

# The specific inhibitory effect of demethoxydehydroaltenusin, a derivative of dehydroaltenusin, on mammalian DNA polymerase $\alpha$

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**Abstract.** In the screening of selective inhibitors of eukaryotic DNA polymerases (pols), dehydroaltenusin from the fungus *Acremonium* sp. was found to be an inhibitor of pol  $\alpha$ . The present study succeeded in chemically synthesizing dehydroaltenusin, and the compound strongly inhibited calf pol  $\alpha$  activity and weakly suppressed rat pol  $\beta$  activity, with  $IC_{50}$  values of 0.68 and 64  $\mu$ M, respectively. We purified or synthesized various slightly modified derivatives of dehydroaltenusin, and using these, investigated the relationship between chemical structure and the inhibitory effects. These results suggest that the ketone group at the 5'-position in dehydroaltenusin is essential for pol inhibitory activity, and the group at the 5-position is important for the specificity of pol  $\alpha$  inhibition. Demethoxydehydroaltenusin was found to be the most specific pol  $\alpha$  inhibitor among the prepared derivatives, and the  $IC_{50}$  values for pols  $\alpha$  and  $\beta$  were 0.24 and 89  $\mu$ M, respectively. This compound did not influence the activities of other replicative pols such as pols  $\delta$  and  $\epsilon$ , and also demonstrated no effect on pol  $\alpha$  activity from another vertebrate, fish and a plant species. Demethoxydehydroaltenusin also had no influence on the other pols and DNA metabolic enzymes tested. Therefore, demethoxydehydro-

altenusin is of interest as a mammalian pol  $\alpha$ -selective inhibitor as a 'chemical knockout agent' *in vitro* and *in vivo*.

## Introduction

The human genome encodes 16 DNA polymerases (pols) that control cellular DNA synthesis (1). Eukaryotic cells reportedly contain three replicative types: pols  $\alpha$ ,  $\delta$  and  $\epsilon$ , mitochondrial pol  $\gamma$ , and at least twelve repair types: pols  $\beta$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$ ,  $\theta$ ,  $\iota$ ,  $\kappa$ ,  $\lambda$ ,  $\mu$ , and  $\sigma$  and REV1 (2). Selective inhibitors of each pol are useful tools and molecular probes for distinguishing pols and for clarifying their biological and *in vivo* functions (3). For example, aphidicolin is a selective inhibitor of both pol  $\alpha$  and eukaryotic DNA replicative pols  $\delta$  and  $\epsilon$ , indicating that these pols are essential for DNA replication (4), and this inhibitor has been very useful for studying the DNA replication system (5); however, aphidicolin is not capable of distinguishing pols  $\alpha$ ,  $\delta$  and  $\epsilon$ .

Therefore, we established an assay method to detect pol inhibitors, and have screened for natural sources of inhibitors for more than 10 years. An inhibitor was isolated that selectively influences the activity of mammalian pol  $\alpha$ , dehydroaltenusin, from a fungus (*Acremonium* sp.) collected from fields in the vicinity of Noda city in Chiba prefecture, Japan (6). A total chemical synthesis method was established for dehydroaltenusin and succeeded in completely synthesizing the compound (7-9).

Subsequently, the slightly modified derivatives were purified or chemically synthesized to examine the structural relationship between dehydroaltenusin and pols. Dehydroaltenusin and its analogues represent a group of potentially useful agents to examine the precise role of each pol *in vivo*, and to develop a drug design strategy for cancer chemotherapy agents. The compounds can also be useful tools as molecular probes to study the three-dimensional structure of mammalian pol  $\alpha$  protein.

## Materials and methods

**Materials.** Nucleotides and chemically synthesized DNA template-primers such as [<sup>3</sup>H]-2'-deoxythymidine 5'-tri-

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**Abbreviations:** pol, DNA-directed DNA polymerase (EC 2.7.7.7);  $IC_{50}$ , 50% inhibitory concentration; dTTP, 2'-deoxythymidine 5'-triphosphate; dNTP, 2'-deoxyribonucleotide 5'-triphosphate; HIV-1, human immunodeficiency virus type-1

**Key words:** demethoxydehydroaltenusin, dehydroaltenusin, DNA polymerase  $\alpha$ , enzyme inhibitor, chemical knockout agent, DNA replication

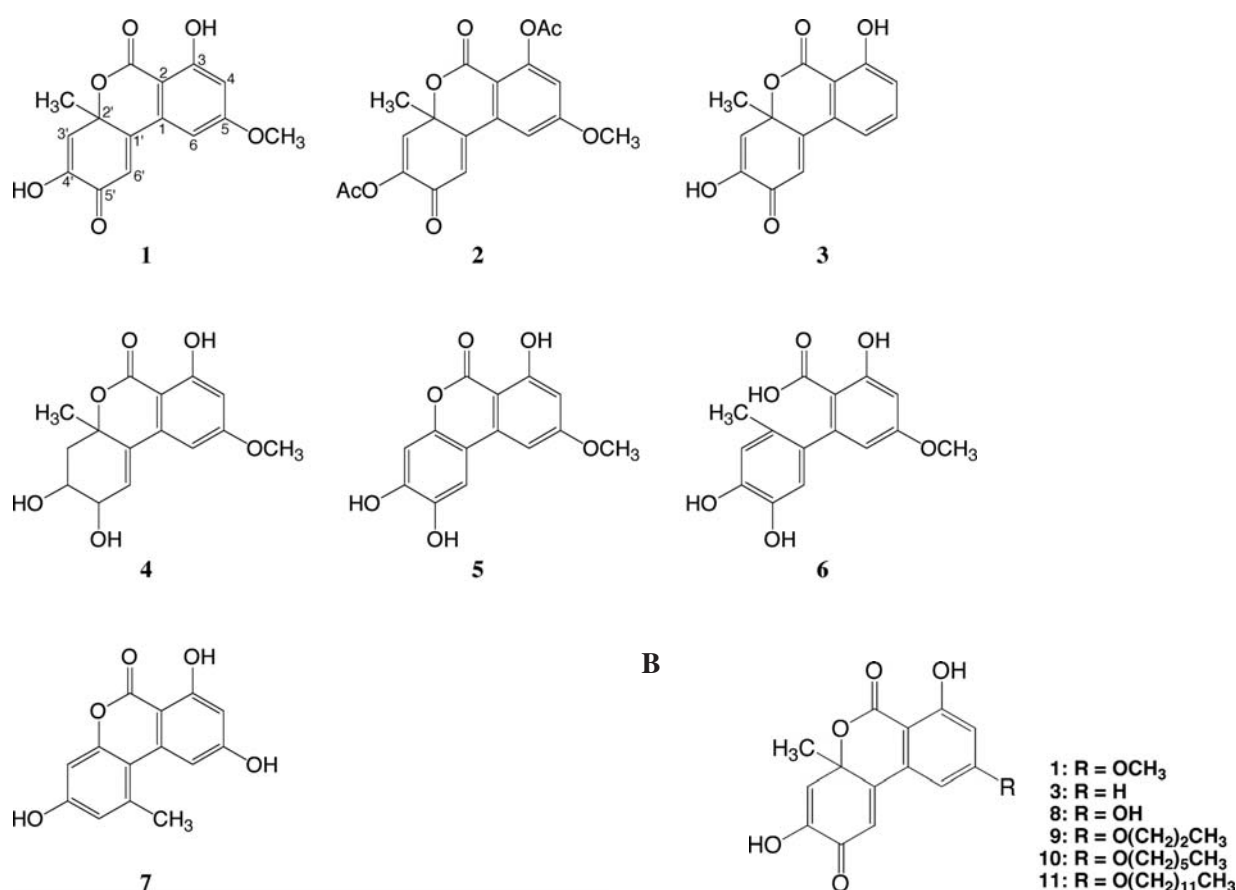


Figure 1. Chemical structures of dehydroaltenusin and its derivatives. (A) Core-modified dehydroaltenusin analogues. (B) Demethoxydehydroaltenusin derivatives modified at the 5-position. 1, Dehydroaltenusin; 2, acetyl-dehydroaltenusin; 3, demethoxydehydroaltenusin; 4, altenuene; 5, 6H-benzo[*c*]chromen-6-one derivative; 6, altenusin; 7, alternariol; 8, demethyldehydroaltenusin; 9, dehydroaltenusin-C3; 10, dehydroaltenusin-C6; and 11, dehydroaltenusin-C12.

phosphate (dTTP, 43 Ci/mmol), poly(dA), and oligo(dT)<sub>12-18</sub> were purchased from GE Healthcare Bio-Science Co. (Buckinghamshire, UK). All other reagents were of analytical grade and were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

**Preparation of dehydroaltenusin and its derivatives.** Dehydroaltenusin **1** and altenusin **6** were synthesized chemically according to a previously reported method (7,8). Acetyl-dehydroaltenusin **2** was prepared by acetylation of dehydroaltenusin-C3 **9**. Demethoxydehydroaltenusin **3** and a 6H-benzo[*c*]chromen-6-one derivative **5** were newly synthesized starting from 2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl trifluoromethanesulfonate (10,11) and 4,5-bis(benzyloxy)-2-iodophenyl acetate (12), respectively. Altenuene **4** and alternariol **7** were isolated from the extract of *Acremonium* sp. (13). Demethyldehydroaltenusin **8**, dehydroaltenusin-C3 derivative **9**, dehydroaltenusin-C6 derivative **10** and dehydroaltenusin-C12 derivative **11** were prepared from a common intermediate, 7-hydroxy-2,2-dimethyl-5-(6-methyl-1,3-benzodioxol-5-yl)-4H-1,3-benzodioxin-4-one. All new synthetic products were fully characterized by <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR), infrared spectroscopy (IR) and high resolution mass spectrometry (HRMS). The chemical structures of dehydroaltenusin derivatives **1-11** are

shown in Fig. 1. The synthetic details and characterization for all chemical derivatives will be reported elsewhere (Kuramochi *et al.*, unpublished data).

**DNA polymerases and other DNA metabolic enzymes.** Pol  $\alpha$  was purified from calf thymus by immuno-affinity column chromatography as described previously (14). Pol  $\beta$  was purified from a recombinant plasmid expressing rat pol  $\beta$  (15). The gene encoding the human pol  $\gamma$  catalytic subunit was cloned into pFastBac. The histidine-tagged enzyme was expressed using the BAC-TO-BAC HT Baculovirus Expression System according to the manufacturer's instructions (Life Technologies, MD, USA) and purified using ProBoundresin (Invitrogen Japan, Tokyo, Japan) (16). Human pols  $\delta$  and  $\epsilon$  were purified by the nuclear fractionation of human peripheral blood cancer cells (Molt-4) using the second subunit of pols  $\delta$ - and  $\epsilon$ -conjugated affinity column chromatography, respectively (17). Recombinant human pol  $\eta$  and  $\iota$  tagged with His<sub>6</sub> at their C-terminal ends were expressed in SF9 insect cells using a baculovirus expression system, and were purified from the cells as described previously (18,19). A truncated form of pol  $\kappa$  (i.e. hDINB1DC) with a 6 x His-tag attached at the C-terminus was overproduced using the BAC-to-BAC Baculovirus Expression System Kit (Gibco BRL) and purified as described by Ohashi *et al.* (20).

Table I. IC<sub>50</sub> values of core-modified dehydroaltenusin analogues on the activities of mammalian DNA polymerase  $\alpha$  and  $\beta$ .

Compound	IC <sub>50</sub> value ( $\mu$ M)	
	Pol $\alpha$	Pol $\beta$
<b>1</b>	0.68	64
<b>2</b>	7.40	48
<b>3</b>	0.24	89
<b>4</b>	>100	>100
<b>5</b>	>100	>100
<b>6</b>	>100	>100
<b>7</b>	>100	>100

**1**, Dehydroaltenusin; **2**, acetyl-dehydroaltenusin; **3**, demethoxy-dehydroaltenusin; **4**, altenuene; **5**, 6*H*-benzo[*c*]chromen-6-one derivative; **6**, altenusin; **7**, alternariol. These compounds were incubated with calf pol  $\alpha$  or rat pol  $\beta$  (0.05 units each). Enzyme activity in the absence of the compound was taken as 100%.

Recombinant His-pol  $\lambda$  was overexpressed and purified according to the method described previously (21). Fish pols  $\alpha$  and  $\delta$  were purified from the testes of cherry salmon (*Oncorhynchus masou*) (22). Pol I ( $\alpha$ -like) from a higher plant, cauliflower inflorescence, was purified according to the methods outlined by Sakaguchi *et al* (23). Recombinant rice (*Oryza sativa* L. cv. Nipponbare) pol  $\lambda$  tagged with His<sub>6</sub> at the C-terminal was expressed in *E. coli* and purified from the cells as described by Uchiyama *et al* (24). The Klenow fragment of pol I from *E. coli* and human immunodeficiency virus type-1 (HIV-1) reverse transcriptase were purchased from Worthington Biochemical Corp. (Freehold, NJ, USA). *Taq* pol, T4 pol and T4 polynucleotide kinase were purchased from Takara (Kyoto, Japan). Purified human placental DNA topoisomerases I and II $\alpha$  were obtained from TopoGen, Inc. (Columbus, OH, USA). T7 RNA polymerase and bovine pancreas deoxyribonuclease I were purchased from Stratagene Cloning Systems (La Jolla, CA, USA).

**DNA polymerase assay.** The reaction mixtures for pol  $\alpha$ , pol  $\beta$ , fish pols, plant pols and prokaryotic pols were described previously (25,26). Those for pol  $\lambda$  and pols  $\delta$  and  $\epsilon$  were as described by Umeda *et al* (16) and Ogawa *et al* (27), respectively. The reaction mixtures for pol  $\eta$ ,  $\iota$  and  $\kappa$  were the same as that for pol  $\alpha$ , and the reaction mixture for pol  $\lambda$  was the same as that for pol  $\beta$ . For the pols, poly(dA)/oligo(dT)<sub>12-18</sub> (A/T=2/1) and [<sup>3</sup>H]-dTTP were used as the DNA template-primer and nucleotide (i.e., 2'-deoxyribonucleotide 5'-triphosphate, dNTP) substrate, respectively. The substrates of HIV-1 reverse transcriptase used were poly(rA)/oligo(dT)<sub>12-18</sub> and dTTP as the template-primer and nucleotide substrate, respectively. The dehydroaltenusin derivative was dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 sec. Four microliters of each sonicated sample was mixed with 16  $\mu$ l 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  $\mu$ l) were added to 16  $\mu$ l of each enzyme standard reaction mixture and incubated at 37°C for 60 min, except for *Taq* pol, which was incubated at 74°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 (25,26).

**Other enzyme assays.** DNA primase activity of pol  $\alpha$  and the activities of T7 RNA polymerase, human DNA topoisomerases I and II, T4 polynucleotide kinase and bovine deoxyribonuclease I were measured using standard assays according to the manufacturer's specifications as described by Tamiya-Koizumi *et al* (28), Nakayama and Saneyoshi (29), Mizushima *et al* (30), Soltis and Uhlenbeck (31) and Lu and Sakaguchi (32), respectively.

## Results

**Effects of dehydroaltenusin and its analogues on the activities of mammalian pols  $\alpha$  and  $\beta$ .** First, the inhibitory effect of chemically synthesized dehydroaltenusin **1** and its analogues **2-7** (Fig. 1A) on mammalian pols  $\alpha$  and  $\beta$  was investigated. Dehydroaltenusin dose-dependently inhibited the activity of calf pol  $\alpha$ , with 50% inhibition observed at a dose of 0.68  $\mu$ M (Table I) and almost complete inhibition at 4  $\mu$ M (**7**). Although we reported previously that the naturally purified dehydroaltenusin from a fungus (*Acremonium* sp.) was a specific inhibitor of mammalian pol  $\alpha$  (**6**), this compound was also effective at inhibiting rat pol  $\beta$  with an IC<sub>50</sub> value of 64  $\mu$ M.

In the prepared dehydroaltenusin analogues **2-7**, acetyl-dehydroaltenusin **2** and demethoxydehydroaltenusin **3** inhibited pol  $\alpha$  and  $\beta$  activities (Table I), and especially demethoxydehydroaltenusin was an ~3-fold stronger pol  $\alpha$  inhibitor than dehydroaltenusin. The inhibitory effect of demethoxydehydroaltenusin on pol  $\beta$  was ~1.4-fold weaker than that of dehydroaltenusin; therefore, the specificity of pol  $\alpha$  inhibition by demethoxydehydroaltenusin was greater than that by dehydroaltenusin (i.e., the IC<sub>50</sub> value ratio against pol  $\beta$ /pol  $\alpha$  for demethoxydehydroaltenusin was 371, and for dehydroaltenusin, 94). Acetyl-dehydroaltenusin was a >10-fold weaker pol  $\alpha$  inhibitor than dehydroaltenusin, and compounds **4-7** did not influence the activities of pols  $\alpha$  and  $\beta$ .

**Inhibitory effects of demethoxydehydroaltenusin derivatives on mammalian pols  $\alpha$  and  $\beta$  activities.** Since the structural difference between dehydroaltenusin **1** and demethoxydehydroaltenusin **3** is the group at the 5-position of these compounds, four modified groups were synthesized chemically at the 5-position, **8-11**, as shown in Fig. 1B. Fig. 2A shows the inhibition dose-response curves of demethoxydehydroaltenusin derivatives, and all compounds (0-1  $\mu$ M) dose-dependently inhibited the activity of calf pol  $\alpha$ .

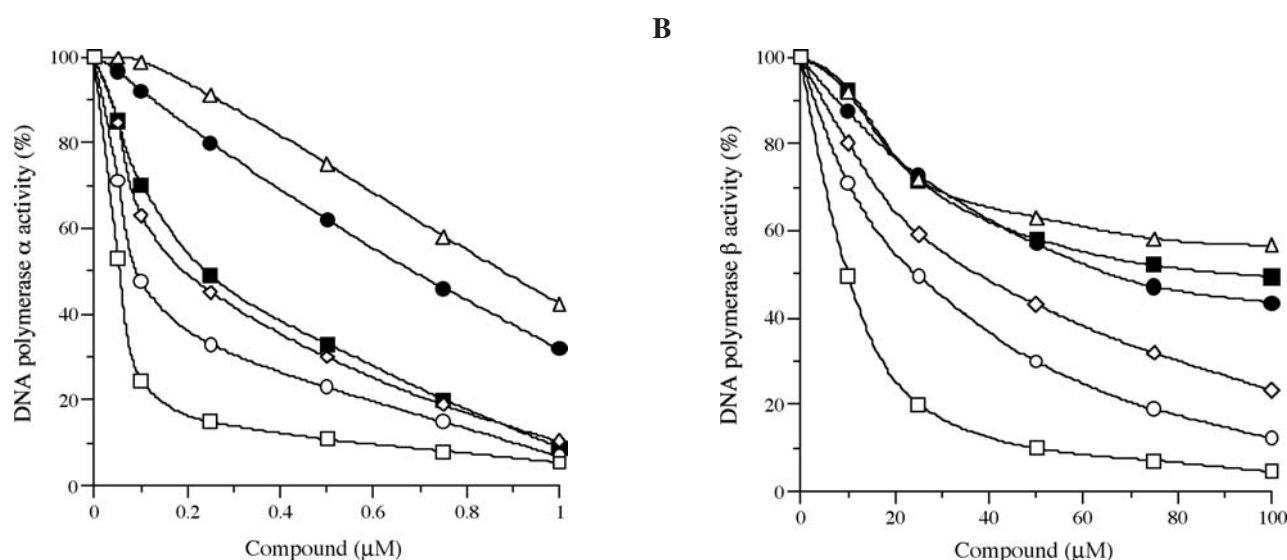


Figure 2. Dose-response curves of demethoxydehydroaltenusin derivatives. (A) Calf thymus pol  $\alpha$  activity (0–1  $\mu$ M) and (B) rat recombinant pol  $\beta$  activity (0–100  $\mu$ M). **1**, Dehydroaltenusin ( $\bullet$ ); **3**, demethoxydehydroaltenusin ( $\blacksquare$ ); **8**, demethyldehydroaltenusin ( $\triangle$ ); **9**, dehydroaltenusin-C3 ( $\diamond$ ); **10**, dehydroaltenusin-C6 ( $\circ$ ); and **11**, dehydroaltenusin-C12 ( $\square$ ). The amount of each enzyme in the assay mixture was 0.05 units. The enzymatic activity was measured as described in Materials and methods. Enzyme activity in the absence of the compound was taken as 100%.

Dehydroaltenusin-C12 **11** had the strongest inhibitory effect among the compounds tested, and in order of their effect, the derivatives were ranked as follows: dehydroaltenusin-C12 **11** > dehydroaltenusin-C6 **10** > dehydroaltenusin-C3 **9** > demethoxydehydroaltenusin **3** > dehydroaltenusin **1** > demethyldehydroaltenusin **8**.

On the other hand, the inhibitory effects on rat pol  $\beta$  activity by all the compounds were weaker than those on calf pol  $\alpha$ , and the inhibitory effect of each derivative varied markedly in the following order: dehydroaltenusin-C12 **11** > dehydroaltenusin-C6 **10** > dehydroaltenusin-C3 **9** > dehydroaltenusin **1** > demethoxydehydroaltenusin **3** > demethyldehydroaltenusin **8** (Fig. 2B). The  $IC_{50}$  value ratio against pol  $\beta$ /pol  $\alpha$  ranked as follows: demethoxydehydroaltenusin **3** (371) > dehydroaltenusin-C6 **10** (267) > dehydroaltenusin-C3 **9** (195) > dehydroaltenusin-C12 **11** (180) > demethyldehydroaltenusin **8** (142) > dehydroaltenusin **1** (94). These results suggested that demethoxydehydroaltenusin **3** was the most specific pol  $\alpha$  inhibitor of the synthesized derivatives, and the specificity of demethoxydehydroaltenusin was ~4-fold stronger than that of dehydroaltenusin; therefore, demethoxydehydroaltenusin was investigated for the inhibitory effects against other pol species and DNA metabolic enzymes in the later part of this study.

*Effects of demethoxydehydroaltenusin on the activities of various pols and other DNA metabolic enzymes.* The selective inhibition of demethoxydehydroaltenusin **3** was compared with that of dehydroaltenusin **1** and aphidicolin, which is a known inhibitor of replicative pols such as pols  $\alpha$ ,  $\delta$  and  $\epsilon$  (33). As shown in Table II, dehydroaltenusin and demethoxydehydroaltenusin were 94-fold and 371-fold, respectively, more effective against the activity of calf pol  $\alpha$  than that of rat pol  $\beta$ . Dehydroaltenusin also inhibited human pol  $\lambda$  activity, and the inhibitory concentration on pol  $\lambda$  was almost

the same as that for pol  $\beta$ . On the other hand, demethoxydehydroaltenusin did not suppress other mammalian pols including pol  $\lambda$ ; therefore, this compound could be a more potent pol  $\alpha$  inhibitor than dehydroaltenusin.

Since aphidicolin exhibited inhibition of calf pol  $\alpha$  with an  $IC_{50}$  value of 20  $\mu$ M, the effect of demethoxydehydroaltenusin on this enzyme was ~83-fold stronger than that of aphidicolin (Table II). Aphidicolin also inhibited eukaryotic pol activities such as human pols  $\delta$  and  $\epsilon$ , fish (cherry salmon) pols  $\alpha$  and  $\delta$ , and plant (cauliflower) pol  $\alpha$ , although dehydroaltenusin and demethoxydehydroaltenusin did not show any influence on these pols.

These three compounds had no inhibitory effect on human pols  $\gamma$ ,  $\eta$ ,  $\iota$  and  $\kappa$ , prokaryotic pols such as the Klenow fragment of *E. coli* pol I (Klenow fragment), *Taq* pol and T4 pol, or other DNA metabolic enzymes such as calf primase of pol  $\alpha$ , HIV-1 reverse transcriptase, T7 RNA polymerase, human DNA topoisomerases I and II, T4 polynucleotide kinase and bovine deoxyribonuclease I (Table II). The  $IC_{50}$  values for pols in Tables I and II did not change when the DNA template-primer was activated DNA (i.e., double-stranded DNA) digested by deoxyribonuclease I instead of poly(rA)/oligo(dT)<sub>12-18</sub>.

Demethoxydehydroaltenusin did not effect the thermal transition of melting temperature (data not shown); thus, none of the compound bound to double-stranded DNA, suggesting that this must inhibit enzyme activities by interacting with pol  $\alpha$  directly. It was investigated whether an excessive amount of nucleic acid [i.e., poly(rC)] or protein [i.e., bovine serum albumin (BSA)] could prevent the inhibitory effect of demethoxydehydroaltenusin to determine whether the effect resulted from their non-specific adhesion to pol  $\alpha$  or selective binding to specific sites. Poly(rC) and BSA had little or no influence on the effect of demethoxydehydroaltenusin, suggesting that the binding to pol  $\alpha$  occurs selectively.



Table II. IC<sub>50</sub> values of demethoxydehydroaltenusin derivatives modified at the 5-position on the activities of various DNA polymerases and other DNA metabolic enzymes.

Enzyme	IC <sub>50</sub> value (μM)		
	1	3	Aphidicolin
Mammalian DNA polymerases			
Calf DNA polymerase α	0.68	0.24	20
Rat DNA polymerase β	64	89	>100
Human DNA polymerase γ	>100	>100	>100
Calf DNA polymerase δ	>100	>100	13
Human DNA polymerase ε	>100	>100	16
Human DNA polymerase η	>100	>100	>100
Human DNA polymerase ι	>100	>100	>100
Human DNA polymerase κ	>100	>100	>100
Human DNA polymerase λ	69	>100	>100
Fish DNA polymerases			
Cherry salmon DNA polymerase α	>100	>100	28
Cherry salmon DNA polymerase δ	>100	>100	24
Plant DNA polymerases			
Cauliflower DNA polymerase α	>100	>100	32
Rice DNA polymerase λ	>100	>100	>100
Prokaryotic DNA polymerases			
<i>E. coli</i> DNA polymerase I (Klenow fragment)	>100	>100	>100
<i>Taq</i> DNA polymerase	>100	>100	>100
T4 DNA polymerase	>100	>100	>100
Other DNA metabolic enzymes			
Calf DNA primase of DNA polymerase α	>100	>100	>100
HIV-1 reverse transcriptase	>100	>100	>100
T7 RNA polymerase	>100	>100	>100
Human DNA topoisomerase I	>100	>100	>100
Human DNA topoisomerase II	>100	>100	>100
T4 polynucleotide kinase	>100	>100	>100
Bovine deoxyribonuclease I	>100	>100	>100

1, Dehydroaltenusin; 3, demethoxydehydroaltenusin. These compounds were incubated with each enzyme (0.05 units). The enzymatic activity was measured as described in Materials and methods. Enzyme activity in the absence of the compound was taken as 100%.

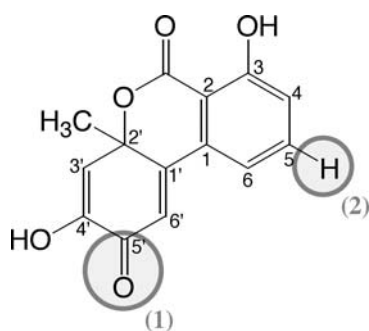


Figure 3. The chemical structure of a dehydroaltenusin derivative, demethoxydehydroaltenusin 3, an inhibitor of mammalian pol α. Groups (1) and (2) were essential for mammalian pol α inhibitory activity of the derivatives.

These results suggested that demethoxydehydroaltenusin, which is a derivative of dehydroaltenusin, could be a selective inhibitor of mammalian pol α.

## Discussion

We previously reported an inhibitor of mammalian pol α from a fungus, *Acremonium* sp., and this compound was dehydroaltenusin 1 (6). The inhibitory effect of dehydroaltenusin on pol α was 94-fold stronger than that on pol β, and 10 derivatives 2-11 were prepared to screen for more potent pol α inhibitors than dehydroaltenusin.

As shown in Table I, compounds 1-3 inhibited pol α activity, but compounds 4-7 did not; therefore, the ketone group at the 5'-position (1 of Fig. 3), as contained by

compounds **1-3** but not by compounds **4-7** in these analogues of dehydroaltenuin, must be essential for the inhibition. Since the specificity of pol  $\alpha$  inhibitory activity of demethoxydehydroaltenuin **3** was higher than that of the other dehydroaltenuin derivatives (i.e., compounds **1** and **8-11**) (Fig. 2), the group at the 5-position (**2** of Fig. 3) could be important for the selective inhibition of pol species. These results show that the dehydroaltenuin derivative, which has no group at the 5-position (i.e., demethoxydehydroaltenuin **3**) is the best inhibitor of mammalian pol  $\alpha$ .

Demethoxydehydroaltenuin-induced inhibition of calf pol  $\alpha$  activity was competitive with the template-primer and non-competitive with the dNTP substrate (data not shown). BIAcore analysis demonstrated that this compound bound to the core domain of the largest subunit, p180, of mouse pol  $\alpha$ , which has catalytic activity (34), but did not bind to the smallest subunit or the DNA primase p46 of mouse pol  $\alpha$  (35) (data not shown). These results suggested that the demethoxydehydroaltenuin molecule competes with the template-primer molecule at its binding site on the catalytic domain of mammalian pol  $\alpha$ , binds to the site, and simultaneously disturbs dNTP substrate incorporation into the template-primer. It was considered that demethoxydehydroaltenuin binds three-dimensionally in a special pocket in mammalian pol  $\alpha$ . The structural relationship between the pol  $\alpha$  catalytic subunit and demethoxydehydroaltenuin should be investigated by NMR analysis and computer simulation similarly to our previous studies using fatty acids and pol  $\beta$  (36-38). Such studies are currently in process.

Demethoxydehydroaltenuin will be used to elucidate the biochemical and biomedical functions of pol  $\alpha$  from two different focal points; to understand the precise role of each pol *in vivo*, and to develop drug design strategies for cancer chemotherapy agents, as pol  $\alpha$  is an essential enzyme for DNA replication and subsequently for cell division (1,2). Inhibitors of mammalian pol  $\alpha$  are not only molecular tools useful for analyzing pols as a 'chemical knockout agent' instead of 'gene knockout method', but should also be considered as a group of potentially useful cancer chemotherapy agents. Demethoxydehydroaltenuin did not influence the activities of mammalian pols  $\delta$  and  $\epsilon$ , which are other replicative pols, but also showed no effect on pol  $\alpha$  of another vertebrate, cherry salmon. Demethoxydehydroaltenuin is a type of antibiotic produced by a fungus and is chemically stable under *in vivo* conditions, indicating that it may be useful for analyzing the replication system within cells and for clinical use. Aphidicolin, once believed to be a pol  $\alpha$ -specific inhibitor, is now known to also inhibit the activities of pols  $\delta$  and  $\epsilon$  (5). No pol  $\alpha$  inhibitors with such a limited spectrum of action have been reported to date, and demethoxydehydroaltenuin can be a key agent for analyzing both the *in vitro* and *in vivo* functions of pol  $\alpha$  in more detail. Thus, this compound can be a useful tool with which to study the *in vitro* functions of pol  $\alpha$  and as a molecular probe to distinguish the structure of pol  $\alpha$ .

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