BIOLOGICAL PROPERTIES OF STREPTONIGRIN DERIVATIVES

I. ANTIMICROBIAL AND CYTOCIDAL ACTIVITIES

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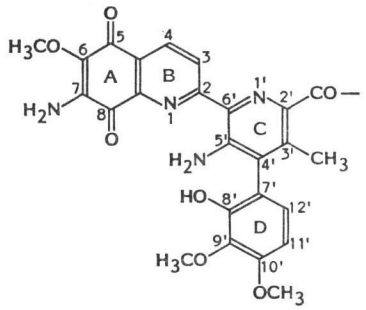
In the course of our screening for inhibitors against reverse transcriptase of avian myeloblastosis virus, two inhibitors have been isolated from the cultured broth of *Streptomyces re-*

Table 1. Structures of streptonigrin derivatives.

STN-:



1	STN-OH	
2	STN-OCH_3	
3	STN-NH_2	A
4	STN-$NHCH_2CH_2OH$	A, B
5	STN-$NHCH_2CH_2CH_2N(CH_3)_2$	B
6	STN-$NHCH_2COOH$	B*
7	STN-$NHCH_2COOCH_3$	B
8	STN-NHOH	B
9	STN-$NHNH_2$	B
10	STN-$NHNHCONH_2$	A, B
11	STN-$NHNHCSNH_2$	A, B
12	STN-$NH(CH_2)_3NH(CH_2)_4$NH-STN	B
13	STN-$NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3$NH-STN	B

* **6** was obtained by hydrolysis of **7**.

Table 2. Antimicrobial spectra of streptonigrin derivatives.

Test microbe	MIC (μg/ml)						
	1	**2**	**3**	**4**	**5**	**8**	**11**
Staphylococcus aureus FDA 209 P	0.78	>25	>25	>25	>25	25	>25
S. aureus Smith	0.39	>25	25	>25	>25	3.12	6.25
Micrococcus flavus FDA 16	6.25	>25	>25	>25	>25	>25	>25
M. luteus PCI 1001	1.56	>25	>25	>25	>25	>25	>25
Bacillus anthracis	<0.05	>25	6.25	12.5	12.5	1.56	6.25
B. subtilis PCI 219	<0.05	25	>25	>25	25	3.12	6.25
Corynebacterium bovis 1810	0.78	>25	25	>25	>25	>25	>25
Escherichia coli K-12	3.12	>25	>25	>25	>25	>25	>25
E. coli K-12 ML 1629	6.25	>25	>25	>25	>25	>25	>25
E. coli NIHJ	0.39	>25	>25	>25	>25	>25	>25
Shigella sonnei 191-66	3.12	>25	>25	>25	>25	>25	>25
Proteus vulgaris OX 19	6.25	>25	>25	>25	>25	>25	>25
Klebsiella pneumoniae PCI 602	<0.05	25	>25	12.5	25	3.12	3.12
Salmonella typhosa T-63	6.25	>25	>25	>25	>25	>25	>25
Serratia marcescens	0.78	>25	>25	>25	>25	>25	>25
Candida tropicalis NI 7495	25	>25	>25	>25	>25	>25	>25
C. pseudotropicalis NI 7494	25	>25	>25	>25	>25	>25	>25
C. albicans 3147	>25	>25	>25	>25	>25	>25	>25
Saccharomyces cerevisiae	>25	>25	>25	>25	>25	>25	>25

Antimicrobial spectra of **8** and **9** were the same. **10** and **13** showed the same antimicrobial spectra with that of **4**. **5** and **12** had the same antimicrobial spectrum. **6** and **7** did not inhibit the growth of above microbes at a concentration of 25 μg/ml.

trostaticus[1]. One of them was identified with streptonigrin, the aminoquinolinequinoide antibiotic produced by *S. flocculus*[2~4], and the other was proved to be novel and named retrostatin[1]. Streptonigrin (**1**) showed strong antitumor activity but the clinical application of **1** was limited by its extraordinary strong side effects mainly due to bone marrow depression[5,6]. Chemical modifications of the amino group on C7 or C5′, or the hydroxyl group on C8′ of **1** usually resulted in substantial loss of the antitumor activity[7]. For example, the *in vitro* and *in vivo* antitumor activities of isopropyridine azastreptonigrin were reported to be approximately 0.01% and 1.0%, respectively, of those of **1**[8]. While streptonigrin methyl ester (**2**) showed marked antitumor activity by parental administration as well as **1**, the maximum tolerated dose of **2** for humans was 5~6 times that of **1**[9,10]. Therefore, the carboxyl group on C2′ of **1** was modified by the acid chloride method using acid chloride of **1** as an intermediate (Method A) or by the reaction of an amine with **1** in the presence of phenyl bis(2-

Fig. 1. Effects of streptonigrin derivatives on the growth of L5178Y/S and L5178Y/ADR cells.

A test sample dissolved in DMSO (2.5 mg/ml) was diluted with serum-free FISCHER's medium to make a test solution. A mixture of the test solution (0.2 ml) and the cell suspension (5.0~6.0×10^4 cells/1.8 ml) in FISCHER's medium containing 10% horse serum (Gland Island Biological Co.) was incubated in a tightly capped test tube at 37°C for 72 hours[12].

The cell numbers were counted by using a hemocytometer after the incubation.

A, L5178Y/S; B, L5178Y/ADR.

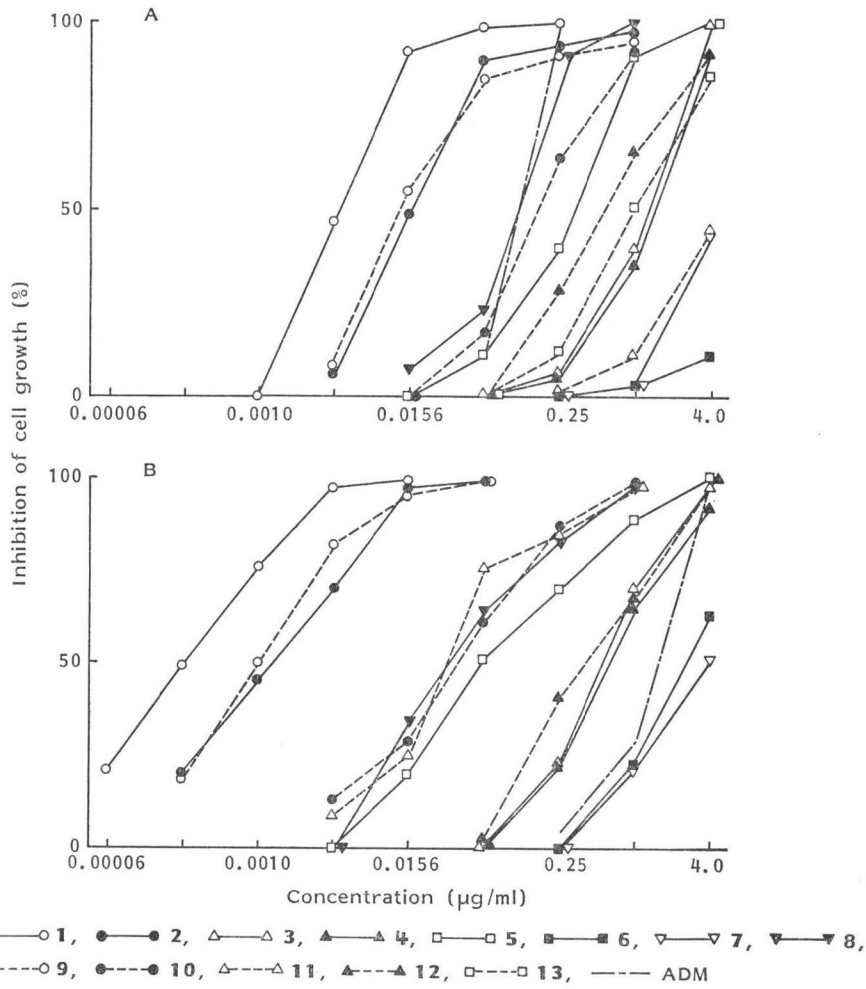


Fig. 2. Effects of streptonigrin derivatives on the growth of P388/S and P388/ADR cells.

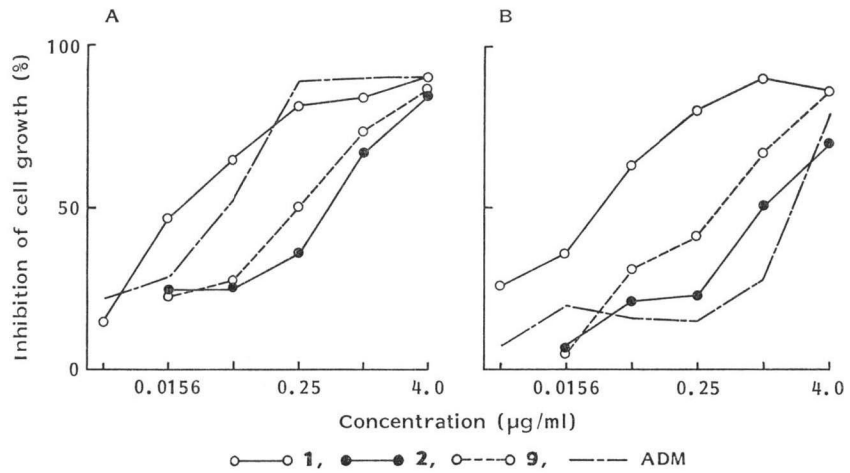
Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum (GIBCO), 10 μM 2-hydroxyethyl disulfide (Aldrich Chemical Co.) and kanamycin (100 μg/ml) was used as a culture medium.

P388/S and P388/ADR ascites cells were harvested from the peritoneal cavity of each tumor-bearing DBA/2NCrj mouse 6 days after transplantation and subcultured twice at 37°C in Falcon No. 1007 plastic dishes (Falcon Plastics) in a humidified atmosphere of 5% CO_2.

A mixture of the test solution (0.1 ml) prepared as described in the legend to Fig. 1 and the cell suspension (5.0×10^4 cells/0.9 ml) in the medium was cultured in a loosely capped test tube at 37°C for 48 hours[14].

The cell numbers were counted in a Model Dn Coulter counter.

A, P388/S; B, P388/ADR.



thioxo-1,3-thiazolidine-3-yl)phosphine oxide (Method B)[11]. Biological properties of **1** and its derivatives (**2**~**13**, Table 1) are described in this paper.

Antimicrobial spectra of the streptonigrin derivatives were determined by the agar dilution method on glucose nutrient agar. Streptonigrin hydroxamic acid (**8**) and streptonigrin hydrazide (**9**) showed rather weak antimicrobial activities when compared with that of **1** as seen in Table 2. The other derivatives showed very weak or faint activities as exemplified by lack of activity observed for **2**, **6** or **7** at a concentration of 25 μg/ml.

Growth inhibitory activities of the streptonigrin derivatives against various tumor cells were determined using a parental line of lymphosarcoma L5178Y cells (L5178Y/S), an adriamycin (ADM)-resistant subline of L5178Y cells (L5178Y/ADR)[12,13], a parental line of P388 leukemia cells (P388/S) and an ADM-resistant subline of P388 cells (P388/ADR)[14~17]. **1**, **2** and **9** showed 32, 8 and 8 times, respectively, stronger cytotoxicities than ADM against L5178Y/S (Fig. 1). Furthermore, it is worthwhile to emphasize that the ID_{50} of **1**, **2** and **9** against L5178Y/S (4.0×10^{-3}, 1.6×10^{-2} and 1.3×10^{-2} μg/ml, respectively, Fig. 1A) are remarkably higher than those against L5178Y/ADR (2.7×10^{-4}, 1.3×10^{-3} and 0.9×10^{-3} μg/ml, respectively, Fig. 1B). In fact, NISHIMURA *et al.*[12] observed that none of the tested antitumor agents showed higher cytotoxicity against L5178Y/ADR than against L5178Y/S. The sensitivity of L5178Y/ADR to **1** and its derivatives is apparently higher than that of L5178Y/S. The collateral sensitivity of this type has been reported mainly in *in vivo* tumor systems[16,18]. INABA *et al.*[19] observed, however, the *in vitro* collateral sensitivity of 6-mercaptopurine-resistant sublines of P388 and L1210 leukemia to the purine antagonists, 5-carbamoyl-1*H*-imidazol-4-yl piperonylate and 4-carbamoylimidazolium-5-olate. The ID_{50} of **1**, **2**, **9** and ADM against P388/S were 1.6×10^{-2}, 5.0×10^{-1}, 2.5×10^{-1} and 6.0×10^{-2} μg/ml, respectively (Fig. 2A). Though P388/ADR had been reported to show wide cross-resistance against various antitumor

agents of different groups[16,17], no significant difference was observed between the ID_{50} of **1**, **2** and **9** against P388/S and the corresponding values of P388/ADR as shown in Fig. 2. According to the previous findings by INABA *et al.*[14,15], P388/ADR acquired resistance to a variety of antitumor agents due to the enhanced active efflux of these substances, and **1**, **2** and **9** seemed not to be affected to the common efflux system of P388 leukemia cells.

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