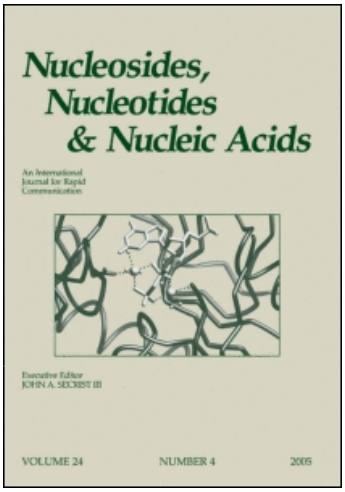

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POTENT *IN VITRO* ANTICANCER ACTIVITIES OF RING-EXPANDED ("FAT") NUCLEOSIDES CONTAINING THE IMIDAZO[4,5-*E*][1,3]DIAZEPINE RING SYSTEM

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POTENT *IN VITRO* ANTICANCER ACTIVITIES OF RING-EXPANDED ("FAT") NUCLEOSIDES CONTAINING THE IMIDAZO[4,5-*E*][1,3]DIAZEPINE RING SYSTEM

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ABSTRACT

The ring-expanded ("fat") nucleoside, 4,8-diamino-6-imino-6*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepine (**1**) and its 2′,3′,5′-tri-*O*-benzoyl derivative (**2**) exhibited potent broad spectrum anticancer activities *in vitro* against a wide variety of human tumor cell lines. The tribenzoyl derivative **2** was found to be considerably more active than the parent nucleoside **1**. Further studies using human prostate cancer cells PC-3 and DU-145 suggest that the treatment of exponentially growing culture cells with **1** and **2** leads to marked loss of cell viability in a dose-dependent manner.

Ring-expanded ("Fat") purine nucleosides and nucleotides are of chemical, biochemical, biophysical and medicinal interest (1–3). A number of such nucleoside analogues were recently discovered to possess potent *in vitro* anticancer activities against a wide variety of human tumor cell lines. In particular, we report here the broad spectrum anticancer activities of our two leading "fat" nucleosides,

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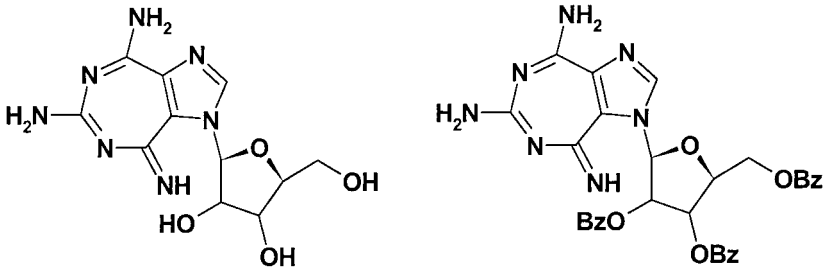

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4,8-diamino-6-imino-6*H*-1-β-D-ribofuranosylimidazo[4,5-*e*][1,3]diazepine (**1**) and its 2′,3′,5′-tri-*O*-benzoyl derivative (**2**). While the activity profiles for the two nucleosides were similar, the latter was considerably more active than the parent nucleoside **1** in almost all the human tumor cell lines screened. The benzoyl protecting groups are believed to enhance the cell permeability of **2**.

Nucleosides **1** and **2** were synthesized using the procedure that we reported previously (4). 4,5-Dicyanoimidazole was condensed with guanidine to prepare the required heterocyclic base. The latter was ribosylated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose by the Vorbrüggen method (5), employing *N*, *O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (for silylation) and trimethylsilyl trifluoromethanesulfonate (TMS-triflate) (as a catalyst for condensation), to obtain the tribenzoyl derivative **2**. The latter, upon treatment with a solution of sodium methoxide in methanol afforded **1**.

Nucleosides **1** and **2** were screened (6) for antitumor activity against 54 different human tumor cell lines *in vitro*. The assay was conducted according to the published procedure (7). Briefly, cell suspensions were diluted according to the particular cell type and the expected target cell density (5000–40,000 cells per well based on cell growth characteristics), and were added by pipette (100 μL) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 hours at 37°C for stabilization. Dilutions at twice the intended test concentrations of compounds were added at time zero in 100 μL aliquots to the microliter plate wells. The plates were incubated for 48 hours in 5% CO_2 atmosphere and 100% humidity. The cells were assayed by using the sulforhodamine B assay (8). The plates were read by plate reader. The GI_{50}, TGI, and LC_{50} were calculated from the optical densities. The test results showed potent anticancer activities for **1** and **2** against a wide variety of human tumor cell lines grouped under leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer with GI_{50} values ranging between $10^{-5}-10^{-6}$ *M* for **1** and $10^{-5}-10^{-7}$ *M* for **2**.

In light of the broad spectrum *in vitro* anticancer activities of **1** and **2**, we decided to focus our further efforts on prostate cancer using our most active nucleoside **2**. Prostate cancer is the second leading cause of cancer mortality in American males (9). Our initial goal was to explore the *in vitro* effect of **2** in two androgen-independent human prostate cancer cell lines, PC-3 and DU-145, in which

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the NCI screening data (6). had already exhibited marked anticancer activities. The *in vitro* effect studies concentrated on exploring the dose-dependent induction of apoptosis (programmed cell death) in treated cancer cells.

We investigated the dose response profile of the cytotoxic effects of nucleoside **2** against human prostate cancer cells. Specifically, PC-3 and DU-145 cells were each treated with increasing concentrations of **1** or **2** (0–50 μM) for 2 days, and the cell viability was determined using the trypan blue exclusion assay (10). The results in each case indicated that the treatment of exponentially growing culture of cells with **1** or **2** for 2 days leads to marked loss of cell viability in a dose-dependent manner.

We then investigated the time course of cytotoxicity of **1** and **2** against androgen-independent prostate cancer cells. After 6 days of treatment of PC-3 and DU-145 cells each with 30 μM concentrations of **1** or **2**, $\geq$98% cell killing was observed.

In conclusion, ring-expanded nucleosides bearing the skeletal structure of **1** hold good promise as potential chemotherapeutic agents against cancer, including but not limited to, human prostate cancer.

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