

teins were detected by chemiluminescence (ECL, Amersham, Arlington Heights, IL, USA), or by incubation with the colorgenic substrates, nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP), as suggested by the manufacturer (Sigma Chemical, St Louis, MO, USA).

RESULTS AND DISCUSSION

PTDS Inhibits Cell Proliferation

In a previous study, PDTC and PTDS (Fig. 1) were found to arrest cell growth in a human hepatoma

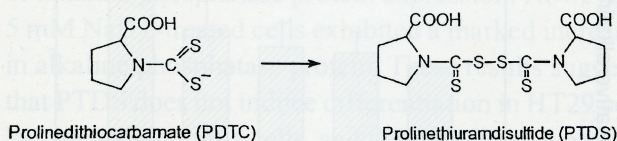


Fig. 1. Structures of PDTC and PTDS.

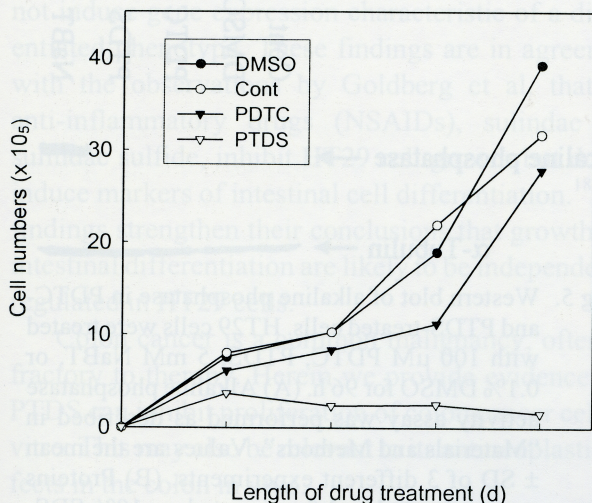


Fig. 2. Effect of PDTC and PTDS on cell proliferation. HT29 cells were treated with 0.1% DMSO, or 100 μ M PDTC or PTDS at 37 °C continuously until trypsinization and counting at the times indicated. Each point represents the mean of quadruplicate samples $\pm$ SD. This is a representative example of 2 experiments, each of which was conducted with quadruplicate samples.

HepG2 cell line.¹⁴ Here we studied the effect of PDTC and PTDS on the proliferation of HT29 adenocarcinoma cells. We determined the capacity of PDTC- or PTDS-treated cells to proliferate by counting them at various stages of treatment. The result is shown in Fig. 2. After 1-day exposure to 100 μ M PTDS, HT29 cells apparently could not divide at all, whereas PDTC showed less of an inhibitory effect at the same concentration. The inhibitory activity by PTDS was maintained for up to 4 days of treatment. By contrast, after 3 days of treatment with PDTC, the HT29 cells began to proliferate more normally than before treatment. This result indicates that the effective time period of PDTC is shorter than that of PTDS. However, HT29 cells proliferated normally despite the continuous presence of DMSO in the medium.

As shown in Fig. 3, DNA synthesis in HT29 cells was determined by the incorporation of [³H] thymidine. The synthesis of DNA in DMSO-exposed cells was not affected after 24 h of treatment. Comparison of control cells and DMSO-treated cells showed that incorporation of [³H] thymidine in PDTC-treated

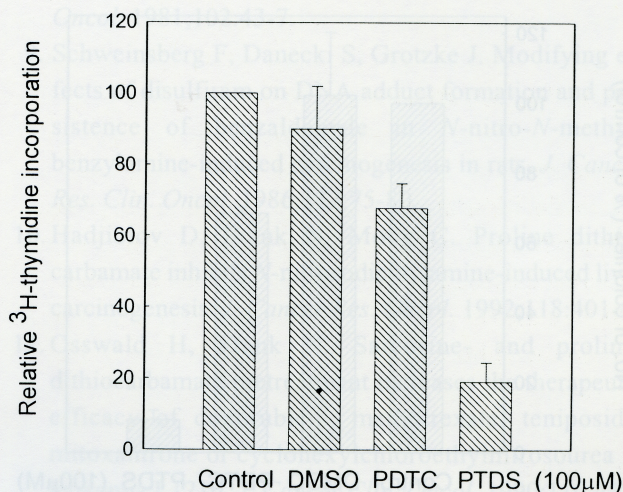


Fig. 3. DNA synthesis in PDTC- and PTDS-treated cells. HT29 cells were treated with 100 μ M PDTC or PTDS for 24 h. [³H]thymidine (1 μ Ci/mL) was present during the final 4 h, and its incorporation in DNA was determined as detailed in "Materials and Methods". Mean values $\pm$ SD of 3 different experiments.