Isothiazolone Derivatives Selectively Inhibit Telomerase from Human and Rat Cancer Cells *in Vitro*

Nobuki Hayakawa,[‡] Katsura Nozawa,[§] Akio Ogawa,[∥] Naoki Kato,[∥] Kenji Yoshida,[∥] Ken-ichi Akamatsu,[∥] Masayuki Tsuchiya,[∥] Akio Nagasaka,[‡] and Shonen Yoshida*[,§]

Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan, Chugai Pharmaceutical Co. Ltd, Fuji Gotemba Research Laboratories, 135 Komakado-1 chome, Gotemba-shi, 412-8513, Japan, and Laboratory of Cancer Cell Biology, Research Institute for Disease Mechanism and Control and First Department of Surgery, Nagoya University School of Medicine, Showa-ku, Nagoya 466-8550, Japan

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ABSTRACT: The telomere hypothesis postulates stabilization of telomere length and telomerase activation as key events in cellular immortalization and carcinogeneses. Accordingly, telomerase has been suggested as a novel and highly selective target for design of antitumor drugs. Screening of a chemical library including 16 000 synthetic compounds yielded six that strongly inhibited telomerase activity in extracts of cultured human cells, including four isothiazolone derivatives and two unrelated compounds. The most potent inhibitor was 2-[3-(trifluoromethyl)phenyl]isothiazolin-3-one (TMPI), a concentration of 1.0 μM inhibited telomerase activity by 50% according to a telomere repeat amplification protocol (TRAP) assay. Analysis using partially purified telomerase from AH7974 rat hepatoma cells demonstrated noncompetitive inhibition with the telomere-repeat primer and mixed inhibition with the dNTPs; the inhibition constant was 2.5 μM. TMPI did not inhibit eukaryotic DNA polymerase α, β, or human immunodeficiency virus reverse transcriptase (HIV RT). Thus, inhibition by TMPI was highly selective for telomerase. Inhibition by TMPI was quenched by 1 mM of dithiothreitol or glutathione, suggesting that TMPI inhibits telomerase by acting at a cystein residue. TMPI inhibition of this enzyme may find application as an antineoplastic agent.

Telomerase is an RNA-dependent DNA polymerase that adds telomeric repetitive DNA to the 3′ ends of eukaryotic chromosomes. The enzyme includes an RNA subunit that functions as a template (*1*). In most human somatic cells, telomerase activity is low or undetectable and telomere length progressively shortenes with successive cell divisions (*2*, *3*). On the other hand, telomerase activity can be detected in immortalized cell lines and in cells of over 90% of human tumors. In these cells, telomere lengths are stabilized (*4*, *5*). These findings suggest that telomerase is required for the ongoing proliferation of neoplastic cells and that telomerase inhibition represents a new therapeutic approach against cancer (*1*, *6*, *7*). In the present study, we set out to identify inhibitors of telomerase using a modification of the telomeric repeat amplification protocol (TRAP) (*5*) to permit performance of a large number of assays in a short time. The TRAP was combined with a scintillation proximity assay (SPA) (*8*) using [^{3}H]dTTP and scintillator beads as described by Savoysky et al. (*9*). We identified six chemicals as telomerase inhibitors. Further studies were performed to characterize the activity of the most potent of these.

* Correspondence should be addressed to: Shonen Yoshida, M. D., Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Showa-ku, Nagoya, 466-8550, Japan. Telephone: +81-52-744-2453. Fax: +81-52-744-2457. E-mail: syoshida@tsuru.med.nagoya-u.ac.jp.

[‡] Department of Internal Medicine.

[§] Laboratory of Cancer Cell Biology.

[⊥] First Department of Surgery.

[∥] Chugai Pharmaceutical Co., Ltd.

MATERIALS AND METHODS

Chemicals and Enzymes. Oligodeoxyribonucleotide primers were synthesized by Takara-Shuzo, Kyoto, Japan. Deoxyribonucleoside triphosphates also were obtained from the same source. Other reagents were of the highest obtainable grade and were purchased from commercial sources. The chemical library screened for telomerase inhibiton in this study was composed of organic compounds synthesized in Chugai Pharmaceutical Co. and those obtained from Maybridge Chemical Co. (Cornwall, UK), including 16 000 compounds with molecular weights of approximately 400. Calf thymus DNA polymerase α was purified by immunoaffinity-column chromatography as described previously (*10*). DNA polymerase β was purified from rat liver using a conventional method (*11*) or was purified from *E. coli* carrying a rat DNA polymerase β-cDNA recombinant plasmid (a gift from Dr. Akio Matsukage, Aichi Cancer Center Research Institute). HIV RT was obtained commercially from Seikagaku Kogyo Co. (Tokyo, Japan).

Animals and Cancer Cells. All animals received humane care in compliance with the Guidelines for Animal Experiments established at Nagoya University. Rat ascites hepatoma cells (AH 7974) were maintained by intraperitoneal transplantation in male Donryu rats. When cell growth reached the stationary phase, ascitic fluid was withdrawn and cells were harvested by centrifugation. A human leukemic cell line (HL 60) was cultured in RPMI medium supplemented with 10% fetal calf serum.

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Extraction of Telomerase. Cells were washed twice in ice-cold phosphate-buffered saline (PBS) by centrifuging at 3800 ($\times g$) for 5 min at 4 °C and then washed once with a buffer containing 10 mM *N*-2-hydroxyethyl-piperazine-*N*-7-2-ethanesulfonic acid (HEPES) (pH 7.5), 1.5 mM $MgCl_2$, 10 mM KCl, and 1 mM dithiothreitol (DTT). Then cell pellets were suspended in three volumes of 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) lysis buffer (*5*), containing 10 mM Tris-HCl (pH 7.5); 1 mM $MgCl_2$; 1 mM ethylene glycol-*O*,*O*′-bis(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA); 0.1 mM phenyl methylsulfonylfluoride (PMSF); 5 mM 2-mercaptoethanol; 0.5% CHAPS; and 10% glycerol. Homogenates were maintained on ice for 30 min and then centrifuged at 10000 ($\times g$) for 30 min. Supernatants were collected and stored at −80 °C until used as CHAPS extract.

Partial Purification of Rat Telomerase. The extract from 30 g of AH7974 cells (30 mL) was subjected to $(NH_4)_2SO_4$ fractionation. The precipitate following 30 to 60% saturation with $(NH_4)_2SO_4$ was dissolved in 5 mL of a buffer containing 20 mM Tris-HCl (pH 7.5), 1 mM ethylenediamine tetraacetate (EDTA), 1 mM 2-mercaptoethanol, 1 mM sodium bisulfite, 0.01% Nonidet P-40, 1 mM benzamidine, and 10% glycerol and then was applied to a gel filtration column of Sephacryl S-500 (4.5 × 90 cm). Fractions (10 mL each) containing peaks of telomerase activity were collected, and to these a 0.25 volume of glycerol was added. Fractions then were divided into aliquots and stored at −80 °C until used. The protein concentration of extract was adjusted to 1.6 μg protein/mL as determined by the method of Bradford (*12*).

Assay of Telomerase. The telomere repeat amplification protocol (TRAP) (*5*) was used for analysis of telomerase inhibition, with minor modifications. The assay was performed in two steps. The first step was telomerase-mediated extension of the first primer. The second step was polymerase chain reaction (PCR) amplification of 4μL aliquots, containing 6.4 μg protein, of products from the first reaction. These were added to 46 μL of reaction mixture containing 20 mM Tris-HCl (pH 8.3); 1.5 mM $MgCl_2$; 63 mM KCl, 0.005% Tween 20; 1 mM EGTA; 50 μM each of dATP, dTTP, and dGTP; 0.1 mg/mL bovine serum albumin (BSA); and 0.1 μg of TS (forward) primer (*5*). This mixture was incubated for 40 min at 30 °C. After the reaction mixture containing the telomerase-generated extension product was heated for 10 min at 85 °C, 5 μL of this mixture was mixed with PCR reaction mixture (45 μL) containing 20 mM Tris-HCl (pH 8.3); 1.5 mM $MgCl_2$; 63 mM KCl; 0.005% Tween 20; 1 mM EGTA; 50 μM each of dATP, dTTP, and dCTP; 10 μM of dGTP; 0.1 mg/mL BSA; 0.09 μg of TS primer; 2 units of *Taq* DNA polymerase; 2 μCi of [a-^{32}P]dGTP; and 0.1 μg of ACX (reverse) primer (*13*). An internal PCR standard (ITAS) was routinely added to the PCR reaction mixture. The ITAS consisted of 0.05 μg of NT primer (5′-ATCGCTTCTCGGCCTTTT-3′) and 0.005 amol of TSNT primer (5′-AATCCGTCGAGCAGAGTTAAAAGGCCGA-GAAGCGAT-3′) as described by Kim et al. (*13*). After the mixture were heated at 94 °C for 60 s, it was subjected to 28 cycles of PCR amplification (cycle steps, 94 °C for 30 s and 58 °C for 45 s). PCR products (50 μL) were analyzed by electrophoresis on a 12.5% nondenaturing polyacrylamide gel (PAGE; thickness, 1 mm). Telomerase activity was evident as incorporation of radioactive substrate in ladders of product DNA in multiples of six bases corresponding to the telomere repeat unit. Signal intensity was quantified with a BAS 2000 image analyzer (Fuji Film, Tokyo, Japan). Lysis buffer with no additions and RNase-treated telomerase were used as negative controls. For the RNase contorol, cell extracts with 6.4 μg protein were incubated with 0.64 μg RNase during the first extension reaction.

For the screening of telomerase inhibitors, TRAP was combined with a scintillation proximity assay (SPA) (*8*) using [^{3}H]dTTP and scintillator beads as described by Savoysky et al., designated TRAP−SPA (*9*).

Assay of DNA Polymerases and Reverse Transcriptase. Effects of telomerase inhibitors on 0.2 units of DNA polymerase α (*10*) were measured using activated calf thymus DNA (200 μg/mL) as a template-primer at pH 7.2, with or without 4 mM dithiothreitol. Effects of inhibitors on DNA polymerase β (0.2 units) were assayed (*11*) using activated calf thymus DNA (200 μg/mL) at pH 8.8, without dithiothreitol. Effects of inhibitors on human inmmunodeficiency virus reverse transcriptase (HIV RT, 0.1 or 0.2 units) were assayed in a reaction mixture (25 μL) containing 40 mM Tris-HCl (pH 7.5); 200 μg/mL activated calf thymus DNA; 8 mM $MgCl_2$; 40 μM [^{3}H]dTTP (240 cpm/pmol); 40 μM concentration of each of the other three dNTPs; 100 mM KCl; 200 μg/mL BSA, and an aliquot of the enzyme solution, with or without dithiothreitol. Alternatively, 200 μg/mL activated calf thymus DNA and four dNTPs (each 40 μM) were replaced by 40 μg/mL poly(A)-oligo(dT)12−18, and 40 μM [^{3}H]dTTP (240 cpm/pmol), respectively (*14*). One unit of enzyme activity was defined as the amount which catalyzing incorporation of 1 nmol of deoxyribonucleotides for 60 min at 37 °C under the conditions employed. In all cases, incubation was carried out at 37 °C for 30 min and acid-insoluble radioactivity was measured as described previously (*15*).

RESULTS

Chemical Structures of Telomerase Inhibitors. Screening of the library of the chemical compounds for telomerase inhibition was performed by the TRAP−SPA method as described in Materials and Methods. Among 16 000 compounds, six were selected as candidate telomerase inhibitors on the basis of a 50% inhibition concentration (IC_{50}) < 10 μM. As indicated by structural formula in Figure 1, they were 2-[3-(trifluoromethyl) phenyl]isothiazolin-3-one (TMPI), 2-(2,3,4-trifluorophenyl)isothiazolin-3-one (TFPI), ethyl 4-(3-oxo-3*H*-2-isothiazol-2-yl)benzoate (EOIB), 2-methylthio-4-(3-oxo-3*H*-isothiazol-2-yl)benzonitrile (MOIB), 3,5-di-*tert*-butylbenzene-1,2-diol (DBBD), and 1-[(5-methoxyquinoline-8-ylimino)methyl]naphthalen-2-ol (MQMN).

Inhibition of Telomerase by Various Chemical Compounds. Since the TRAP method involved a PCR protocol, we first tested dose-dependency of the PCR employed. Various amounts of products of the extension by telomerase in the first reaction were amplified by PCR in the second reaction. Incorporation of [^{32}P]dGTP into product DNA increased in a linear manner with increasing amounts of initial first reaction products (Figure 2). Thus, the assay was nearly linear up to 6.4 μg protein in Sephacryl S-500 fractions of rat telomerase. The assay was validated using ddGTP, a

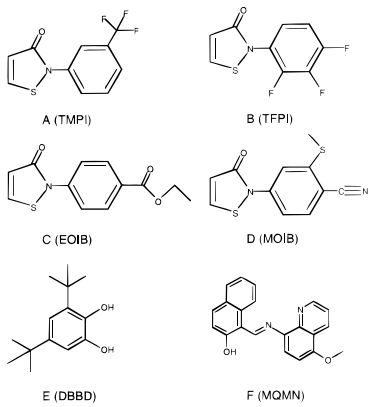


FIGURE 1: Chemical structures of telomerase inhibitors identified by screening a chemical library. A, 2-[3-(trifluoromethyl)phenyl]-isothiazolin-3-one (TMPI);B, 2-(2,3,4-trifluorophenyl)isothiazolin-3-one(TFPI);C,ethyl 4-(3-oxo-3*H*-2-isothiazol-2-yl)benzoate(EOIB);D, 2-methylthio-4-(3-oxo-3*H*-isothiazol-2-yl)benzonitrile(MOIB);E, 3,5-di-*tert*-butylbenzene-1,2-diol(DBBD);F, 1-[(5-methoxyquinoline-8-ylimino)methyl]naphthalen-2-ol (MQMN).

Table 1: IC^{50} of Telomerase Inhibitors[a]

telomerase inhibitors	telomerase inhibition IC_{50} (μM)
TMPI	1.0
TFPI	2.0
EOIB	2.0
MOIB	3.5
DBBD	15.0
MQMN	22.0

[a] IC_{50} values are shown for telomerase inhibition by six chemical compounds (names and structures Figure 1). Inhibition of telomerase activity was measured using a partially purified rat telomerase as in Materials and Methods. Concentrations showing 50% inhibition of the telomerase activity (IC_{50}) were obtained by scanning the ladders of product DNA in the presence of various concentrations of inhibitors. None of these chemical compounds inhibited the PCR reaction when assessed by ITAS bands (data no shown).

known inhibitor of reverse transcriptase and human telomerase (*16*). The IC_{50} for ddGTP, estimated to be 6.5 μM with our assay system, was similar to a previously reported value (4 μM) obtained with human telomerase (*16*).

Having validated the assay system, we further analyzed the effect of the inhibitors using partially purified rat telomerase because purification is much easier for the rat enzyme than for the human enzyme. Reaction products obtained with the rat ascites hepatoma telomerase were exactly the same to those with human telomerase, indicating that the rat enzyme was processive like the human enzymes (Figure 3). Table 1 summarizes the IC_{50} values. Among candidate inhibitors, TMPI (Figure 1A) inhibited telomerase most strongly, with an IC_{50} of 1.0 μM. Concentrations of TMPI exceeding 20 μM completely inhibited telomerase activity (Figure 5). Three other compounds, TFPI (Figure 1B), EOIB (Figure 1C), and MOIB (Figure 1D), also strongly inhibited telomerase, showing with the IC_{50s} of 2−3 μM. Notably, the first four compounds include an isothiazolone structure conjugated to modifications of benzene (Figure 1). The other inhibitors, DBBD (Figure 1E) and MQMN (Figure 1F), were unrelated to the first four and had only moderate effects with IC_{50s} of 15−22 μM, respectively.

Mode of Inhibition by TMPI. We studied the mode of inhibition by TMPI, the isothiazolone derivative showing the strongest inhibition (Table 1). Inhibition was measured as a function of concentration of TS (forward) primer, or of the dNTPs. Lineweaver−Burk plots of these data showed inhibition by TMPI to be noncompetitive with the TS primer (Figures 3A and 3B) and of mixed type with the dNTPs

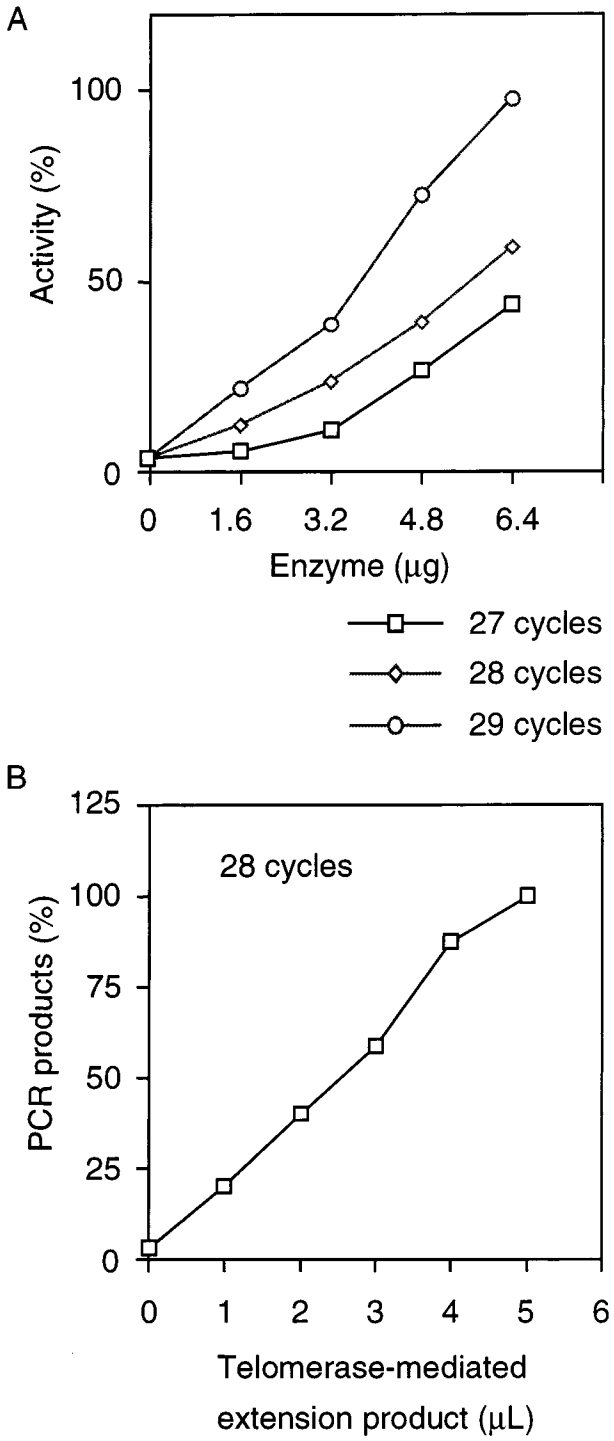
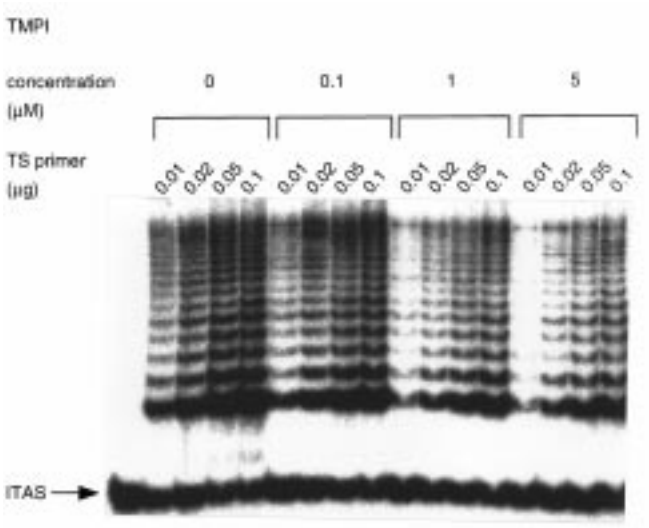
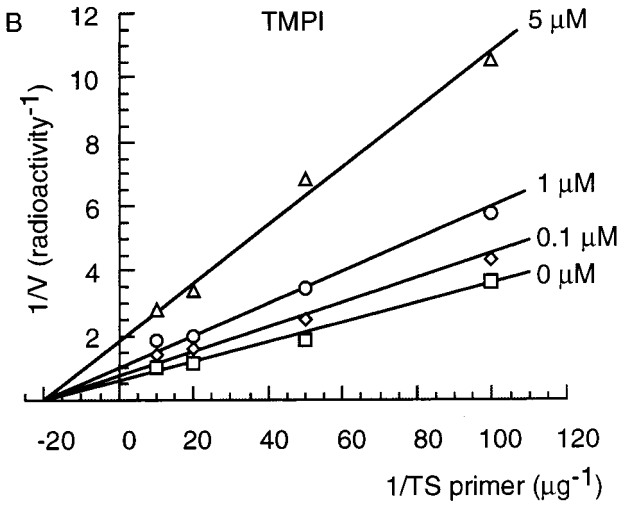
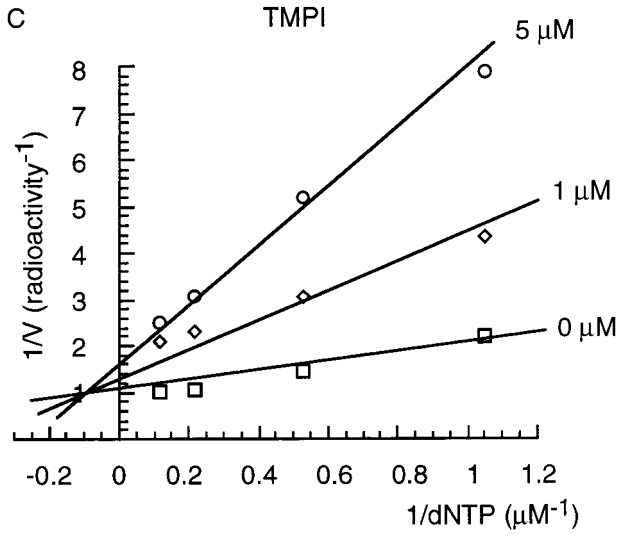


FIGURE 2: Linearity of the telomerase reaction. A, linearity of the overall reaction. Telomerase activity was measured with various amounts of partially purified rat telomerase as indicated. Number of polymerase chain reaction (PCR) cycles was 27, 28, or 29 as indicated. Amounts of PCR products, determined with a radio labeling image analyzing system, are represented in arbitrary units. B, linearity of the PCR reaction. Various amounts of the products of the reaction catalyzed by telomerase (extension of TS primer by reverse transcription) were then subjected to PCR for 28 cycles. Results were shown to be reproducible in three separate experiments.







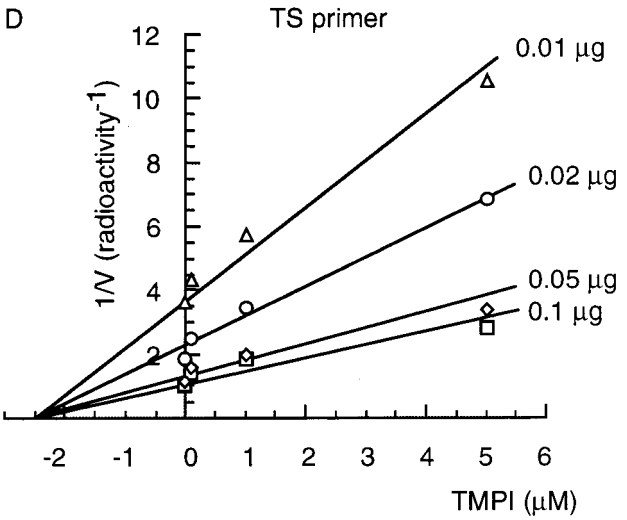
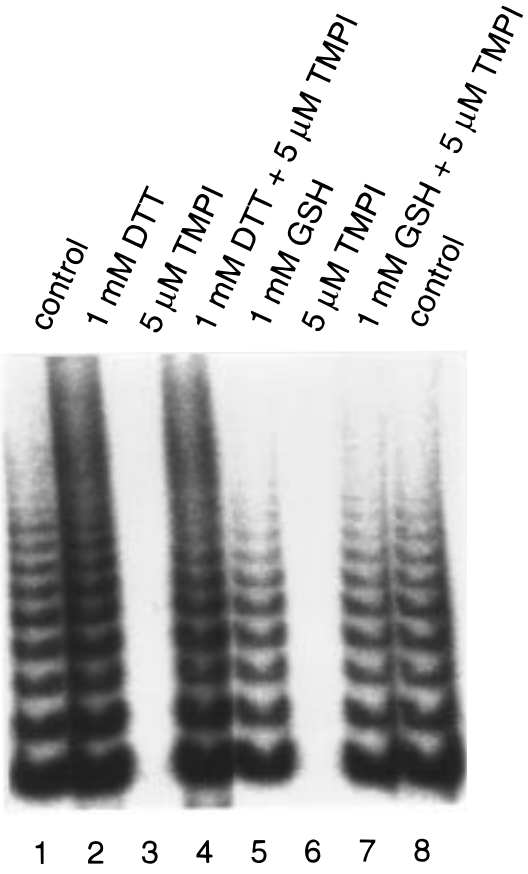
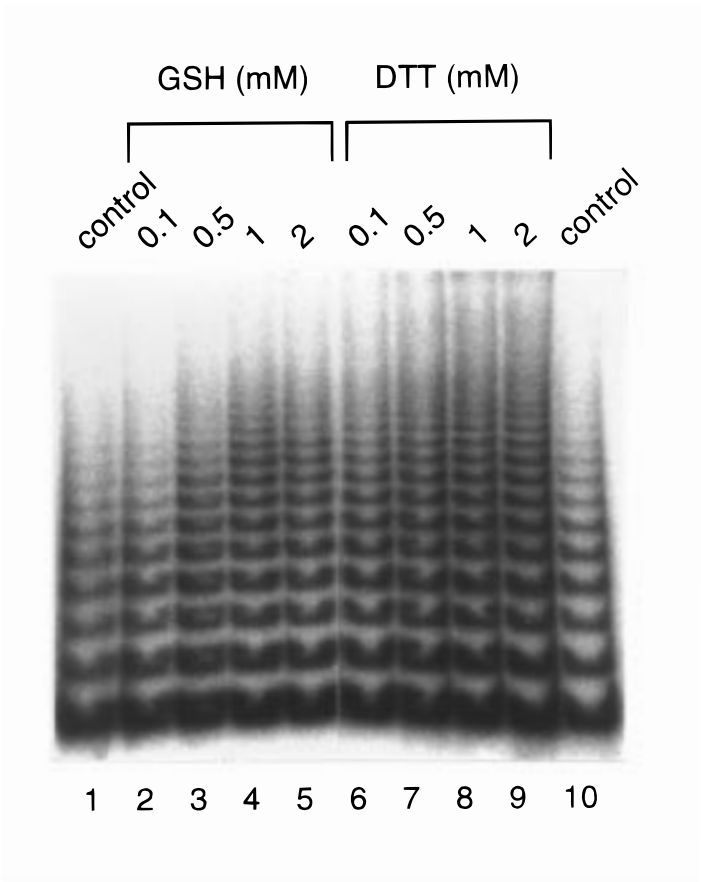


FIGURE 3: Kinetic analysis. A, gel electrophoresis pattern of telomerase inhibition by TMPI. Inhibition of telomerase activity was measured using partially purified rat telomerase as described in Materials and Methods. Various concentrations of TMPI were added before extension of TS (forward) primer by telomerase. The concentration of TS primer also was varied as indicated. B, noncompetitive inhibition with TS primer. Telomerase activity was measured by image analysis of radioactive bands on gel shown in panel A. Reactions were carried out with the indicated concentrations of TS (forward) primer in the absence (□) or presence of TMPI at concentrations of 1.0 (◇), and 5.0 μM (○). A Lineweaver−Burk plot was then constructed. C, mixed type inhibition with dNTPs. Telomerase activity was assayed with indicated concentrations of three dNTPs (dATP, dTTP, and dGTP), in the absence (□) or presence of TMPI at the concentrations of 1.0 (◇) and 5.0 μM (○). A Lineweaver−Burk plot was constructed. D, Dixon plots. Telomerase was assayed with indicated concentrations of TS (forward) primer in the absence (□) or presence of TMPI at 0.1(◇), 1.0 (○), and 5.0 μM (△). The K_i was estimated as 2.5 μM.





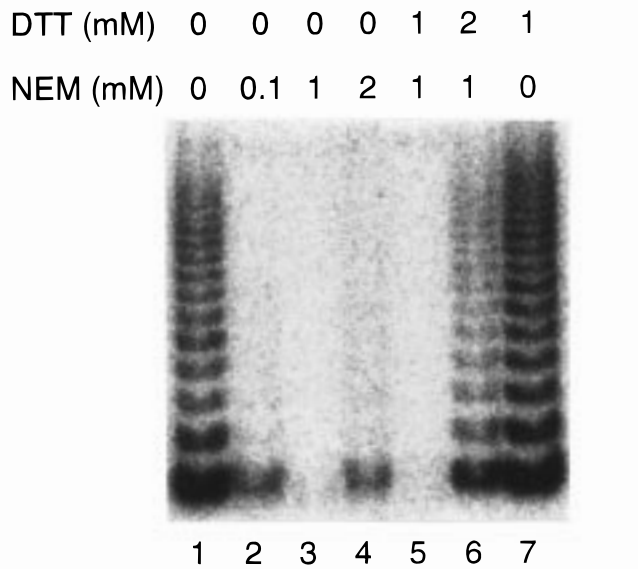


FIGURE 4: Effects of thiol reagents. A, reversal of TMPI inhibition by thiol reagents. Assay of telomerase was carried out in the presence or absence of 1 mM of DTT or glutathione (GSH), and of 5 μM TMPI, as indicated at the top of each lane (lanes 2−7). Control represents the reaction without these additions (lanes 1−8). Other conditions were as described in Materials and Methods. B, inhibition of telomerase by *N*-methylmaleimide (NEM). Assay was carried out in the presence of NEM (0.1, 1.0, and 2.0 mM; lanes 2−6)), with (lanes 5−7) or without DTT (lanes 1−4), as indicated at the top of each lane. C, effects of glutathione and DTT on telomerase activity. Glutathione (GSH; lanes 2−5) and DTT (lanes 6−9) at concentrations of 0.1, 0.5, 1.0, and 2.0 mM were added to the assay system as indicated at the top of each lane. Control (lanes 1 and 10) represent the assays without thiol reagents.

(Figure 3C). The K_m value for telomerase was 180 nM for the TS primer and 1.7 μM for the dNTPs considered together. K_m values for individual dNTPs were difficult to obtain given limitations of the assay procedure. The K_i value for telomerase inhibition by TMPI was estimated as 2.5 μM from a Dixon plot (Figure 3D). It was noted that the inhibition by TMPI or other inhibitors reduced the overall rate of reaction of telomerase, not affecting the relative length of product DNA (Figure 3A and data not shown).

Effects of Thiol Reagents on the Inhibitory Activity of TMPI. As shown in Figure 4A, addition of thiol reagents, 1 mM of DTT or glutathione, completely quenched telomerase

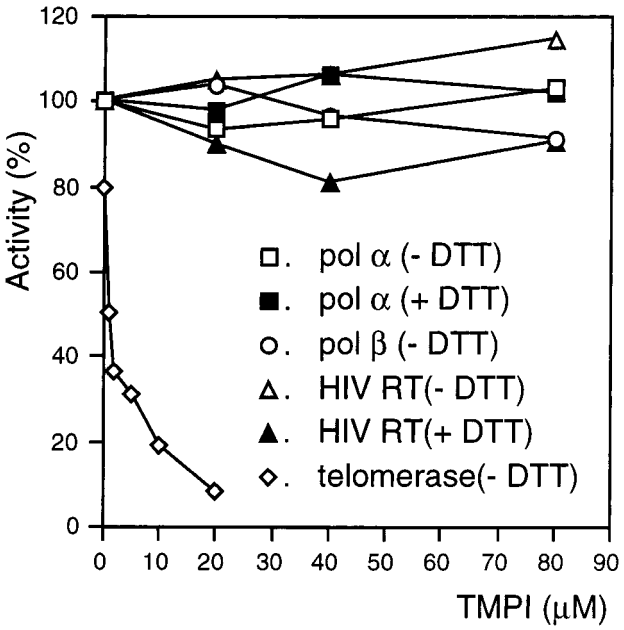


FIGURE 5: Effects of TMPI on activities of DNA polymerases α and β and on that of HIV reverse transcriptase. Activities of DNA polymerase α with (■) or without DTT (□), those of HIV reverse transcriptase with (▲) or without DTT (△), and that of DNA polymerases β (○) were assayed as described in Materials and Methods. TMPI was added to the reaction mixtures at concentrations of 0, 20, 40, and 80 μM. For comparison, inhibition of partially purified rat telomerase (◇) by TMPI (0, 0.1, 1.0, 2.0, 5.0, 10, and 20 μM) without DTT also is shown.

inhibition by 5 μM TMPI. A sulfhydryl-blocking reagent, *N*-ethylmaleimide (NEM), inhibited the telomerase reaction and this effect was reversed by DTT (Figure 4B). These results indicate TMPI inhibits telomerase by interacting with cysteine in the active center. However, telomerase exerted its activity without thiol reagent and the addition of DTT or glutathione (0.1−2 mM) to the reaction mixture stimulated only slightly (approximately 2-fold; Figure 4C).

Effects of Telomerase Inhibitors on DNA Polymerases α, β, and HIV RT. Although TPMI strongly inhibited telomerase activity, it did not affect the activities of DNA polymerase α or β, or that of HIV RT (Figure 5) under the assay conditions described in Materials and Methods. Under the same reaction conditions, ddGTP strongly inhibited the HIV RT with an IC_{50} of 0.04 μM (*17*).

Since the inhibition of telomerase by TMPI was completely quenched by 1 mM DTT, inhibition of other DNA polymerases was examined both in the presence and absence of DTT (Figure 5). No obvious inhibition of these enzymes was observed under these two conditions, indicating that resistances to TMPI and other isothiazolone derivatives of these enzymes were not due to the presence of the thiol reagent in the reaction mixture.

DISCUSSION

As telomerase could be a novel and highly selective target for antitumor drug design, a number of reports have examined substances believed to inhibit telomerase. Since telomerase belongs to the reverse transcriptase (RT) family, inhibitors of retroviral RT have been tested. Among these, the 2′,3′-dideoxynucleosides undergo phosphorylation to triphosphate forms (d_2NTPs) that strongly inhibit RT and also inhibit telomerase from various sources (*16*, *18*). However, phosphorylation of 2′,3′-dideoxynucleosides in cells is inefficient and unfortunately d_2NTPs inhibit cellular DNA polymerases β and γ (*19*) much more strongly than telomerase. The anti-human immunodeficiency virus (HIV) drug, 3′-azidothymidine (AZT) inhibits telomerase when in its triphosphate form (AZT-TP), but AZT-TP also inhibits cellular DNA polymerases (*20*). While telomerase activity in cultured cancer cells is dramatically suppressed by a number of differentiation inducers (*21*), the enzyme itself is not directly inhibited by these agents. Therefore, the specificity of inhibition by these drugs may be relatively low or variable. A peptide nucleic acid (PNA) (*22*, *23*) with a sequence complementary to the telomerase RNA or a hammer-head ribozyme targeted for telomerase RNA (*24*) may offer more specific inhibiton, but presently efficiency of transduction efficiency of these oligonucleotides or their cDNAs into cell is poor.

Seeking more practical inhibitors, we screened a chemical library for inhibition of mammalian telomerase using a TRAP−SPA assay (*9*), identifying six inhibitors among 16 000 synthetic chemical compounds. Four of these inhibitors contained an isothiazolone moiety conjugated to a derivative of benzene. One of these, a compound designated TMPI (Figure 1A), showed the strongest inhibition (IC_{50}, 1.0 μM; estimated K_i, 2.5 μM), which was noncompetitive with primer DNA and of mixed type with the substrate dNTPs. Inhibition by TMPI appeared selective for telomerase, given its minimal effect on mammmalian DNA polymerases α and β and HIV reverse transcriptase.

Isothiazolone derivatives previously have been reported to have effects as antimicrobial agents or metabolic inhibitors. For examples, benzisothiazol-3-one (BIT) has shown lethal activity against *Escherichia coli* and *Shizosaccaharomyces pombe*, presumably by interference with initiation of DNA replication (*25*). Growth-inhibitory activity of BIT is rapidly quenched by addition of thiol-containing compounds such as cysteine. Isothiazolone derivatives have been shown to interact oxidatively with thiols to form disulfides (*26*). Further, *N*-phenyl heteroaryl-fused isothiazolones inhibit

cartilage proteoglycan breakdown in arthritis (*27*, *28*) by blockcade of a matrix metalloproteinases activation pathway at the IL-1β receptor (*27*). Other isothiazolones block the IL-5 receptor by covalently binding at a cysteine residue (*29*). Notably in this context, telomerase inhibition by TMPI was quenched by thiol reagents, DTT and glutathione, as that by NEM (Figure 4). We believe that the isothiazolone moiety of TMPI may interact with the sulfhydryl group of one or more cysteine residues at or near the active site of the target molecule, even though activities of both DNA polymerase α and HIV RT, which are little affected by TMPI, are strongly inhibited by conventional sulfhydryl-blocking reagents (*19*). The halogenated phenyl group of TMPI may facilitate access of the isothiazolone moiety to a cysteine residue in the active center of telomerase. Similarly, isothiazolone derivatives may be found to exert a variety of biologic activities that reflect their specific conjugates, e.g. modified phenyl or benzyl groups.

The isothiazolone derivatives that we have described herein may be important tools for in vitro. analysis of telomerase reaction mechanisms. Effects of these compounds *in vivo* are now under study in our laboratory.

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REFERENCES

1. Feng, J., Funk, W. D., Wang, S.-S., Weinrich, S. L., Avilion, A. A., Chiu, C. P., Adams, R. R., Chang, E., Allsopp, R. C., Yu, J., Le, S., West, M. D., Harley, C. B., Andrews, W. H., Greider, C. W., and Villeponteau, B. (1995) *Science 269*, 1236−1241.
2. Harley, C. B., Futcher, A. B., and Greider, C. W. (1990) *Nature 345*, 458−460.
3. Harley, C. B., Kim, N. W., Prowse, K. R., Weinrich, S. L., Hirsch, K. S., West, M. D., Bacchetti, S., Hirte, H. W., Counter, C. M., Greider, C. W., Wright, W. E., and Shay, J. W. (1994) *Cold Spring Harbor Symp. Quantum Biol. 59*, 307−315.
4. Counter, C. M., Avilion, A. A., LeFeuvre, C. E., Stewart, N. G., Greider, C. W., Harley, C. B., and Bacchetti, S. (1992) *EMBO J. 11*, 1921−1929.
5. Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. (1994) *Science 66*, 2011−2015.
6. Rhyu, M. S. (1995) *J. Natl. Cancer Inst. 87*, 884−894.
7. Parkinson, E. K. (1996) *Br. J. Cancer 73*, 1−4.
8. Bosworth, N., and Towers, P. (1989) *Nature 341*, 167−168.
9. Savoysky, E., Akamatsu, K., Tsuchiya, M., and Yamazaki, T. (1996) *Nucleic Acids Res. 24*, 1175−1176.
10. Tamai, K., Kojima, K., Hanaichi, T., Masaki, S., Suzuki, M., Umekawa, H., and Yoshida, S. (1988) *Biochim. Biophys. Acta 950*, 263−273.
11. Yoshida, S., Yamada, M., and Masaki, S. (1979) *J. Biochem. 85*, 1387−1395.
12. Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
13. Kim, N. W., and Wu, F. (1997) *Nucleic Acids Res. 25*, 2595−2597.
14. Izuta, S., Shimada, N., Kitagawa, M., Suzuki, M., Kojima, K., and Yoshida, S. (1992) *J. Biochem. 112*, 81−87.
15. Yoshida, S., Kondo, T., and Ando, T. (1974) *Biochim. Biophys. Acta 353*, 463−474.
16. Strahl, C., and Blackburn, E. H. (1996) *Mol. Cell. Biol. 16*, 53−65.
17. Prasad, V. R., Lowy, I., de los Santos, T., Chiang, L., and Goff, S. P. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 11363−11367.
18. Prowse, K. R., Avilion, A. A., and Greider, C. W. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1493−1497.
19. T. S.-F., Wang (1996) in *DNA Replication in Eukalyotic Cells* (M. L. DePamphilis, Ed.) pp 461−493, Cold Spring Harbor Laboratory Press: Plainview, NY.
20. Inoue, T., Tsushita, K., Itoh, T., Ogura, M., Hotta, T., Saneyoshi, M., Yoshida, S., Saitoh, H., Tomoda, Y., and Nagai, Y. (1989) *Antimicrob. Agents Chemother. 33*, 576−579.
21. Savoysky, E., Yoshida, K., Ohtomo, T., Yamaguchi, Y., Akamatsu, K., Yamazaki, T., Yoshida, S., and Tsuchiya, M. (1996) *Biochem. Biophys. Res. Commun. 226*, 329−334.
22. Hamilton, S. E., Pitts., A. E., Katipally, R. R., Jia, X., Rutter, J. P., Davies, B. A., Shay, J. W., Wright, W. E., and Corey, D. R. (1997) *Biochemistry 36*, 11873−11880.
23. Norton, J. C., Piatyszek, M. A., Wright, W. E., Shay, J. W., and Corey, D. R. (1996) *Nat. Biotechnol. 14*, 615−619.
24. Kanazawa, Y., Ohkawa, K., Ueda, K., Mita, E., Takehara, T., Sasaki, Y., Kasahara, A., and Hayashi, N. (1996) *Biochem. Biophys. Res. Commun. 225*, 570−576.
25. Collier, P. J., Ramsey, A. J., Austin, P., and Gilbert, P. (1990) *J. Appl. Bacteriol. 69*, 569−577.
26. Collier, P. J., Ramsey, A., Waigh, R. D., Douglas, K. T., Austin, P., and Gilbert, P. (1990) *J. Appl. Bacteriol. 69*, 578−584.
27. Wright, S. W., Petraitis, J. J., Abelman, M. M., Batt, D. G., Bostrom, L. L., Corbett, R. L., Decicco, C. P., Di Meo, S. V., Freimark, B., Giannaras, J. V., Green, A. M., Jetter, J. W., Nelson, D. J., Orwat, M. J., Pinto, D. J., Pratta, M. A., Sherk, S. R., Williams, J. M., Magolda, R. L., and Arner, E. C. (1994) *J. Med. Chem. 37*, 3071−3078.
28. Wright, S. W., Petraitis, J. J., Batt, D. G., Corbett, R. L., Di Meo, S. V., Freimark, B., Giannaras, J. V., Orwat, M. J., Pinto, D. J., Pratta, M. A., Sherk, S. R., Stampfli, H. F., Williams, J. M., Magolda, R. L., and Arner, E. C. (1995) *Bioorg. Med. Chem. 3*, 227−234.
29. Devos, R., Guisez, Y., Plaetinck, G., Cornelis, S., Tavernier, J., van der Heyden, J., Foley, L. H., and Scheffler, J. E. (1994) *Eur. J. Biochem. 225*, 635−640.

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