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Structure–activity relationships of untenone A and its derivatives for inhibition of DNA polymerases

Fumiyo Saito,[a,b] Ryo Takeuchi,[a,c] Tomoyuki Kamino,[a,b] Kouji Kuramochi,[a] Fumio Sugawara,[a,c] Kengo Sakaguchi[a,c] and Susumu Kobayashi[a,b,*]

[a]*Frontier Research Center for Genome and Drug Discovery, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*
[b]*Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*
[c]*Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*

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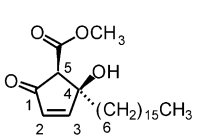
Abstract—We found that untenone A and mannzamenone A inhibit mammalian DNA polymerases α and β, and human terminal deoxynucleotidyl transferase (TdT). The syntheses of both compounds and the structure–activity relationships of untenone A derivatives are described.

1. Introduction

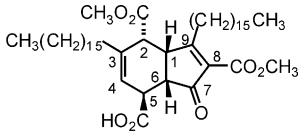
Untenone A (**1**), which was isolated from an Okinawan sponge, *Plakortis* sp., inhibits the cell proliferation of L1210 leukemia.[1] The related manzamenone A (**2**) was also isolated from an Okinawan *Plakortis* sponge.[2] Manzamenone A was reported to display inhibitory activity against protein kinase C. Untenone A has been considered to be an intermediate in the biosynthetic pathway of manzamenone A.[1–3]

Plakevulin A (**3**) has been recently isolated from extracts of an Okinawan *Plakortis* sponge (SS-973).[4] Plakevulin A, which has a cyclopentene ring and a levulinyl ester, exhibited cytotoxicity against murine leukemia L1210 and carcinoma KB cells. Plakevulin A also exhibited inhibitory activity against DNA polymerases α and γ.

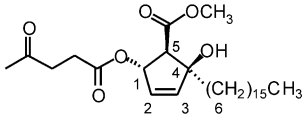
We have been studying DNA polymerses because we are interested in the activities of these enzymes, for example, DNA replication, repair, cell divisions and so on. From our screenings, we have found both natural and synthetic products which inhibit activities of DNA polymerases.[5] The recent report on plakevulin A prompted us to investigate inhibitory activities of the related untenone A and manzamenone A. We also became interested in the structure–activity relationships of such cyclopentene derivatives for inhibitory activity against DNA polymerases.



Untenone A (1)



Manzamenone A (2)



Plakevulin A (3)

2. Chemistry

The syntheses of (±)-untenone (**1**) and (±)-manzamenone (**2**) were followed by the previously published procedures by Yamada[6] and Whitehead[3] as shown in Scheme 1. (±)-Untenone (**1**) was synthesized from *cis*-4-cyclopentene-1,3-diol (**5**), which was prepared by singlet oxygenation of 1,3-cyclopentadiene (**4**) in the presence

* Corresponding author. Tel./fax: +81-4-7121-3671; e-mail: kobayash@rs.noda.tus.ac.jp

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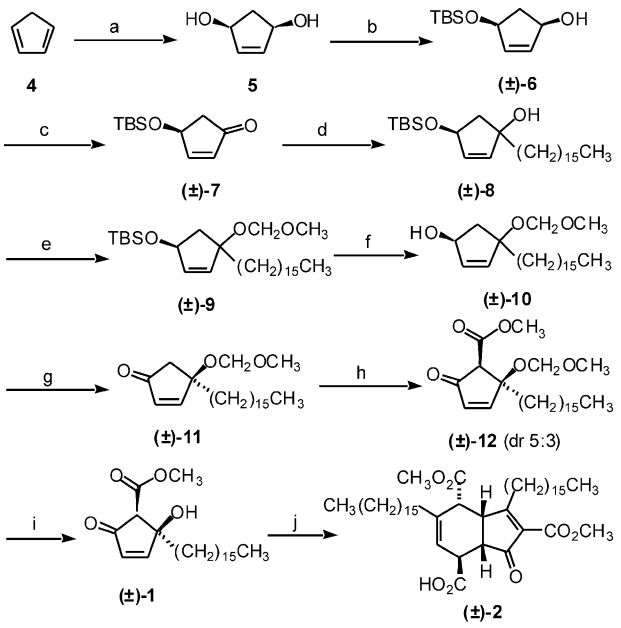
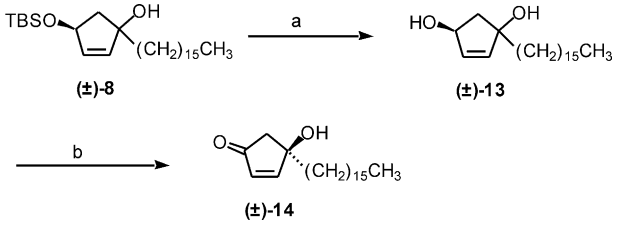
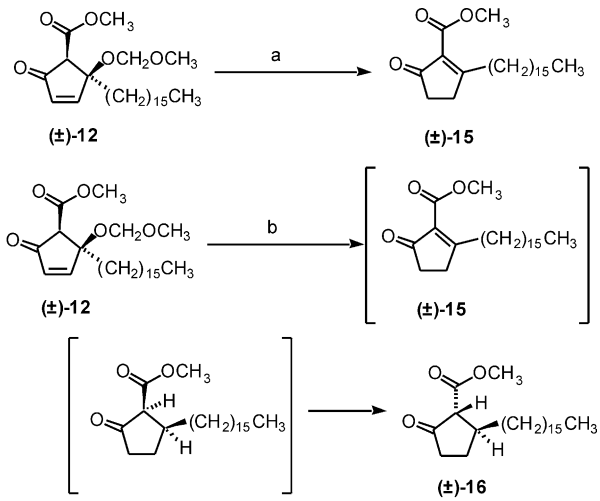


Scheme 1. Syntheses of (±)-**1** and (±)-**2**. Reagents and conditions: (a) *hν*, O_2, rose bengal, thiourea, MeOH, 0 °C; (b) *n*-BuLi, TBSCl, THF, −78 °C–0 °C; (c) PCC, MS4A, CH_2Cl_2; (d) $CH_3(CH_2)_{15}Br$, SmI_2, THF–HMPA; (e) MOMCl, *i*-Pr_2NEt, $(CH_2Cl)_2$; (f) TBAF, THF; (g) Jones reagent, acetone, 0 °C; (h) LDA, THF–HMPA then $NCCO_2CH_3$; (i) AcOH–conc. HCl (50:1); (j) Δ.

of rose bengal and thiourea.[7] Monosilylation of **5** by using *n*-BuLi (1.0 equiv) and TBSCl (1.2 equiv) gave (±)-**6**. Oxidation of (±)-**6**, followed by addition of an alkylsamarium reagent gave (±)-**8**. The hydroxy group of (±)-**8** was protected as methoxymethyl ether (±)-**10**. Oxidation of the secondary alcohol followed by methoxycarbonylation with LDA and $NCCO_2CH_3$ gave methoxymethyl protected untenone (±)-**12**. Deprotection of (±)-**12** with AcOH–conc. HCl afforded (±)-untenone A (**2**). (±)-Manzamenone A (**3**) was prepared from (±)-**2** by heating via a unique biogenetic pathway.[3c] We also prepared several analogues in order to examine structure–activity relationships. Hydroxy ketone **14** was synthesized by Jones oxidation of diol **13** in 88% yield (Scheme 2). Hydrogenation of the double bond and elimination of the methoxymethyloxy group occurred by the treatment of (±)-**12** with 10% Pd on carbon under H_2 atmosphere to yield (±)-**15** (Scheme 3).[8] Meanwhile, the treatment of (±)-**12** with $Pd(OH)_2$ under H_2 atmosphere gave (±)-**16** by further hydrogenation and isomerization of the β-ketoester. The stereochemistry of (±)-**16** was established by a NOESY experiment.[9]



Scheme 2. Synthesis of (±)-**14**. Reagents and conditions: (a) TBAF, THF, quant; (b) Jones reagent, acetone, 0 °C, 88%.



Scheme 3. Syntheses of (±)-**15** and **16**. Reagents and conditions: (a) H_2, Pd–C, EtOAc, quant; (b) H_2, $Pd(OH)_2$, EtOAc, quant.

3. Results and discussion

All synthetic derivatives were subjected to enzymatic inhibition assays of mammalian DNA polymerases α (pol. α) and β (pol. β), and human terminal deoxynucleotidyl transferase (TdT). The assay methods employed here were previously described by Mizushina et al.,[10] and the results are shown in Table 1.

We found that synthetic untenone A (**1**) inhibited DNA polymerases α and β, and TdT. Comparing with the inhibitory activity of plakevulin A against DNA polymerase α (IC_{50} 57 μM) and DNA polymerase β (IC_{50} 146 μM),[4] untenone A was found to possess stronger and more selective inhibition of pol. α. And manzamenone A (**2**) was found to be a strong inhibitor of pol. α, pol. β and TdT. Methoxymethyl-protected untenone **12** was also active in the enzyme assay to exhibit decreased selectivity compared to untenone A. On replacement of a methoxycarbonyl group with hydrogen (**14**), the inhibitory activity against both pol. α and pol. β and TdT was completely lost. The α,β-unsaturated β-ketoester **15** showed lower inhibition than untenone A. The saturated deoxygenated derivative **16** showed similar activity against pol. α, pol. β and TdT with **14**. Thus, the β-ketoester group was very important for the activity. And the substituents of the 4-position affected the inhibitory activity.

Table 1. IC_{50} values of enzymatic inhibition against mammalian DNA polymerases α (pol. α) and β (pol. β), and human terminal deoxynucleotidyl transferase (TdT)

Compd	Pol. α	IC_{50} (μM) Pol. β	TdT
(±)-**1**	4.3	57	16
(±)-**2**	1.9	3.2	2.5
(±)-**12**	5.9	9.3	18
(±)-**14**	>200	>200	>200
(±)-**15**	17	107	129
(±)-**16**	20	90	84

In conclusion, we have found that synthetic (±)-untenone A (**1**) and (±)-manzamenone A (**2**) inhibited mammalian DNA polymerases α and β, and human terminal deoxynucleotidyl transferase (TdT). We also found that untenone A was a relatively selective inhibitor of DNA polymerase α. In order to examine the structure–activity function relationships of untenone A, we synthesized untenone derivatives **12**, **14**, **15** and **16** and investigated the inhibitory activities for each enzyme.[11] And we found that the β-ketoester moiety was essential for these activities. The substituents at the 4-position influenced the inhibitory activity of DNA polymerases α and β, and TdT as well as selective inhibition of DNA polymerase α.

Acknowledgements

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8. Reduction of the double bond of compound (±)-**12** was unsuccessful by both hydrogenation and 1,4-reduction. Hydrogenation of **12** using catalytic Rh-Al_2O_3 and PtO_2-C gave (±)-**16**. The use of other reductants ($NaBH_4$ in MeOH, Mg in MeOH and CuCl, $PhMe_2SiH$ in DMF, etc) gave complex mixture.
9. The NOESY correlation was observed for H-5/H-6.
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11. All new compounds were fully characterized by ^1H NMR, ^{13}C NMR, IR spectra and satisfactory high-resolution MS were obtained. **14**: mp = 44–49 °C. IR (film) 3418, 3017, 2925, 2854, 1715, 1589, 1465, 1404, 1339, 1215, 1067, 801, 759, 668 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.8 Hz), 1.19–1.27 (28H, brm), 1.74 (2H, m), 1.80 (1H, brs), 2.44 (1H, d, J = 18.3 Hz), 2.56 (1H, d, J = 18.3 Hz), 6.13 (1H, d, J = 5.6 Hz), 7.41 (1H, d, J = 5.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 24.2, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7 (×5), 29.8, 31.9, 40.3, 48.8, 79.2, 133.3, 165.8, 206.9. HRMS calcd for $C_{21}H_{38}O_2Na$ ($M + Na^+$) 345.2764, found 345.2765. **15**: IR (film) 3020, 2926, 2854, 1739, 1714, 1620, 1465, 1437, 1361, 1295, 1259, 1216, 1155, 1026, 758, 667 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 7.0 Hz), 1.26–1.32 (23H, brm), 1.36 (1H, m), 1.57 (4H, m), 2.49 (2H, m), 2.68 (2H, m), 2.76 (2H, t, J = 7.9 Hz), 3.84 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 27.7, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7 (×7), 30.4, 31.9, 32.7, 34.9, 51.8, 163.8, 189.0, 203.8. HRMS calcd for $C_{23}H_{40}O_3Na$ ($M + Na^+$) 387.2869, found 387.2868. **16**: mp = 38–41 °C. IR (film) 3021, 2927, 2855, 1754, 1726, 1463, 1439, 1216, 1129, 927, 759, 669 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.9 Hz), 1.23–1.31 (27H, brm), 1.36 (1H, m), 1.43 (1H, m), 1.47 (1H, m), 1.54 (1H, m), 2.23 (1H, m), 2.32 (1H, m), 2.42 (1H, dd, J = 8.3 Hz, 18.7 Hz), 2.57 (1H, m), 2.83 (1H, d, J = 11.2 Hz), 3.76 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 27.2, 27.4, 29.4, 29.5, 29.6, 29.6, 29.7 (x 7), 31.9, 35.0, 38.5, 41.5, 52.4, 61.9, 170.1, 212.1. HRMS calcd for $C_{23}H_{42}O_3Na$ ($M + Na^+$) 389.3026, found 389.3039.