4, 99.7, 56.0, 28.5. HRMS (EI) calcd for $C_{15}H_{12}O_5$ ([M]$^+$) 272.0685, found 272.0692.

#### 5.4.16. 7-Hydroxy-4a-methyl-4a*H*-benzo[*c*]chromene-2,6-dione (32)

A solution of **30** (55.2 mg, 0.194 mmol) and LiOH·$H_2O$ (164 mg, 3.90 mmol) was stirred at 50 °C for 18 h. The mixture was diluted with EtOAc. The reaction was quenched by the addition of 1 M HCl solution. The layers were separated. The organic layer was washed with water, brine, dried ($Na_2SO_4$) and concentrated.

To a solution of the crude carboxylic acid (33.1 mg) in MeOH (3 mL) was added PhI(OAc)$_2$ (75.6 mg, 0.235 mmol) at 0 °C, and the mixture was stirred at room temperature for 1.5 h. The mixture was concentrated. The residue was chromatographed over silica gel. Elution with $CHCl_3$ gave **32** (30.2 mg, 64% in two steps) as yellow solids. Mp = 124–129 °C; IR $\nu_{max}$ (KBr): 3348, 3055, 2925, 2925, 2854, 1674, 1635, 1577, 1493, 1460, 1367, 1281, 1213, 1149, 1067, 1032, 930, 906, 822, 730, 698 cm$^{-1}$. $^1$H NMR (270 MHz, $CDCl_3$): $\delta$ *7.64 (1H,* br t, $J$ = 8.4 Hz), 7.23 (1H, dd, $J$ = 7.6 Hz, 0.8 Hz), 7.17 (1H, dd, $J$ = 8.4 Hz, 0.8 Hz), 7.11 (1H, d, $J$ = 10.0 Hz), 6.66 (1H, d, $J$ = 1.6 Hz), 6.35 (1H, dd, $J$ = 10.0 Hz, 1.6 Hz), 1.72 (3H, s). $^{13}$C NMR (68 MHz, $CDCl_3$): $\delta$ 184.3, 167.1, 162.1, 149.1, 146.7, 137.0, 133.8, 127.9, 123.4, 120.6, 115.5, 106.2, 28.7. HRMS (EI) calcd for $C_{14}H_{10}O_4$ ([M]$^+$) 242.0579, found 242.0581.

#### 5.4.17. 3′-{[2-(Acetylamino)-3-methoxy-3-oxopropyl]sulfanyl}-3,4′,5′-trihydroxy-2′-methyl-5-methoxybiphenyl-2-carboxylic acid (34) and 2′-{[2-(acetylamino)-3-methoxy-3-oxopropyl]-sulfanyl}-3,3′,4′-trihydroxy-6′-methyl-5-methoxybiphenyl-2-carboxylic acid (35)

A solution of **1** (21.2 mg, 0.074 mmol) and **33** (21.8, 0.123 mmol) in MeOH–$H_2O$ (1:1, 2 mL) was stirred at room temperature for 30 min and then concentrated. The residue was purified by silica gel chromatography ($CHCl_3$–MeOH = 9:1 with 1% AcOH) to give the crude product (35.3 mg). This crude products was further purified by silica gel chromatography (EtOAc–MeOH = 10:1 with 0.5% AcOH) to give a 1:1 inseparable mixture of **34** and **35** (30.2 mg, 91%) as amorphous solids.[33] IR $\nu_{max}$ (KBr): 3367, 3089, 3010, 2954, 2850, 1739, 1653, 1608, 1423, 1375, 1281, 1255, 1205, 1157, 1072, 1039, 983, 910, 842, 800, 756, 723, 667 cm$^{-1}$. $^1$H NMR (270 MHz, $CDCl_3$ with 1% TFA at 40 °C). Compound **34**: $\delta$ 6.84 (1H, s), 6.54 (1H, d, $J$ = 2.7 Hz), 6.22 (1H, d, $J$ = 2.7 Hz), 4.65 (1H, m), 3.83 (3H, s), 3.74 (3H, s), 2.76 (2H, m), 2.18 (3H, s), 1.98 (3H, s). Compound **35**: $\delta$ 6.84 (1H, s), 6.54 (1H, d, $J$ = 2.7 Hz), 6.14 (1H, d, $J$ = 2.7 Hz), 4.75 (1H, m), 3.85 (3H, s), 3.73 (3H, s), 2.88 (2H, m), 2.18 (3H, s), 1.93 (3H, s). HRMS (ESI) calcd for $C_{21}H_{23}NO_9NaS$ ([M+Na]$^+$) 488.0985, found 488.0979.

### 5.5. DNA polymerase assay

In mammalian DNA polymerases, DNA polymerase α was purified from calf thymus by immuno-affinity column chromatography as described previously.[34] DNA polymerase β was purified from a recombinant plasmid expressing rat DNA polymerase β.[35] The reaction mixtures for DNA polymerases α and β were described previously.[36,37] For the DNA polymerases, poly(dA)/oligo(dT)$_{12-18}$ (A/T = 2/1) and [$^3$H]-dTTP were used as the DNA template-primer and nucleotide (i.e., 2′-deoxyribonucleotide 5′-triphosphate, dNTP) substrate, respectively. The dehydroaltenusin derivative was dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Four microlitres of each sonicated sample were mixed with 16 μL of each enzyme (final 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM ethylenediamine tetraacetic acid (EDTA), and kept at 0 °C for 10 min. These inhibitor–enzyme mixtures (8 μL) were added to 16 μL of each enzyme standard reaction mixture, and incubated at 37 °C for 60 min. Activity without the inhibitor was considered 100%, and the remaining activity at each concentration

of the inhibitor was determined relative to this value. One unit of pol activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of dNTP (i.e., dTTP) into synthetic DNA template-primer in 60 min at 37 °C under the normal reaction conditions for each enzyme.[36,37]

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