BIOLOGICAL PROPERTIES OF STREPTONIGRIN DERIVATIVES II. INHIBITION OF REVERSE TRANSCRIPTASE ACTIVITY

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Reverse transcriptase of RNA tumor viruses plays an important role in the integration of the viral genome into host cell DNA[1,2] and specific inhibitors of this enzyme might provide chemotherapeutic agents against infection by retro viruses.

In the course of our screening for enzyme inhibitors against reverse transcriptase of avian myeloblastosis virus (AMV), the novel substances, retrostatin[3] and chromostin[4], and limocrocin[5] which had been reported as a pigment produced by *Streptomyces limosus*[6], were isolated as specific enzyme inhibitors. In addition, another potent inhibitor coproduced with retrostatin by *S. retrostaticus* was found to be identical with streptonigrin (**1**)[3], an antitumor antibiotic produced by *S. floculus*[7]. Although **1** showed strong antitumor activity[8,9], clinical application of **1** was discontinued due to its marked side effects on the gastrointestinal tract and especially on the bone marrow. As the methyl ester of **1** (**2**) showed some improvement in chemotherapeutic coefficient[10,11], modification of the carboxyl group on C2′ was carried out[12] (Table 1).

The antibacterial and cytocidal activities of the derivatives against various bacteria and tumor cells were reported previously[13] and the results are summarized in part in Table 2.

1 was also recognized by CHIRIGOS *et al.* to be an inhibitor of reverse transcriptase[14]. Several known antibiotics of different groups were tested for their inhibitory activities against AMV reverse transcriptase and **1** showed the most potent activity. In this note, the effects of **1** and its derivatives (**2**～**11**, Table 1) on the activities of various DNA and RNA polymerases including AMV reverse transcriptase are described in comparison with other biological properties.

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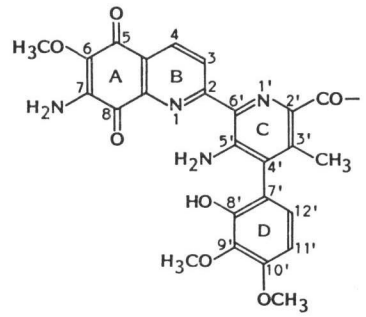
Table 1. Structures of streptonigrin derivatives.

STN-:



1	STN-OH
2	STN-OCH_3
3	STN-NH_2
4	STN-$NHCH_2CH_2OH$
5	STN-$NHCH_2CH_2CH_2N(CH_3)_2$
6	STN-$NHCH_2COOH$
7	STN-NHOH
8	STN-$NHNH_2$
9	STN-$NHNHCONH_2$
10	STN-$NH(CH_2)_3NH(CH_2)_4NH$-STN
11	STN-$NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH$-STN

The inhibition of reverse transcriptase activity was measured by the method described previously[5] with the following modification: The assay solution contained 100 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 5 mM dithiothreitol, 60 mM NaCl, 0.2 mM [^{3}H]TTP (12.5 μCi/ml), 20 μg/ml poly(rA), 0.02 U/ml oligo(dT)$_{12\sim18}$ and 3.0 U/ml AMV reverse transcriptase (Takara Shuzo Co., Ltd., Kyoto). The sample was dissolved in DMSO at 5 mg/ml and diluted with distilled water to provide the test solution. A mixture of the assay solution (50 μl) and the test solution (50 μl) was incubated at 37°C for 1 hour. After the reaction was terminated by cooling in an ice bath, 50 μl aliquot of the reaction mixture was soaked into a 2.4 cm-round piece of DEAE-cellulose paper which was washed three times with 5% $Na_2HPO_4 \cdot 12H_2O$ and once each with distilled water and ethanol. The detailed assay methods for DNA-directed DNA polymerases of *Escherichia coli* and calf thymus and DNA-directed RNA polymerase of *E. coli* have been described previously[5].

In contrast to the remarkable decrease in

Table 2. Biological activities of streptonigrin derivatives.

Compound	MIC (μg/ml)	ID_{50} (μg/ml)					
	B. subtilis[a]	L5178Y/S[a]	L5178Y/ADM^{r}[a]	RDDP[b] (AMV)	DDDP[c] (*E. coli*)	DDDP[c] (Calf thymus)	DDRP[d] (*E. coli*)
1	< 0.05	0.0043	0.00026	3	>160	>160	100
2	25	0.017	0.0013	>40	>160	>160	>160
3	>25	1.3	0.55	5	>160	160	33
4	>25	1.4	0.62	2	>160	>160	NT
5	25	0.33	0.058	3	37	43	NT
6	>25	>4.0	2.5	2	160	>160	NT
7	3.12	0.12	0.33	5	140	>160	20
8	3.12	0.014	0.00098	6	80	>160	27
9	>25	0.17	0.038	2	>160	>160	NT
10	25	0.57	0.41	7	86	150	NT
11	>25	1.0	NT	3	42	53	NT
ADM	NT	0.12	1.5	22	4	7	24

[a] Data calculated from the results reported previously[13]. [b] RNA-directed DNA polymerase (reverse transcriptase). [c] DNA-directed DNA polymerase. [d] DNA-directed RNA polymerase.
NT: Not tested.

Table 3. Classification of streptonigrin derivatives.

Group	Inhibition*			Compound
	Bacterial growth	Cell growth	RDDP	
A	++	++	++	**1**
B	−	+	−	**2**
C	±	+	++	**8**
D	− or ±	− or ±	++	The others

* ++, Strong inhibition; +, inhibition; ±, trace inhibition; −, no inhibition.

antibacterial activity exemplified by the results against *Bacillus subtilis*, some of the derivatives showed rather strong cytotoxicities against mouse lymphosarcoma L5178Y cells. The cytotoxicity of **8** (ID_{50} 0.014 μg/ml) against a parental line of L5178Y cells (L5178Y/S) was comparable to that of **2** (0.017 μg/ml) which was approximately one fourth of that of **1** (0.0043 μg/ml), whereas ID_{50} of **6** was over 4 μg/ml. In general, an doxorubicin-resistant subline of L5178Y cells (L5178Y/ADM^{r}) showed a collateral sensitivity to **1** and its derivatives (Table 2).

According to CHIRIGOS *et al.*[14], the inhibitory activity of **1** against AMV reverse transcriptase was completely lost in **2**. As evident in Table 2, however, the derivatives **3**~**11** retained this activity. The inhibitory activity of **1** against AMV reverse transcriptase was markedly reversed by an increase in enzyme concentration, suggesting a direct interaction with the enzyme (unpublished observation). Further kinetic studies are now in progress in our laboratory. As well as **1**, the newly synthesized derivatives lacked inhibitory activity against DNA-directed DNA polymerases of *E. coli* and calf thymus, while ADM, a known inhibitor of DNA-directed DNA and RNA polymerases, gave a lower ID_{50} for these enzymes (4 and 7 μg/ml, respectively) than for AMV reverse transcriptase (22 μg/ml). However, **3**, **7** and **8** inhibited *E. coli* DNA-directed RNA polymerase activity to the same extent as ADM. **1** and its derivatives (**2**~**11**) were classified by their biological activities into 4 groups, as shown in Table 3. Since the decrease in cytocidal activity was not accompanied by a decrease in inhibitory activity against reverse transcriptase, as typically shown by **6**, it would be worthwhile to conduct further studies to evaluate the antiviral activities of streptonigrin derivatives both *in vivo* and *in vitro*.

On the basis of the cytotoxicity of **1**, LASZLO and his colleagues[15~17] suggested that **1** caused a catalytic oxidation of reduced nicotinamide adenine dinucleotide by mitochondrial dia-

phorase, concomitant generation of hydrogen peroxide and a resultant decrease in ATP synthesis. We are interested in elucidating whether this property of **1** is shared by its derivatives, and results of such studies will be reported shortly.

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