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Penicilliols A and B, novel inhibitors specific to mammalian Y-family DNA polymerases

Takuma Kimura [a], Toshifumi Takeuchi [a], Yuko Kumamoto-Yonezawa [b], Eiji Ohashi [c,†], Haruo Ohmori [c], Chikahide Masutani [d], Fumio Hanaoka [e], Fumio Sugawara [a], Hiromi Yoshida [b,f], Yoshiyuki Mizushina [b,f,*]

[a] Department of Applied Biological Science, Science University of Tokyo, Noda, Chiba 278-8510, Japan
[b] Laboratory of Food and Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan
[c] Institute For Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan
[d] Cellular Biology Laboratory, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, Japan
[e] Faculty of Science, Gakushuin University, Toshima-ku, Tokyo 171-8588, Japan
[f] Cooperative Research Center of Life Sciences, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan

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ABSTRACT

Penicilliols A (**1**) and B (**2**) are novel 5-methoxy-3(2*H*)-furanones isolated from cultures of a fungus (*Penicillium daleae* K.M. Zalessky) derived from a sea moss, and their structures were determined by spectroscopic analyses. These compounds selectively inhibited activities of eukaryotic Y-family DNA polymerases (pols) (i.e., pols η, ι and κ), and compound **1** was a stronger inhibitor than compound **2**. Among mammalian Y-family pols, mouse pol ι activity was most strongly inhibited by compounds **1** and **2**, with IC_{50} values of 19.8 and 32.5 μM, respectively. On the other hand, activities of many other pols, such as A-family (i.e., pol γ), B-family (i.e., pols α, δ and ε) or X-family (i.e., pols β, λ and terminal deoxynucleotidyl transferase), and some DNA metabolic enzymes, such as calf primase of pol α, human immunodeficiency virus type-1 (HIV-1) reverse transcriptase, human telomerase, T7 RNA polymerase, mouse IMP dehydrogenase (type II), human topoisomerases I and II, T4 polynucleotide kinase or bovine deoxyribonuclease I, are not influenced by these compounds. In conclusion, this is the first report on potent inhibitors of mammalian Y-family pols.

1. Introduction

We have long been interested in the integrity of the genome of eukaryotes and its relation to cell differentiation. DNA replication, recombination and repair in eukaryotes are key systems to maintain these processes,[1] and DNA polymerases (pols) have important roles. In this regard, we have concentrated our efforts on investigating eukaryotic pols associated with these processes.[2]

The human genome encodes at least 14 pols to conduct cellular DNA synthesis.[3,4] Eukaryotic cells contain three replicative pols (α, δ and ε), mitochondrial pol γ, and at least twelve non-replicative pols [β, ζ, η, θ, ι, κ, λ, μ, ν, terminal deoxynucleotidyl transferase (TdT) and REV1].[3–5] Pols have a highly conserved structure, which means that their overall catalytic subunits vary, on the whole, very little from species to species. Conserved structures usually indicate important, irreplaceable functions of the cell, the maintenance of which provides evolutionary advantages. Based on sequence homology, eukaryotic pols can be divided into four main different families, A, B, X, and Y.[6] Family A includes mitochondrial pol γ, and pol θ, and family B includes three replicative pols (α, δ, and ε) and pol ζ. Family X is pols β, λ, μ, and terminal deoxynucleotidyl transferase (TdT), and family Y includes pols η, ι, κ, and REV1. Because not all functions of eukaryotic pols have been fully elucidated, selective inhibitors of pol families are useful tools for distinguishing pols and clarifying their biological functions. We have therefore been searching for natural compounds that selectively inhibit each of these eukaryotic pols.[7–18]

In this study, we report novel compounds **1** and **2**, isolated from a fungal strain derived from a sea moss, and named penicilliols A and B, respectively (Fig. 1). These compounds selectively inhibited activities of mammalian Y-family pols. To our knowledge, this is the first report on such inhibitors specific to Y-family pols.

2. Results

2.1. Isolation and cultivation of fungus

Fungal strains were isolated from mosses collected on the beach in Nichinan, Miyazaki prefecture, Japan, and the strains were trea-

* Corresponding author. Tel.: +81 78 974 1551x3232; fax: +81 78 974 5689.
E-mail address: mizushin@nutr.kobegakuin.ac.jp (Y. Mizushina).
† Present address: Department of Biology, School of Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8581, Japan.

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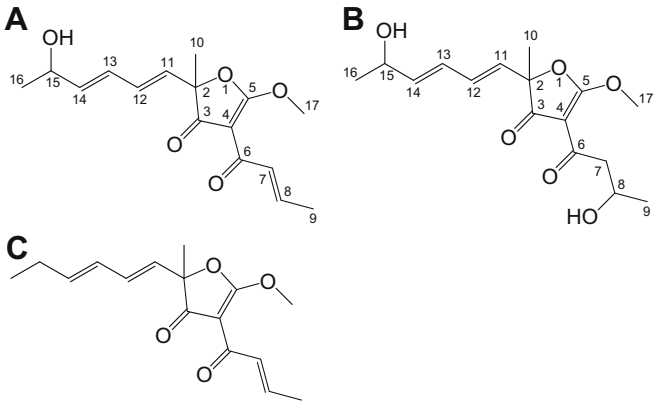

Figure 1. Structure of compounds **1** (penicilliol A) (A), **2** (penicilliol B) (B) and gregatin A (C).

ted with 5% acetic acid. The fungi were isolated using standard isolation techniques on corn meal agar (Sigma–Aldrich Corp., MO, USA) plates, including rose Bengal (Junsei Chemical Co., Ltd, Tokyo, Japan), and then cultured on potato dextrose agar (Difco & BBL, NJ, USA) plates at 27 °C. The fungus, which was screened as an inhibitor of pols, was identified as *Penicillium daleae* K.M. Zalessky by Techno Suruga Laboratory Co., Ltd (Shizuoka, Japan). This isolated fungus was cultured by transferring a small agar piece of the cultured plate into a 3 L Erlenmeyer flask containing potato dextrose broth (24 g) (Difco & BBL, NJ, USA) in H_2O (1 L). The culture was kept under static conditions in the dark for 14 days.

2.2. Extraction and purification of compounds

The cultured broth was filtered through cheesecloth to remove fungal mycelia. The filtrate was extracted with CH_2Cl_2. The organic layer was evaporated in vacuo to obtain crude extract (22.9 mg). This crude extract was separated by silica gel column chromatography with $CHCl_3$–MeOH (99:1–0:100) to give fractions 1–7. Fraction 2 was purified by silica gel column chromatography with *n*-hexane–EtOAc (4:1–2:1) to give compound **1** (1.8 mg) as a colorless oil, and fraction 6 was purified by silica gel column chromatography with *n*-hexane–EtOAc (2:1–1:1) to give compound **2** (4.5 mg) as a colorless oil.

2.3. Structure determination of isolated compounds

The molecular formula of compound **1** was determined to be $C_{16}H_{20}O_5$ by a high resolution electron spray ionization mass spectrometer (HR-ESIMS). The IR spectrum indicated the presence of a hydroxyl group (3450 cm^{-1}) and a conjugated ketone group (1643 cm^{-1}). ^1H and ^{13}C NMR spectra suggested that compound **1** and gregatin A (Fig. 1C)[19] had similar structures and differed only in C15 of their side chains. The ^1H signal of $H_2$15 in gregatin A was replaced in compound **1** by that of a more downstream H15 (δ 4.35) that was correlated with C15 (δ 68.2) in the HMQC spectrum (Fig. 2A), suggesting the presence of C15 alcohol, in agreement with the HR-ESIMS identification of an oxygen atom in the molecular formula. Thus, the structure of compound **1** was determined to be 4-[(2*E*)-but-2-enoyl]-2-[(1*E*,3*E*)-5-hydroxyhexa-1,3-dien-1-yl]-5-methoxy-2-methylfuran-3(2*H*)-one (Fig. 1A), and was named penicilliol A.

The molecular formula of compound **2** was determined to be $C_{16}H_{22}O_6$ by HR-ESIMS. A comparison of the 1D- and 2D NMR data and the molecular formulae obtained for compounds **1** and **2** showed that they had almost identical structures but differed in C7 and C8 on their side chains. The ^1H signals of H7 (δ 7.21) and H8 (δ 7.34) in compound **1** were replaced in compound **2** by those of the more upstream $H_2$7 (δ 3.19) and H8 (δ 4.33), respectively (Fig. 2B). These signals correlated with C7 (δ 40.3) and C8 (δ 66.3), respectively, suggesting the presence of C7 methylene and C8 alcohol, supporting the HR-ESIMS identification; therefore, the structure of compound **2** was determined to be 4-(3-hydroxybutanoyl)-2-[(1*E*,3*E*)-5-hydroxyhexa-1,3-dien-1-yl]-5-methoxy-2-methylfuran-3(2*H*)-one (Fig. 1B), and was named penicilliol B.

2.4. Effect of isolated compounds on the activities of DNA polymerases and other DNA metabolic enzymes

First, the novel isolated compounds **1** and **2** were investigated as to whether they inhibited the activities of ten mammalian pols, such as families A (pol γ), B (pols α, δ and ε), X (pols β and λ and TdT), and Y (pols η, ι and κ). As shown in Figure 3A, these compounds at 100 μM were found to significantly inhibit the activities of all the three Y-family pols, and mouse pol ι was most strongly inhibited among the three pols. Compounds **1** and **2** inhibited the activity of these pols dose-dependently, and 50% inhibition of pol ι was observed at concentrations of 19.8 and 32.5 μM, respectively (Fig. 4). Thus, compound **1** is an approximately 1.6-fold stronger inhibitor than compound **2**. On the other hand, activities of the other families (i.e., families A, B and X) of mammalian pols (Fig. 3A), fish pol (cherry salmon pol δ), insect pols (fruit fly pols α, δ and ε), higher plant pols (cauliflower pol α and rice pol λ), and prokaryotic pols (the Klenow fragment of *Escherichia coli* pol I, T4 pol and *Taq* pol) (Fig. 3B) were not influenced.

These compounds at the same concentration (i.e., 100 μM) did not suppress activities of other DNA metabolic enzymes, such as calf primase pol α, human immunodeficiency virus type-1 (HIV-1) reverse transcriptase, human telomerase, T7 RNA polymerase, mouse IMP dehydrogenase (type II), human topoisomerases I and II, T4 polynucleotide kinase, and bovine deoxyribonuclease I (DNase I). These results suggest that compounds **1** and **2** may be selective inhibitors of mammalian Y-family pols, especially pol ι.

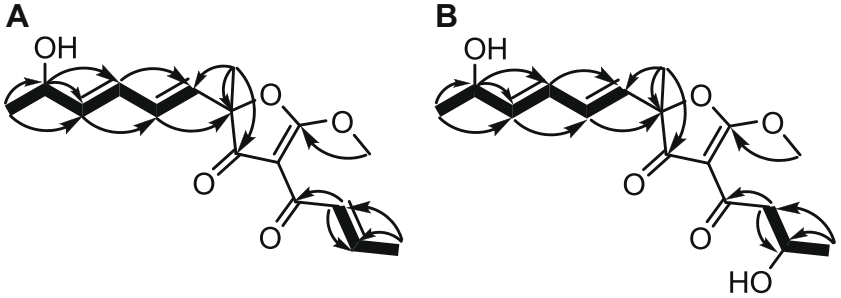

Figure 2. Selected COSY (bold lines) and HMBC ($^1H \rightarrow ^{13}C$) (arrows) correlations in compounds **1** (penicilliol A) (A) and **2** (penicilliol B) (B).

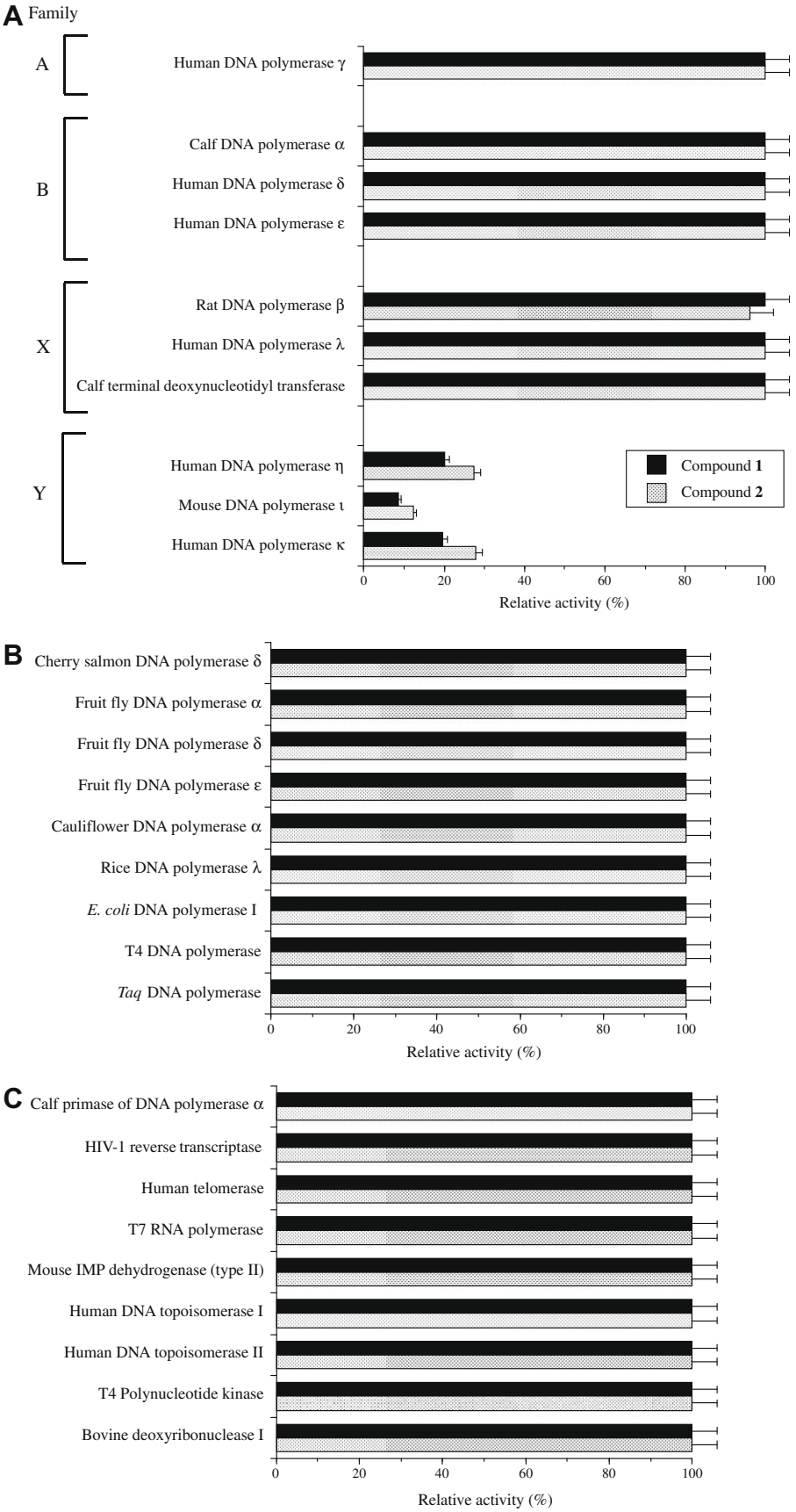

Figure 3. Effect of compounds **1** (penicilliol A) and **2** (penicilliol B) on the activities of various DNA polymerases and other DNA metabolic enzymes. (A) Mammalian pols, (B) fish, insect, plant and prokaryotic pols, and (C) other DNA metabolic enzymes. Compound **1** (black bars) and compound **2** (gray bars) (100 μM each) were incubated with each enzyme (0.05 units). % of relative activity. Enzymatic activities were measured as described in the Section 4. Activities in the absence of the compounds were taken as 100%. Data are shown as the means ± SEM of four independent experiments.

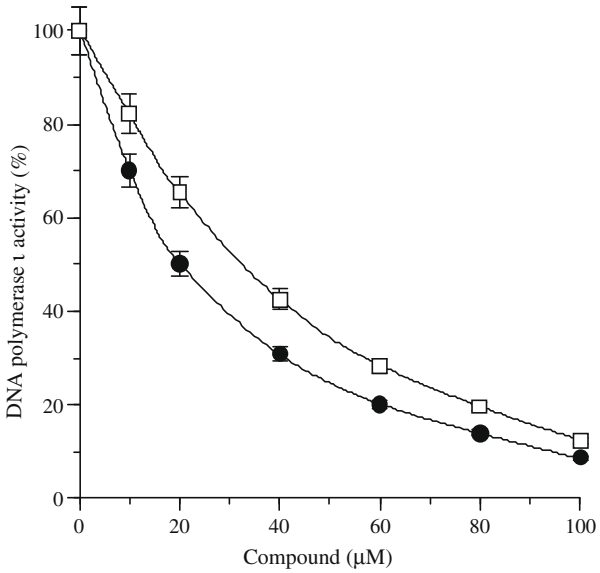

Figure 4. Y-family DNA polymerase inhibition dose-response curves of compounds **1** (penicilliol A) and **2** (penicilliol B). Compounds **1** (closed circle) and **2** (open square) were incubated with mouse pol ι (0.05 units of each). Pol activity was measured as described in the Section 4. Activity in the absence of the compounds was taken as 100%. Data are shown as the means ± SEM of three independent experiments.

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for compounds **1** (penicilliol A) and **2** (penicilliol B)

Position	1		2	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
2	90.2		91.5	
3	198.1		197.6	
4	103.6		107.7	
5	163.4		164.1	
6	185.3		196.0	
7	145.0	7.21 (m)	40.3	3.19 (m)
8	120.7	7.34 (dd, 15.6, 1.2)	66.3	4.33 (m)
9	19.4	2.07 (dd, 6.8, 1.2)	24.0	1.36 (dd, 4.0, 2.4)
10	22.5	1.57 (s)	22.4	1.56 (d, 2.0)
11	128.9	5.69 (d, 15.2)	127.6	5.66 (dd, 15.6, 2.0)
12	130.3	6.29 (dd, 15.2, 10.4)	130.9	6.33 (ddd, 15.6, 10.4, 5.8)
13	127.7	6.15 (dd, 15.2, 11.6)	127.9	6.15 (dd, 15.2, 10.4)
14	139.7	5.81 (dd, 15.2, 6.4)	140.1	5.82 (ddd, 15.2, 5.8, 2.8)
15	68.2	4.35 (m)	68.2	4.35 (m)
16	23.2	1.27 (d, 6.4)	23.2	1.28 (d, 6.4)
17	51.7	3.84 (s)	51.9	3.84 (s)

Recorded in CD_3OD and chemical shifts are expressed as δ ppm. s, singlet; d, doublet; dd, double of doublets; t, triplet; m, multiple.

2.5. Mode of inhibition of pol ι by compound 1

Next, to elucidate the mechanism by which compound **1** inhibited mouse pol ι, the extent of inhibition as a function of the DNA template-primer or dNTP substrate concentration was studied (Table 2). In kinetic analysis, poly(dA)/oligo(dT)$_{12-18}$ and 2′-deoxythymidine 5′-triphosphate (dTTP) were used as the synthetic DNA template-primer and 2′-deoxynucleoside 5′-triphosphate (dNTP) substrate, respectively. Double reciprocal plots of the obtained data showed that the compound **1**-induced inhibition of pol ι activity was non-competitive with respect to both the DNA template-primer and the dNTP substrate. For the DNA template-primer, the apparent Michaelis constant (K_m) was unchanged at 2.60 μM, whereas 54.6%, 70.8% and 78.4% decreases in maximum velocity (V_{max}) were observed in the presence of 5, 10 and 15 μM of compound **1**, respectively. The K_m for the dNTP substrate was unchanged at 3.09 μM, and the V_{max} for the dNTP substrate decreased from 50.2 to 22.5 pmol/h in the presence of 15 μM of compound **1**. Inhibition constant (K_i) values, obtained from Dixon plots, were found to be 4.35 μM and 6.48 μM for the DNA template-primer and dNTP substrate, respectively. Because the K_i value for the DNA template-primer was approximately 1.5-fold smaller than that for the dNTP substrate, the affinity of compound **1** was greater for the enzyme-DNA template-primer binary complex than for the enzyme-nucleotide substrate complex. When activated DNA (i.e., DNA with gaps digested by bovine DNase I) and four dNTPs were used as the DNA template-primer and dNTP substrates, respectively, the mode of inhibition of pol ι by compound **1** was the same as that with the above synthetic DNA template-primer (data not shown). The mode of inhibition of pol ι by compound **2** was the same as by compound **1** (data not shown).

Table 2
Kinetic analysis of the inhibitory effects of compound **1** (penicilliol A) on the activities of mouse DNA polymerase ι as a function of the DNA template-primer dose and the nucleotide substrate concentration

DNA substrate	Compound **1** (μM)	K_m[a] (μM)	V_{max}[a] (pmol/h)	K_i[b] (μM)	Inhibitory mode[a]
Template-primer[c]	0	2.60	68.5	4.35	Non-competitive
	5		31.1		
	10		20.0		
	15		14.8		
Nucleotide substrate[d]	0	3.09	50.2	6.48	Non-competitive
	5		35.7		
	10		27.6		
	15		22.5		

[a] These data were obtained from Lineweaver–Burk plot.
[b] These data were obtained from Dixon plot.
[c] That is, poly(dA)/oligo(dT)$_{12-18}$.
[d] That is, dTTP.

3. Discussion

As described in this report, we found potent inhibitors specific to mammalian Y-family pols from a fungal strain derived from a sea moss. These were novel natural compounds named penicilliols A (**1**) and B (**2**). Compound **1** showed stronger effects in inhibiting the pols than compound **2**, therefore; the double bond at position 7 and hydrogen group at position 8 in compound **1**, which are the sole structural differences between compounds **1** and **2**, may be important for these bio-activities.

Y-family pols differ from pols belonging to other families in their ability to replicate through damaged DNA. Members of this family are hence called translesion synthesis (TLS) pols.[20] Depending on the lesion, TLS pols can bypass the damage in an error-free or error-prone fashion, the latter resulting in elevated mutagenesis. Xeroderma pigmentosum variant (XPV) patients, for instance, have mutations in the gene encoding pol η, which is able to bypass cyclobutane pyrimidine dimmers (CPDs), the most frequent UV-induced lesions, in an error-free fashion. In XPV patients, alternative error-prone pols, for example, pol ζ (a B-family pol), are thought to be involved in errors which result in the cancer predisposition of these patients. Other members of Y-family in humans (pols ι, κ, and Rev1) are thought to be involved in bypassing different lesions.[6,20] The inhibitors of Y-family pols may be useful as anti-cancer drugs for clinical radiation therapy.

In conclusion, this is the first report on potent inhibitors of mammalian Y-family pols, especially pol ι. Since compounds **1**

and **2** have extremely high specificity for pol families, these compounds could be useful molecular tools as Y-family pol-specific inhibitors in studies to determine the precise roles of the pol family in vitro, and also may provide valuable information for developing drug design strategy for anti-cancer chemotherapy agents.

4. Experimental

4.1. Materials

Nucleotides and chemically synthesized template-primers, such as poly(dA), oligo(dT)$_{12-18}$, and [^{3}H]-dTTP (43 Ci/mmol), were purchased from GE Healthcare Bio-Sciences (Little Chalfont, UK). All other reagents were of analytical grade and were purchased from Wako Chemical Industries (Osaka, Japan).

4.2. Enzymes

Pol α was purified from calf thymus by immuno-affinity column chromatography, as described by Tamai et al.[21] Recombinant rat pol β was purified from *E. coli* JMpβ5, as described by Date et al.[22] The human pol γ catalytic gene was cloned into pFastBac. Histidine-tagged enzyme was expressed using the BAC-TO-BAC HT Baculovirus Expression System according to the supplier's manual (Life Technologies, MD, USA) and purified using ProBoundresin (Invitrogen Japan, Tokyo Japan).[23] Human pols δ and ε were purified by the nuclear fractionation of human peripheral blood cancer cells (Molt-4) using the second subunit of pols δ and ε-conjugated affinity column chromatography, respectively.[24] A truncated form of human pol η (residues 1–511) tagged with His$_6$ at its C-terminal was expressed in *E. coli* cells and was purified as described previously.[25] A recombinant mouse pol ι tagged with His$_6$ at its C-terminal was expressed and purified by Ni-NTA column chromatography as described elsewhere (Masutani et al., in preparation). A truncated form of pol κ (residues 1–560) with 6 × His-tags attached at the C-terminus was overproduced in *E. coli* and purified as described previously.[26] Recombinant human His-pol λ was overexpressed and purified according to a method described previously.[27] Fish pol δ was purified from the testis of cherry salmon (*Oncorhynchus masou*).[28] Fruit fly pols α, δ and ε were purified from early embryos of *Drosophila melanogaster*, as described previously.[29,30] Pol α from a higher plant, cauliflower inflorescence, was purified according to the methods outlined by Sakaguchi et al.[31] Recombinant rice (*Oryza sativa* L. cv. Nipponbare) His-pol λ was overexpressed and purified according to a method described previously.[32] Calf thymus TdT and bovine pancreas DNase I were obtained from Stratagene Cloning Systems (La Jolla, CA, USA). HIV-1 reverse transcriptase (recombinant) and the Klenow fragment of pol I from *E. coli* were purchased from Worthington Biochemical Corp. (Freehold, NJ, USA). T4 pol, *Taq* pol, T7 RNA polymerase and T4 polynucleotide kinase were purchased from Takara Bio (Tokyo, Japan). Purified human placenta DNA topoisomerases I and II were purchased from TopoGen, Inc. (Columbus, OH).

4.3. DNA polymerase assays

The reaction mixtures for pol α, pol β, plant pols and prokaryotic pols were described previously.[7,8] Those for pol γ, and pols δ and ε were as described by Umeda et al.[23] and Ogawa et al.[33], respectively. The reaction mixtures for pols η, ι and κ were the same as for pol α, and the reaction mixture for pol λ was the same as for pol β. For pols, poly(dA)/oligo(dT)$_{12-18}$ (A/T = 2/1) and dTTP were used as the DNA template-primer and nucleotide (i.e., dNTP) substrate, respectively. For HIV-1 reverse transcriptase, poly(rA)/oligo(dT)$_{12-18}$ (A/T = 2/1) and dTTP were used as the template-primer and nucleotide substrate, respectively. For TdT, oligo(dT)$_{12-18}$ (3′-OH) and dTTP were used as the DNA primer and nucleotide substrate, respectively.

The compounds were dissolved in distilled dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Aliquots of 4 μl sonicated samples were mixed with 16 μl of each enzyme (final amount 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM EDTA, and kept at 0 °C for 10 min. These inhibitor–enzyme mixtures (8 μl) were added to 16 μl of each of the enzyme standard reaction mixtures, and incubation was carried out 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 dNTP (i.e., dTTP) into synthetic DNA template-primers in 60 min at 37 °C under the normal reaction conditions for each enzyme.[7,8]

4.4. Other DNA metabolic enzymes assays

The activities of calf primase of pol α, human telomerase, T7 RNA polymerase, mouse IMP dehydrogenase, human DNA topoisomerases I and II, T4 polynucleotide kinase and bovine DNase I were measured in standard assays according to the manufacturer's specifications, as described by Tamiya-Koizumi et al.[34], Oda et al.[35], Nakayama et al.[36], Mizushina et al.[37], Mizushina et al.[38], Soltis et al.[39] and Lu and Sakaguchi[40], respectively.

4.5. Instrumental analyses

All reactions were monitored by TLC, which was carried out on Silica Gel 60 F_{254} plates (Merck, Germany).

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

Optical rotations were recorded on a JASCO P-1010 digital polarimeter at room temperature.

Infrared spectra (IR) were recorded on a JASCO FT/IR-410 spectrometer using NaCl (neat), and were reported as wave numbers (cm^{-1}).

Mass spectra (MS) were obtained on an Applied Biosystems mass spectrometer (APIQSTAR pulsar i) under conditions of high resolution, using poly (ethylene glycol) as the internal standard.

4.6. Structure determination

4.6.1. Penicilliol A (4-[(2*E*)-but-2-enoyl]-2-[(1*E*,3*E*)-5-hydroxyhexa-1,3-dien-1-yl]-5-methoxy-2-methylfuran-3(2*H*)-one) (1)

Colorless oil: $[\alpha]_D^{26} = +60.7$ (*c* 0.09, $CHCl_3$); IR (film) ν_{max} = 3450, 2973, 1693, 1643, 1587, 1441, 1390, 1201, 1128, 1043, 991 cm^{-1}; HR ESIMS *m*/*z* found 293.1386 $[M+H]^+$, calcd for $C_{16}H_{20}O_5H$ 293.1388; ^{13}C and ^{1}H data, see Table 1.

4.6.2. Penicilliol B (4-(3-hydroxybutanoyl)-2-[(1*E*,3*E*)-5-hydroxyhexa-1,3-dien-1-yl]-5-methoxy-2-methylfuran-3(2*H*)-one) (2)

Colorless oil: $[\alpha]_D^{26} = +62.8$ (*c* 0.23, $CHCl_3$); IR (film) ν_{max} = 3421, 2974, 1708, 1643, 1581, 1442, 1394, 1205, 1126, 1043, 993 cm^{-1}; HR ESIMS *m*/*z* found 311.1492 $[M+H]^+$, calcd for $C_{16}H_{22}O_6H$ 311.1494; ^{13}C and ^{1}H data, see Table 1.

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References and notes

1. DePamphilis, M. L. *DNA Replication in Eukaryotic Cells*; Cold Spring Harbor Laboratory Press, 1996.
2. Seto, H.; Hatanaka, H.; Kimura, S.; Oshige, M.; Tsuya, Y.; Mizushina, Y.; Sawado, T.; Aoyagi, N.; Matsumoto, T.; Hashimoto, J.; Sakaguchi, K. *Biochem. J.* **1998**, *332*, 557.
3. Hubscher, U.; Maga, G.; Spadari, S. *Annu. Rev. Biochem.* **2002**, *71*, 133.
4. Bebenek, K.; Kunkel, T. A.. In *DNA Repair and Replication Advances in Protein Chem*; Yang, W., Ed.; Elsevier: San Diego, 2004; Vol. 69, p 137.
5. Takata, K.; Shimizu, T.; Iwai, S.; Wood, R. D. *J. Biol. Chem.* **2006**, *281*, 23445.
6. Friedberg, E. C.; Feaver, W. J.; Gerlach, V. L. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5681.
7. Mizushina, Y.; Tanaka, N.; Yagi, H.; Kurosawa, T.; Onoue, M.; Seto, H.; Horie, T.; Aoyagi, N.; Yamaoka, M.; Matsukage, A.; Yoshida, S.; Sakaguchi, K. *Biochim. Biophys. Acta* **1996**, *1308*, 256.
8. Mizushina, Y.; Yoshida, S.; Matsukage, A.; Sakaguchi, K. *Biochim. Biophys. Acta* **1997**, *1336*, 509.
9. Mizushina, Y.; Takahashi, N.; Ogawa, A.; Tsurugaya, K.; Koshino, H.; Takemura, M.; Yoshida, S.; Matsukage, A.; Sugawara, F.; Sakaguchi, K. *J. Biochem. (Tokyo)* **1999**, *126*, 430.
10. Mizushina, Y.; Ohkubo, T.; Sugawara, F.; Sakaguchi, K. *Biochemistry* **2000**, *39*, 12606.
11. Mizushina, Y.; Kamisuki, S.; Mizuno, T.; Takemura, M.; Asahara, H.; Linn, S.; Yamaguchi, T.; Matsukage, A.; Hanaoka, F.; Yoshida, S.; Saneyoshi, M.; Sugawara, F.; Sakaguchi, K. *J. Biol. Chem.* **2000**, *275*, 33957.
12. Mizushina, Y.; Kamisuki, S.; Kasai, N.; Shimazaki, N.; Takemura, M.; Asahara, H.; Linn, S.; Yoshida, S.; Matsukage, A.; Koiwai, O.; Sugawara, F.; Yoshida, H.; Sakaguchi, K. *J. Biol. Chem.* **2002**, *277*, 630.
13. Mizushina, Y.; Xu, X.; Asahara, H.; Takeuchi, R.; Oshige, M.; Shimazaki, N.; Takemura, M.; Yamaguchi, T.; Kuroda, K.; Linn, S.; Yoshida, H.; Koiwai, O.; Saneyoshi, M.; Sugawara, F.; Sakaguchi, K. *Biochem. J.* **2003**, *370*, 299.
14. Kuriyama, I.; Asano, N.; Kato, I.; Ikeda, K.; Takemura, M.; Yoshida, H.; Sakaguchi, K.; Mizushina, Y. *Bioorg. Med. Chem.* **2005**, *13*, 2187.
15. Kamisuki, S.; Ishimaru, C.; Onoda, K.; Kuriyama, I.; Ida, N.; Sugawara, F.; Yoshida, H.; Mizushina, Y. *Bioorg. Med. Chem.* **2007**, *15*, 3109.
16. Naganuma, M.; Nishida, M.; Kuramochi, K.; Sugawara, F.; Yoshida, H.; Mizushina, Y. *Bioorg. Med. Chem.* **2008**, *16*, 2939.
17. Kimura, T.; Nishida, M.; Kuramochi, K.; Sugawara, F.; Yoshida, H.; Mizushina, Y. *Bioorg. Med. Chem.* **2008**, *16*, 4595.
18. Nishida, M.; Ida, N.; Horio, M.; Takeuchi, T.; Kamisuki, S.; Murata, H.; Kuramochi, K.; Sugawara, F.; Yoshida, H.; Mizushina, Y. *Bioorg. Med. Chem.* **2008**, *16*, 5115.
19. Kobayashi, K.; Ui, T. *Phys. Plant Pathol.* **1977**, *11*, 55.
20. Prakash, S.; Johnson, R. E.; Prakash, L. *Annu. Rev. Biochem.* **2005**, *74*, 317.
21. Tamai, K.; Kojima, K.; Hanaichi, T.; Masaki, S.; Suzuki, M.; Umekawa, H.; Yoshida, S. *Biochim. Biophys. Acta* **1988**, *950*, 263.
22. Date, T.; Yamaguchi, M.; Hirose, F.; Nishimoto, Y.; Tanihara, K.; Matsukage, A. *Biochemistry* **1988**, *27*, 2983.
23. Umeda, S.; Muta, T.; Ohsato, T.; Takamatsu, C.; Hamasaki, N.; Kang, D. *Eur. J. Biochem.* **2000**, *267*, 200.
24. Oshige, M.; Takeuchi, R.; Ruike, R.; Kuroda, K.; Sakaguchi, K. *Protein Exp. Purif.* **2004**, *35*, 248.
25. Kusumoto, R.; Masutani, C.; Shimmyo, S.; Iwai, S.; Hanaoka, F. *Genes Cells* **2004**, *9*, 1139.
26. Ohashi, E.; Murakumo, Y.; Kanjo, N.; Akagi, J-i.; Masutani, C.; Hanaoka, F.; Ohmori, H. *Genes Cells* **2004**, *9*, 523.
27. Shimazaki, N.; Yoshida, K.; Kobayashi, T.; Toji, S.; Tamai, T.; Koiwai, O. *Genes Cells* **2000**, *7*, 639.
28. Yamaguchi, T.; Saneyoshi, M.; Takahashi, H.; Hirokawa, S.; Amano, R.; Liu, X.; Inomata, M.; Maruyama, T. *Nucleosides Nucleotides Nucleic Acids* **2006**, *25*, 539.
29. Aoyagi, N.; Matsuoka, S.; Furunobu, A.; Matsukage, A.; Sakaguchi, K. *J. Biol. Chem.* **1994**, *269*, 6045.
30. Aoyagi, N.; Oshige, M.; Hirose, F.; Kuroda, K.; Matsukage, A.; Sakaguchi, K. *Biochem. Biophys. Res. Commun.* **1997**, *230*, 297.
31. Sakaguchi, K.; Hotta, Y.; Stern, H. *Cell Struct. Funct.* **1980**, *5*, 323.
32. Uchiyama, Y.; Kimura, S.; Yamamoto, T.; Ishibashi, T.; Sakaguchi, K. *Eur. J. Biochem.* **2004**, *271*, 2799.
33. Ogawa, A.; Murate, T.; Suzuki, M.; Nimura, Y.; Yoshida, S. *Jpn. J. Cancer Res.* **1998**, *89*, 1154.
34. Tamiya-Koizumi, K.; Murate, T.; Suzuki, M.; Simbulan, C. G.; Nakagawa, M.; Takamura, M.; Furuta, K.; Izuta, S.; Yoshida, S. *Biochem. Mol. Biol. Int.* **1997**, *41*, 1179.
35. Oda, M.; Ueno, T.; Kasai, N.; Takahashi, H.; Yoshida, H.; Sugawara, F.; Sakaguchi, K.; Hayashi, H.; Mizushina, Y. *Biochem. J.* **2002**, *367*, 329.
36. Nakayama, C.; Saneyoshi, M. *J. Biochem. (Tokyo)* **1985**, *97*, 1385.
37. Mizushina, Y.; Dairaku, I.; Yanaka, N.; Takeuchi, T.; Ishimaru, C.; Sugawara, F.; Yoshida, H.; Kato, N. *Biochimie* **2007**, *89*, 581.
38. Ishimaru, C.; Yonezawa, Y.; Kuriyama, I.; Nishida, M.; Yoshida, H.; Mizushina, Y. *Lipids* **2008**, *43*, 373.
39. Soltis, D. A.; Uhlenbeck, O. C. *J. Biol. Chem.* **1982**, *257*, 11332.
40. Lu, B.; Sakaguchi, K. *J. Biol. Chem.* **1991**, *266*, 21060.