THE QUINOLINE QUINONE AS THE MINIMUM ENTITY FOR REVERSE TRANSCRIPTASE INHIBITORY ACTIVITY OF STREPTONIGRIN

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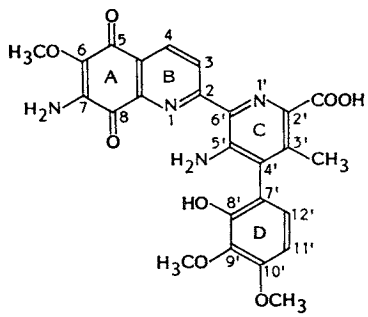
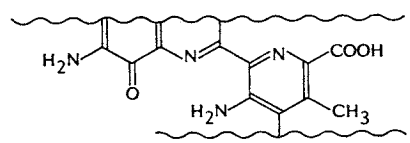
Streptonigrin (**1**) was first isolated by RAO and CULLEN from a culture of *Streptomyces floculus* as an antitumor antibiotic in 1959[1]. Since then, a considerable number of streptonigrin analogs have been synthesized to study the mechanism of action of **1**[2], and a few of the compounds have been tested for antibiotic and cytocidal activities[3,4]. The partial structure (**2**) was suggested as the minimum requisite for these activities[4]. KREMER and LASZLO reviewed various studies of biological properties and biochemical effects of **1**, suggesting that the cytotoxicity of **1** could be due to the depletion of NADH and NADPH, the uncoupling of oxidative phosphorylation, and/or the formation of single strand breakes in DNA caused by radicals generated from streptonigrin and/or oxygen[5]. In bacteria at low concentrations of **1** the lethal sequence involved reduction of **1** by NADH and NADPH followed by a rapid autoxidation which bypassed oxidative phosphorylation and led to depletion of cellular ATP. However, at higher concentrations, **1** exhibited an initial first-order decline in viability, implying that a single hit per cell was lethal and the bacterial chromosome was the most probable candidate for the site of action.

Besides antimicrobial and antitumor activities, **1** showed potent inhibition of reverse transcriptase[6]. In our screening for inhibitors of avian myeloblastosis virus reverse transcriptase, **1** was found to be one of the most potent inhibitors among *ca.* 150 antibiotics tested[7]. Recently, we observed that the amide derivatives of **1** at the carboxyl group on C-2′ showed marked decrease in a cytocidal activity, while no significant change in an inhibitory activity against reverse transcriptase was observed[8,9]. The present study was carried out to clarify the contribution of the 5,8-quinoline quinone moiety to not only a cytocidal activity but also an inhibitory activity against reverse transcriptase by

Fig. 1. Structures of streptonigrin and the quinoline quinone derivatives.



Streptonigrin (**1**)



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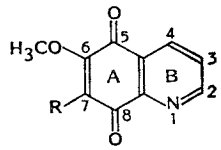


3 R = H

4 R = CH_3

Fig. 2. Inhibition of reverse transcriptase.

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[8] (50 μl) and the test solution (50 μl) was incubated at 37°C for 1 hour. The reaction mixture was processed as described previously[8].

○: **1**, △: **3**, ▲: **4**.

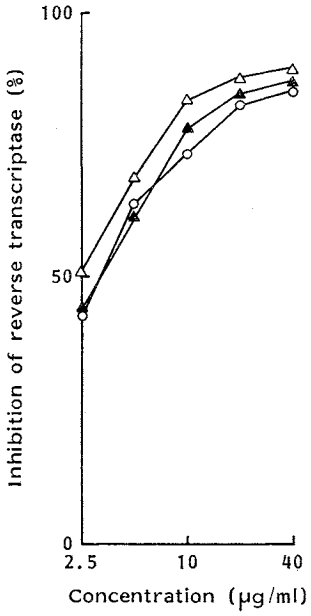
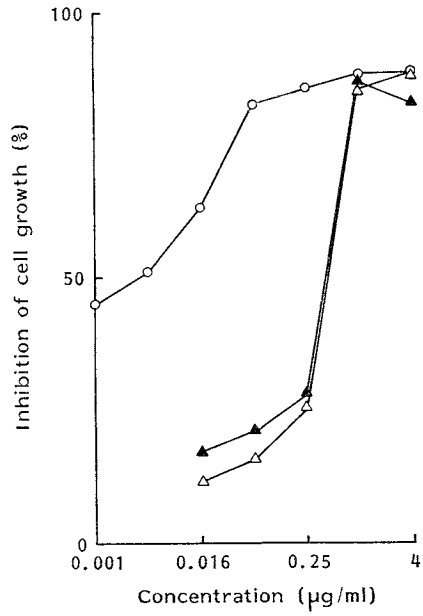


Fig. 3. Inhibition of the growth of L5178Y cells.

The sample was dissolved in DMSO at 2.5 mg/ml and diluted with serum-free FISCHER's medium to provide the test solution. A mixture of the test solution (0.2 ml) and the cell suspension ($5.0\sim 6.0\times10^4$ 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.

○: **1**, △: **3**, ▲: **4**.



comparing the biological activities of **1** and the quinoline quinone derivatives.

Two quinoline quinone derivatives, 6-methoxy-5,8-dihydroquinoline-5,8-dione (**3**) and 6-methoxy-7-methyl-5,8-dihydroquinoline-5,8-dione (**4**), were synthesized as previously reported[10]. The assay method for a cytocidal activity was described in the previous paper[8]. The reverse transcriptase activity and the respiration of rat liver mitochondria were measured according to the previous methods[9,11].

As can be seen in Fig. 2, both **3** and **4** were as active inhibitors of reverse transcriptase as **1**. In contrast, the concentrations of **3** and **4** giving 50% inhibition of the growth of lymphoblastoma L5178Y cells were *ca.* 130-fold that of **1** (Fig. 3). The results given here indicate that the quinoline quinone moiety is the minimum entity to give the inhibition of reverse transcriptase comparable to that of **1**, while the cytotoxicity of **1** is not fully manifested by these compounds.

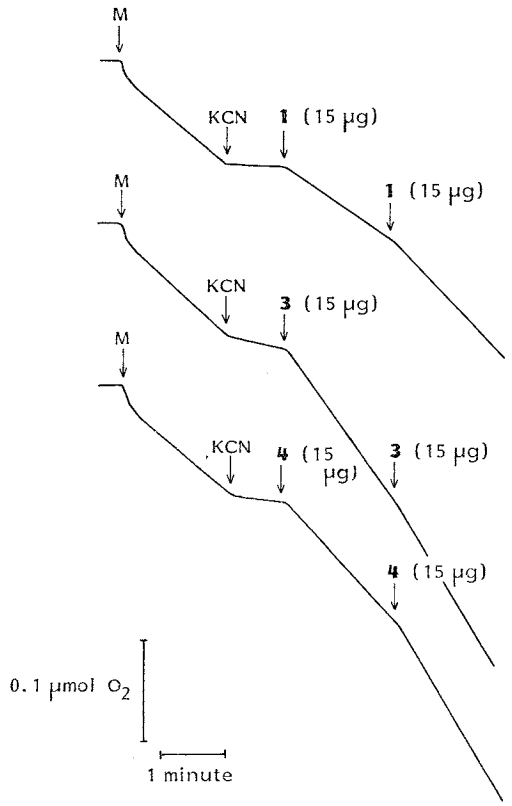
The effects of **1**, **3** and **4** on the respiration of rat liver mitochondria were studied using glutamate as a substrate. **3** and **4** facilitated the oxygen consumption by mitochondria to higher extents than **1** on the weight basis (Fig. 4). The increases in the oxygen consumption rate in the presence of 15 and 30 μg of **1** are 27 and 42 nmol/minute/mg protein, respectively. The corresponding values for **3** are 50 and 64, and those for **4** are 40 and 66, while the basal rate ranges $2\sim10$ nmol/minute/mg protein.

Contrary to the presumption from the results for cytotoxicity, the lack of ability to bypass oxidative phosphorylation was not observed for both **3** and **4**. We reported the importance of this bypass activity to the inhibition of reverse transcriptase using various naphthoquinone derivatives[12] and the results obtained here are in good accordance with the previous findings. Though the cytotoxicity of **1** is due to the uncoupling effect on oxidative phosphorylation, it does not simply result from the electron transfer potential of this antibiotic. In fact, the slopes

Fig. 4. Effect on the oxidation of glutamate by rat liver mitochondria.

The respiration of mitochondria was measured in 3 ml of the basal medium (225 mM sucrose, 5 mM potassium phosphate, 10 mM Tris-HCl, pH 7.4) supplemented with 15 mM glutamate at 30°C.

The sample was dissolved in DMSO at 5 mg/ml and used at the doses indicated in the figure. The other additions were as follows (stock solution): Mitochondria (M), 1.5 mg as protein; and KCN (100 mM in H_2O), 1.0 μmol.



in Fig. 3 were quite different between **1** and the quinoline quinone derivatives (**3** and **4**), implying different mechanisms lying on the basis of lethal effects of these compounds.

Acknowledgment

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