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STRUCTURE–ACTIVITY RELATIONSHIP, SELECTIVITY AND MODE OF INHIBITION OF TERMINAL DEOXYRIBONUCLEOTIDYLTRANSFERASE BY STREPTOLYDIGIN ANALOGS

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Abstract—Three of thirty-one streptolydigin analogs resembled the parent compound in selectively inhibiting terminal deoxyribonucleotidyltransferase (terminal transferase) when compared with cellular DNA polymerases α, β and γ, simian sarcoma virus DNA polymerase, herpes simplex virus-induced DNA polymerase and cellular RNA polymerase II. The other twenty-eight compounds either did not inhibit any of these enzymes or inhibited all of these enzymes without selectivity for terminal transferase. Two analogs that selectively inhibited terminal transferase ($K_i = 0.12$ mM) were 2- to 3-fold more potent than streptolydigin ($K_i = 0.32$ mM). All the selective inhibitors of termininal transferase are 3-acyltetramic acids with various substituent groups at the 1-, 3- and 5-positions. One of these, a less potent inhibitor of terminal transferase than streptolydigin, lacks the 3- and 5-substituents of streptolydigin but has virtually the same 1-substituent. The substituent groups of the other two selective inhibitors are structurally different from those of streptolydigin but essentially identical to each other. The mode of inhibition of terminal transferase by selective inhibitors was the same as for streptolydigin, but different from an analog which non-selectively inhibited terminal transferase. Evidence suggested that the selective inhibitors specifically interacted with terminal transferase and not with initiator (oligo- or polydeoxyribonucleotide), substrate (deoxyribonucleoside 5′-triphosphates) or the divalent cation (Mn^{2+}) required for enzyme activity. The data also implied that these compounds bind to the enzyme at a site(s) other than the initiator or substrate binding sites. In contrast, an analog which non-selectively inhibited terminal transferase apparently interacted with many proteins and polydeoxyribonucleotides non-specifically.

Terminal transferase‖ catalyzes the polymerization of deoxyribonucleotides on the 3′-hydroxyl ends of oligo- or polydeoxyribonucleotide initiators [1]. Initially, this enzyme was found only in thymus [2]. Subsequently, high activities were discovered in leukocytes from patients with acute lymphoblastic leukemia [3, 4] or with rare cases of both acute [5] and chronic [6–8] myelocytic leukemia, while the activity was hardly detectable in normal leukocytes or leukocytes from chronic lymphocytic and most cases of chronic and acute myelocytic leukemia. In acute lymphoblastic leukemia, the specific activity of terminal transferase changes with the status of disease. It is high upon diagnosis, low upon remission and high again upon relapse [4, 9]. Thus, terminal transferase is a valuable marker for certain leukemias. Despite these findings, the intracellular function of terminal transferase is unknown. Therefore, selective inhibitors of terminal transferase might be useful in characterizing its function. Furthermore, such inhibitors may be of value for the treatment of leukemia exhibiting abnormal terminal transferase activity. Previously, we reported [10] that streptolydigin, an antibiotic isolated from *Streptomyces lydicus* [11], selectively inhibited terminal transferase in comparison with cellular DNA polymerases α, β and γ [12], SSV DNA polymerase and cellular RNA polymerase II. In this investigation, we examined thirty-three analogs of streptolydigin (Table 1) to find additional inhibitors of terminal transferase and to determine the structural requirements of compounds needed for selective inhibition.

MATERIALS AND METHODS

Structures of streptolydigin and its analogs are shown in Table 1. Tirandamycin was supplied by The Upjohn Co., Kalamazoo, MI. Streptolydigin analogs II, IV, V, XVI–XVIII, XXIII–XXVII and XXXI [13] and analogs I, III, VI–XV, XIX–XXII, XXVIII–XXX and XXXII (V. J. Lee *et. al.*, *J. med.*

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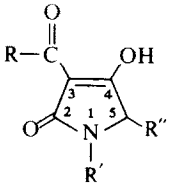
‖ Abbreviations: terminal transferase, terminal deoxynucleotidyltransferase; SSV, simian sarcoma virus; HSV, herpes simplex virus; DEAE, diethylaminoethyl; Buffer A, 25 mM Tris/sulfate (pH 8.3) containing 1 mM $MgSO_4$, 6 mM NaCl, 1 mM dithiothreitol and 0.1 mM (ethylenedinitrilo tetraacetic acid; and I_{50}, concentration of compound yielding 50 per cent inhibition of enzyme activity.

Table 1. Structures of streptolydigin analogs

General formula:



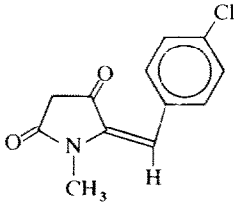
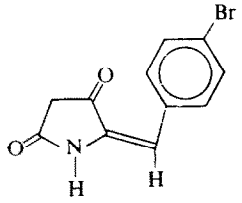
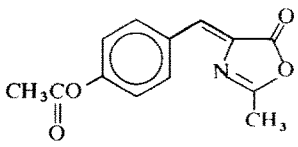
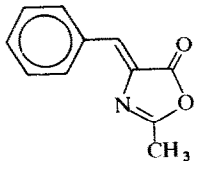
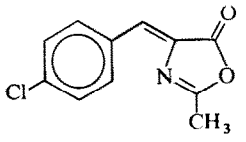
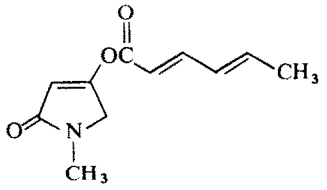
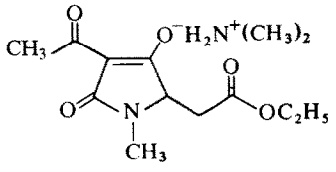
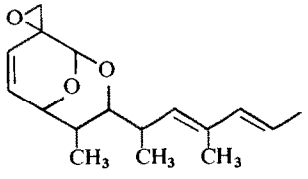
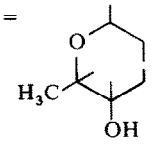
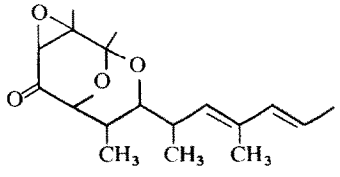
Compound	Structure
I	R = CH_3O; R′ = H; R″ = H_2
II	R = C_2H_5O; R′ = H; R″ = H_2
III	R = C_2H_5O; R′ = H; R″ = $CH_2COOC_2H_5$
IV	R = C_2H_5O; R′ = CH_2C_6H; R″ = H_2
V	R = C_2H_5O; R′ = CH_3; R″ = H_2
VI	R = C_2H_5O; R′ = CH_3; R″ = =CHOH
VII	R = C_2H_5O; R′ = CH_3; R″ = $=CHC_6H_4OH$(p)
VIII	R = C_2H_5O; R′ = $=CHC_6H_4Br$(p)
IX	R = C_2H_5O; R′ = CH_3; R″ = $=CHC_6H_4Cl$(p)
X	
XI	
XII	R = C_6H_4Cl(p)CH = CH; R′ = $CH_2C_6H_5$; R″ = $=CHC_6H_4Cl$(p)
XIII	R = CH_3; R′ = $COCH_3$; R″ = $=CHC_6H_4Cl$
XIV	R = CH_3; R′ = $COCH_3$; R″ = $=CHC_6H_5$
XV	R = CH_3; R′ = $CH_2C_6H_5$; R″ = CH_2COOH
XVI	R = CH_3; R′ = $CH_2C_6H_5$; R″ = H_2
XVII	R = CH_3; R′ = CH_3; R″ = H_2
XVIII	R = CH_3; R′ = CH_3; R″ = $CH_2CONHICH_3$
XIX	R = CH_3; R′ = CH_3; R″ = $CH_2S(=O)CH_2C_6H_5$
XX	R = CH_3; R′ = CH_3; R″ = $=CHS(=O)CH_2C_6H_5$
XXI	R = CH_3; R′ = H; R″ = $CH_2COOC_2H_5$
XXII	R = CH_3; R′ =  ; R″ = H_2
XXIII	R = $CH_3(CH=CH)_2$; R′ =  ; R″ = H_2
XXIV	R = $CH_3(CH=CH)_2$; R′ = $CH_2C_6H_4OCH_3$; R″ = H_2
XXV	R = $CH_3(CH=CH)_2$; R′ = $CH_2C_6H_5$; R″ = H_2
XXVI	R = $CH_3(CH=CH)_2$; R′ = H; R″ = H_2
XXVII	R = $(CH_3)_2CHCH=C(CH_3)CH=CH$; R′ = $CH_2C_6H_4OCH_3$; R″ = H_2
XXVIII	

Table 1—*Continued*

Compound	Structure
XXIX	
XXX	
XXXI	
XXXII	
Streptolydigin	R =  ; R′ =  ; R″ = CHCONHCH$_3$ | CH$_3$
Tirandamycin	R =  R′ = H; R″ = H$_2$

Chem., manscript submitted for publication) were prepared as reported. Other compounds were obtained from the following sources: calf thymus DNA (Worthington Biochemical Corp., Freehold, NJ); poly (A) · $(dT)_{12-18}$ and $(dT)_{12-18}$ (P. L. Biochemicals Inc., Milwaukee, WI; $(dA)_{12-18}$ and $(dG)_{12-18}$ (Collaborative Research Inc., Waltham, MA; tritium-labeled deoxyribonucleoside 5′-triphosphates (Schwartz-Mann, Orangeburg, NY): unlabeled deoxyribonucleoside 5′-triphosphates (Plenum Scientific Research Inc., Hackensack, NJ); and DEAE-Sephadex A-25 (Pharmacia Fine Chemicals Inc., Piscataway, NJ). All other reagents were the highest commercial grade available.

Lymphoblasts from an acute lymphoblastic leukemia patient and phytohemagglutinin-stimulated normal human lymphocytes were obtained as described previously [14]. Molt-4 cells are of acute lymphoblastic leukemia origin. Their culturing and harvesting have been described [14]. Terminal transferase and DNA polymerases α, β and γ were prepared according to our published procedures [14, 15]. DNA polymerases α, β and γ were prepared from all these cell types, whereas terminal transferase was prepared only from leukemic lymphoblasts and Molt-4 cells since phytohemagglutinin-stimulated normal human lymphocytes have little of this enzyme. RNA polymerase II (α-amanitin sensitive) was obtained from Molt-4 cells according to the method of Roeder and Rutter [16] which involves $(NH_4)_2SO_4$ precipitation and DEAE-Sephadex A-25 chromatography. SSV DNA polymerase was prepared according to the method of Abrell and Gallo [17]. All final enzyme preparations were dialyzed

against Buffer A containing 50% (v/v) glycerol and stored at −20°. The specific activities of these enzymes were similar to those reported previously and their properties have been described [14–17]. One unit of enzyme activity equals 1 nmole of tritium-labeled nucleotide polymerized in 1 hr. Homogenous terminal transferase (30,186 units/mg protein) from calf thymus was prepared as described previously [18]. Its properties were the same as those reported for a homogenous terminal transferase from leukemic lymphoblasts [19]. HSV-induced DNA polymerase was obtained from RPMI-8226 cells 12 hr after infection with HSV-type I. RPMI-8226 cells are lymphoid cells of multiple myeloma origin; they were cultured, infected with HSV, and harvested as described by Mizrahi *et al.* [20]. Purified chromatin was obtained from infected cells [21] and the chromatin was extracted with 2 M NaCl, dialyzed against 0.14 M NaCl, and centrifuged to pellet the DNA-histone formed [14]. The supernatant fraction was removed and $(NH_4)_2SO_4$ was added to 70% saturation. The precipitate formed was recovered by centrifugation (25,000 *g*, 15 min), dissolved in Buffer A containing 5% (v/v) glycerol and 0.4 M NaCl, and dialyzed against several changes of the same buffer. The dialysate was clarified by centrifugation, layered onto linear (10–30%) glycerol gradients prepared in Buffer A containing 0.4 M NaCl, and centrifuged as described previously [14]. The gradients were fractionated [14], and the fractions were assayed for various DNA polymerase activities. Two kinds of DNA polymerases were found. The major activity had properties previously reported for HSV-induced DNA polymerase [22–25]. It had a sedimentation coefficient of 8S, was stimulated 3- to 4-fold by 0.15 M KCl, and was inhibited 75 per cent by 7.2 μM phosphonoacetic acid. This activity was well separated and distinguished from a minor DNA polymerase β activity which sedimented at 3–4S, was inhibited by 0.15 M KCl, and was resistant to 7.2 μM phosphonoacetic acid. Neither DNA polymerase α nor γ activities were detected in the gradient fractions. These activities are distinguished from HSV-induced DNA polymerase by resistance to 7.2 μM phosphonoacetic acid, inhibition by 0.15 M KCl (DNA polymerase α) and utilization of the template/primer, poly (A) · $(dT)_{12-18}$, by DNA polymerase γ. Gradient fractions containing HSV-induced DNA polymerase were pooled, dialyzed against Buffer A containing 50% (v/v) glycerol, and stored at −20°. The protein concentration of the enzyme preparation was below the amount detectable by the method of Lowry *et al.* [26], and therefore, specific activity was not determined.

Enzymes were assayed in a final volume of 0.2 ml as follows: (a) for DNA polymerases α or β, 10 μmoles Tris–HCl (pH 8.3), 1.2 μmoles magnesium acetate, 4.0 μmoles dithiothreitol, 0.16 μmole each of dATP, dGTP and dCTP, 0.01 μmole [^{3}H]dTTP (144 c.p.m./pmole), 30 μg of activated [14] calf thymus DNA, and enzyme; (b) for DNA polymerase γ, 10 μmoles Tris–HCl (pH 7.5), 0.1 μmole $MnCl_2$, 20 μmoles KCl, 0.6 μmole dithiothreitol, 8 μg bovine serum albumin, 0.001 μmole [^{3}H]dTTP (1036 c.p.m./pmole), 2.4 μg poly (A) · $(dT)_{12-18}$, and enzyme; (c) for terminal transferase, 10 μmoles Tris–HCl (pH 7.5), 0.1 μmole $MnCl_2$, 20 μmoles KCl, 0.6 μmole dithiothreitol, 8 μg bovine serum albumin, 0.01 μmole [^{3}H]dGTP (115 c.p.m./pmole), 2.4 μg $(dA)_{12-18}$, and enzyme; (d) for SSV DNA polymerase, the same as for DNA polymerase γ except that 0.04 μmole [$_3$H]dTTP (412 c.p.m./pmole) was used; (e) for HSV-induced DNA polymerase, 10 μmoles Tris–HCl (pH 8.0), 0.5 μmole $MgCl_2$, 30 μmoles KCl, 0.2 μmole dithiothreitol, 0.00013 μmole each of dATP, dGTP, dCTP and [^{3}H]dTTP (10,482 c.p.m./pmole), 10 μg of activated calf thymus DNA, and enzyme; and (f) for RNA polymerase II, 10 μmoles Tris–HCl (pH 8.0), 0.4 μmole $MnCl_2$, 0.2 μmole $MgCl_2$, 0.2 μmole dithiothreitol, 0.13 μmole each of ATP, GTP and CTP, 0.0018 μmole [^{3}H]UTP (708 c.p.m./pmole), 20 μg of heat-denatured calf thymus DNA, and enzyme. The addition sequence for assay mixtures was template/primer or initiator, streptolydigin analog, the components of the assay mixture, and finally enzyme. Concentrated stock solutions of streptolydigin analogs were prepared in dimethylsulfoxide and diluted 40-fold into assay mixtures. Controls without streptolydigin analogs contained the same amount of dimethylsulfoxide, which by itself was not inhibitory. Routinely, each enzyme was tested for inhibition at five different duplicate streptolydigin analog concentrations ranging from 0.04 to 0.80 mM. Very active compounds were also tested at lower concentrations. Assay mixtures were incubated at 37° (30° for DNA polymerase γ) for 30 min and kept in an ice bath. Fifty micrograms of yeast RNA and 1 ml of ice-cold 20% trichloroacetic acid containing 3% sodium pyroposphate were added immediately. After 10 min the precipitates were collected on nitrocellulose membrane filters and were washed with ice-cold 5% trichloroacetic acid, dried and counted using a toluene-based scintillation fluid [14]. All enzyme activities were linear with respect to time and protein concentration. Data describing the inhibition of terminal transferase by streptolydigin analogs at various concentrations of dGTP were plotted according to Lineweaver and Burk [27]. Inhibition constants were determined by replotting the *y*-intercepts of these graphs versus inhibitor concentrations [28].

RESULTS

Listed in Table 1 are the structures of streptolydigin analogs which were tested for inhibition of terminal transferase, DNA polymerases α, β and γ, SSV DNA polymerase, HSV-induced DNA polymerase and RNA polymerase II. Compounds I–VII, XV–XVIII, XX–XXIII, XXVIII–XXX and XXXII did not appreciably inhibit any of these enzymes. Compounds VIII, IX, XII, XIX, XXIV–XXVII and XXXI non-selectively inhibited all of these enzymes (e.g. Fig. 1D). The inhibitory actions of compounds X and XI could not be assessed because these compounds, unlike all the others, precipitated nucleoside 5′-triphosphates from solution (data not shown). Three streptolydigin analogs (XIII, XIV and tirandamycin), like streptolydigin, selectively inhibited terminal transferase (Fig. 1)

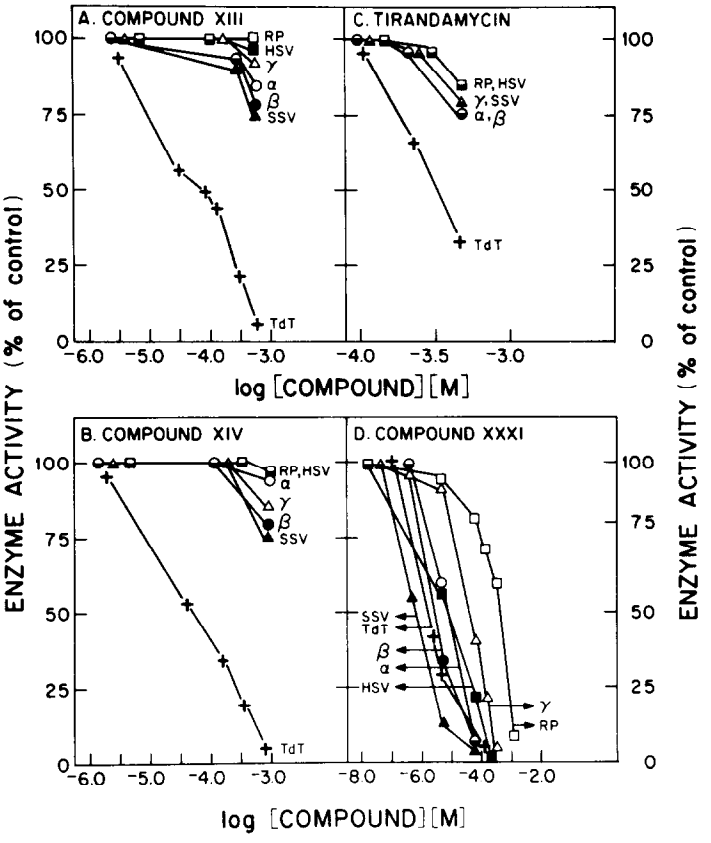


Fig. 1. Inhibition of nucleotide polymerases by streptolydigin analogs. Standard assay mixtures with and without streptolydigin analogs were incubated with either 0.01, 0.16, 0.16, 0.02, 0.03, 0.004 or 0.03 unit of terminal transferase (TdT), DNA polymerases α, β or γ, SSV DNA polymerase, HSV-induced DNA polymerase, or RNA polymerase II (RP), respectively. Controls without streptolydigin analogs incorporated 46, 80, 82, 10, 14, 2 or 14 pmoles of radioactively labeled nucleoside monophosphate for each enzyme, respectively. Structures of compounds are given in Table 1. Concentrations of tirandamycin greater than 0.46 mM could not be used because of solubility problems. Cellular DNA polymerases were obtained from leukemic lymphoblasts but quantitatively identical results were obtained with enzymes from phytohemagglutinin-stimulated normal human lymphocytes and Molt-4 cells.

[10]. Tirandamycin (I_{50} = 0.34 mM) was a weaker inhibitor of terminal transferase than streptolydigin (I_{50} = 0.15 mM), whereas compounds XIII (I_{50} = 0.07 mM) and XIV (I_{50} = 0.04 mM) were more potent inhibitors. Compound XIII and XIV were the only analogs tested that have 1-acetyl substituents. The concentrations (0.65–0.75 mM) of compounds XIII and XIV that inhibited terminal transferase by 95 per cent inhibited the other enzymes by less than 25 per cent.

To determine if the mode of inhibition of terminal transferase by compounds XIII and XIV was similar to that previously reported for streptolydigin [10], experiments involving variation of assay mixture components were conducted. For additional comparison, a non-selective inhibitor of terminal transferase, compound XXXI, was included in these experiments. Inhibition of terminal transferase by compounds XIII and XIV was not substantially affected by the concentration or type of initiator, by the type of substrate, or by the concentration of bovine serum albumin or Mn^{2+} used in the assay mixtures (Table 2). However, inhibition was dependent upon enzyme concentration. In contrast, inhibition of terminal transferase by compound XXXI was dependent upon the type and concentration of initiator and the concentration of bovine serum albumin or enzyme used in assay mixtures, but independent of the type of substrate or concentration of Mn^{2+}. The effect of variation of substrate (dGTP) concentration on the inhibition of terminal transferase by compounds XIII, XIV and XXXI is shown by double reciprocal plots (Fig. 2). Each compound was non-competitive inhibitor, implying that inhibition was independent of substrate concentration. Apparent inhibition constants for compounds XIII, XIV and XXXI were determined to be 0.13, 0.12 and 0.008 mM, respectively, which are lower than that reported for streptolydigin (0.32 mM) [10]. Preincubation of compounds XIII, XIV or XXXI with either terminal transferase, $(dA)_{12-18}$, or dGTP (5 min, 37°), followed by addition of the remaining constituents of the assay mixtures and standard assay conditions, resulted in amounts of inhibition similar

Table 2. Effect of variations of assay mixture components on inhibition of terminal transferase by streptolydigin analogs*

Expt.	Substitution to standard assay mixture	Enzyme activity (% of controls)			
		Inhibitor absent (controls)	Inhibitor present		
			XIII (0.08 mM)	XIV (0.05 mM)	XXXI (0.005 mM)
1	None	100 (43)	39	56	34
	27 μg $(dA)_{12-18}$	100 (40)	43	58	63
	54 μg $(dA)_{12-18}$	100 (39)	41	61	94
	2.4 μg $(dG)_{12-18}$	100 (23)	37	60	76
	7.2 μg $(dT)_{12-18}$	100 (10)	36	54	31
	30 μg Activated DNA	100 (96)	44	60	78
	0.01 μmole [^{3}H]dATP	100 (8)	35	54	38
	0.01 μmole [^{3}H]dCTP	100 (45)	37	49	30
	0.01 μmole [^{3}H]dTTP	100 (16)	39	62	32
	1.0 μmole Mn^{2+}	100 (35)	35	52	36
	100 μg Bovine serum albumin	100 (47)	34	54	94
2	None	100 (79)	41	46	53
	8.8 Enzyme units	100 (4774)	75	83	90

* Standard assay mixtures (0.2 ml final volume) for terminal transferase contained: 10 μmoles Tris–HCl (pH 7.5), 0.1 μmole $MnCl_2$, 20 μmoles KCl, 0.6 μmole dithiothreitol, 8 μg bovine serum albumin, 0.01 μmole [^{3}H]dGTP (115 c.p.m./pmole), 2.4 μg $(dA)_{12-18}$, and either 0.01 unit of enzyme from leukemic lymphoblasts (experiment 1) or 0.16 unit of enzyme from calf thymus (experiment 2). Substitutions of the concentration or type of initiator, substrate, divalent cation, bovine serum albumin or enzyme were made as indicated. When substituted for [^{3}H]dGTP, the specific activities of [^{3}H]dATP, [^{3}H]dCTP and [^{3}H]dTTP were 365, 141 and 306 c.p.m./pmole, respectively. Values in parentheses under the inhibitor absent column signify the pmoles of tritium-labeled nucleoside monophosphate incorporated into DNA by controls.

to controls in which inhibitor alone was preincubated (data not shown).

The degree of inhibition of terminal transferase by compound XIV was independent of time (Fig. 3). This is a classical characteristic of reversible inhibitors [29]. When added at zero time or 10 min after the start of an uninhibited reaction, compound XIV inhibited terminal transferase immediately, suggesting that this compound prevents both the initiation of DNA synthesis and the elongation of DNA chains. Similar results were obtained for compounds XIII and XXXI (data not shown). The reversibility of inhibition of terminal transferase by compounds XIII, XIV and XXXII was also demonstrated by dilution experiments (data not shown) which were conducted as described previously for streptolydigin [10].

DISCUSSION

Only three (compounds XIII, XIV and tirandamycin) of thirty-one streptolydigin analogs tested resembled the parent compound in selectively inhibiting terminal transferase in comparison with other nucleotide polymerases. These selective inhibitors are 3-acyltetramic acids with different 1-, 3- and 5-substituents (Table 1).

Tirandamycin lacks the 1- and 5-substituents of streptolydigin but differs only slightly in the 3-substituent. The similarity of its behaviour to that of streptolydigin suggests that the 1- and 5-substituents are not required for inhibition of terminal transferase. However, the more potent inhibition of terminal transferase by streptolydigin suggests that the 1- and 5-substituents of streptolydigin improve its activity. Four analogs (XXIV–XXVII), which contain 3-dienoyl groups similar to tirandamycin, also inhibited terminal transferase, but not selectively. Thus, inhibition of terminal transferase appears to be a characteristic of 3-dienoyl substituents. On the other hand, related 3-carbalkoxy (I–V) and 3-acetyl (XV–XVIII, XXI, XXII) analogs were inactive.

The other sizable group of potent terminal transferase inhibitors consists of tetramic acids with 5-benzylidene substituents (VIII, IX, XII–XIV). Compounds VIII, IX and XII did not selectively inhibit terminal trensferase, but compounds XIII and XIV not only selectively terminal transferase, but were more inhibitory than streptolydigin. Compound XIII differs from compound XIV by the presence of a *p*-chloro substituent on the benzylidene group, but this difference had little if any effect on the selectivity, degree or mode of inhibition of terminal transferase by these two compounds. Comparison of the struc-

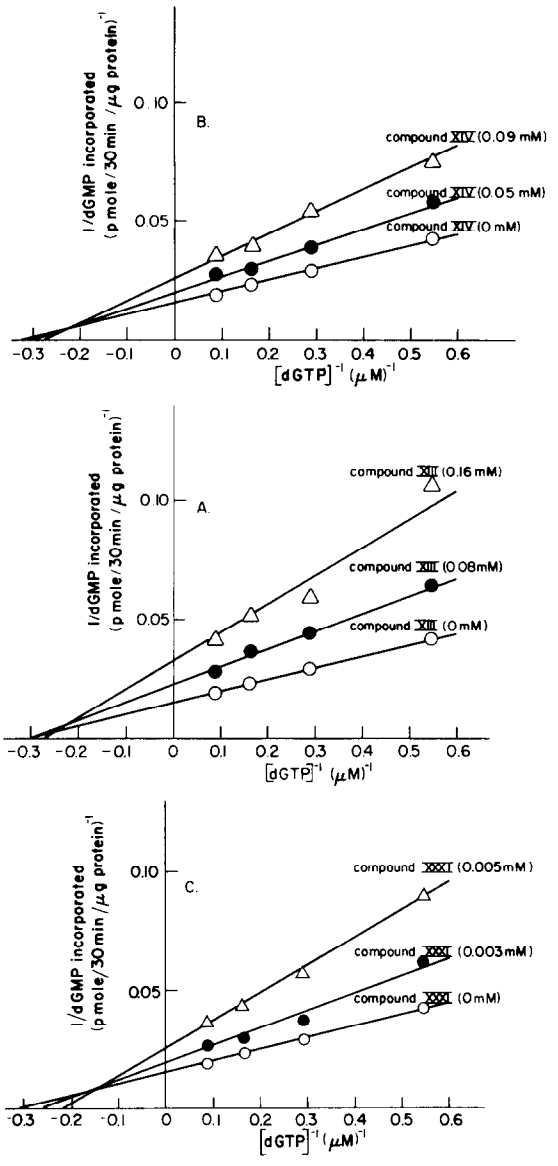


Fig. 2. Inhibition of terminal transferase by streptolydigin analogs at various concentrations of substrate. Terminal transferase (0.36 unit) from leukemic lymphoblasts was assayed in reaction mixtures containing: (A) the indicated concentrations of compound XIII and dGTP, (B) the indicated concentrations of compound XIV and dGTP, or (C) the indicated concentrations of compound XXXI and dGTP. Other components of reaction mixtures and incubation conditions are specified in Materials and Methods. Data were plotted according to Lineweaver and Burk [27].

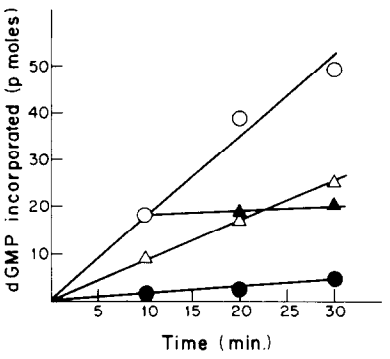


Fig. 3. Time course of inhibition of terminal transferase by compound XIV. Terminal transferase (0.10 unit) from leukemic lymphoblasts was assayed using standard reaction mixtures with or without compound XIV. Key: (○——○) enzyme activity without compound XIV; (△——△) enzyme activity with compound XIV (0.05 mM) added at zero time; (▲——▲) enzyme activity with compound XIV (0.74 mM) added 10 min after the start of an uninhibited reaction; and (●——●), enzyme activity with compound XIV (0.74 mM) added at zero time.

tures of compounds XIII and XIV with compounds VIII, IX and XII, which are non-selective inhibitors of nucleotide polymerases, reveals that while the 5-substituent of these compounds is virtually the same, their 1- and 3-substituents differ (Table 1). Thus, it appears that the 1- and/or 3-acetyl groups of XIII and XIV may be essential for selective inhibition of terminal transferase. Moreover, the 1-acetyl group alone may be the primary determinant of selective inhibition, because many compounds (XV–XXII) which are non-inhibitory or non-selective inhibitors have a 3-acetyl group (Table 1). The only other two analogs which inhibited any of the polymerases were compounds XIX (with a 5-dialkysulfoxide substituent) and XXXI (with a 4-dienoyloxy substituent) and they were non-selective for terminal transferase. In summary, either a 3-dienoyl or a 5-benzylidene group appears to be sufficient for inhibition of one or more polymerases, but selective inhibition of terminal transferase depends on additional factors.

Four lines of evidence indicate that compounds XIII and XIV inhibited terminal transferase by binding reversibly and specifically to the enzyme and not to the initiator, substrate or divalent cation. First, the degree of inhibition was independent of time and reversed by dilution. Second, inhibition was reduced by adding extra amounts of a homogenous enzyme preparation to assay mixtures but not by adding a foreign protein (bovine serum albumin). Third, inhibition was independent of the type or concentration of initiator or substrate. Fourth, inhibition was not affected by excess Mn^{2+}. The third of these also implies that compounds XIII and XIV bind to terminal transferase at a site(s) other than the initiator or substrate binding sites. This mode of inhibition of terminal transferase by compounds XIII and XIV is the same as that previously reported for streptolydigin [10]. In contrast to streptolydigin and compounds XIII and XIV, inhibition of terminal transferase by a non-selective inhibitor (compound XXXI) was dependent upon the type and concentration of initiator and the concentration of bovine serum albumin used in assay mixtures. This indicates that compound XXXI non-specifically binds to proteins and even to nucleic acids. Thus, the mode of inhibition of streptolydigin and its analogs that are selective inhibitors of terminal transferase differs

from that of a streptolydigin analog which non-selectively inhibited terminal transferase.

The discovery of selective inhibitors of terminal transferase provides valuable agents for the characterization of this enzyme. Furthermore, these inhibitors may have potential in the treatment of leukemias which have abnormal terminal transferase activity.

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