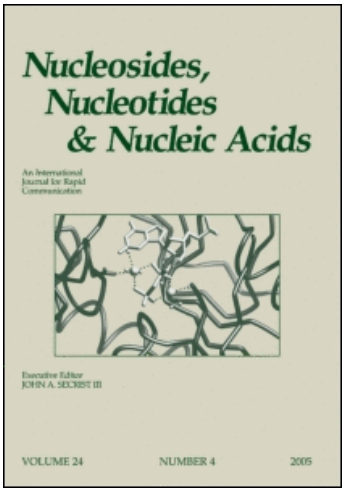

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Sinha, Sarika , Srivastava, Richa , Prusty, Bhupesh , Das, B. C. and Singh, Ramendra K.(2007) 'Some Novel Adenosine Mimics: Synthesis and Anticancer Potential against Cervical Cancer caused by Human Papilloma Virus', Nucleosides, Nucleotides and Nucleic Acids, 26: 6, 773 — 777
To link to this Article: DOI: 10.1080/15257770701501195
URL: http://dx.doi.org/10.1080/15257770701501195

Nucleosides, Nucleotides, and Nucleic Acids, 26:773–777, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701501195

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SOME NOVEL ADENOSINE MIMICS: SYNTHESIS AND ANTICANCER POTENTIAL AGAINST CERVICAL CANCER CAUSED BY HUMAN PAPILLOMA VIRUS

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□ *Two novel adenosine analogs, viz. 9-(1′-β-D-arabinofuranosyl)-6-nitro-1,3-dideazapurine or Ara-NDDP (1) and 9-(5′-deoxy-5′-S-(propionic acid) (1′-β-D-ribofuranosyl) adenine or SAH analog (2), indigenously synthesized, have been found to be potential anticancer agents against cervical cancer caused by human papilloma virus.*

Keywords Nucleosides; cervical cancer; human papilloma virus (HPV)

INTRODUCTION

Cervical cancer is the most common type of cancer afflicting Indian women. Out of the two high-risk viruses, viz. HPV16 and 18, HPV-16 has been the major cause of cervical cancer in Indian pretext.[1] Two novel nucleoside mimics, viz. 9-(1′-ß-D-arabinofuranosyl)-6-nitro-1,3-dideazapurine or Ara-NDDP (**1**) and or SAH analog (**2**) (Figure 1) have been synthesized and studied for their anticancer potential using cell lines, HeLa (HPV-18 positive) and SiHa (HPV-16 positive). Ara-NDDP has already exhibited antiviral activity against HIV-1 (IIIB) with an IC_{50} of 79.4 μg/mL as we reported earlier.[2]

This work was supported by grants from the University Grants Commission and Department of Science and Technology, New Delhi, Government of India.

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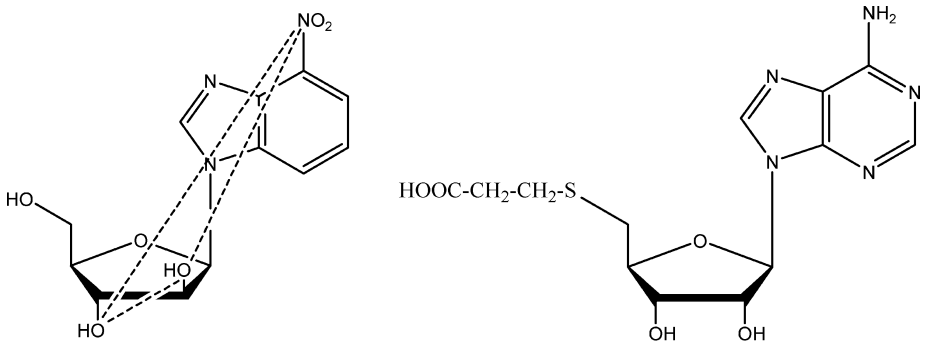

FIGURE 1 Structures of compounds **1** and **2.** Compound **1** showing O-N-O triangulation present in most known anticancer molecules.

RESULTS AND DISCUSSION

For checking anticancer property, a concentration dependent study for compounds **1** and **2** was done in the range 10–200 μg/mL for a period of 24 h. Compound **1** was found to cause necrosis in HeLa cells and membrane blebbing and formation of apoptotic bodies in SiHa cells following treatment at a 200 μg/mL concentration. There was also a reduction in cell viability, as studied by trypan blue assay, with increasing concentrations of **1**. Compound **2** showed no such effect on morphology of the cancer cells in the concentration range chosen.

Further, protein expression studies and AP-1 binding studies with both the compounds were done on HPV-16 positive SiHa cells. In the case of compound **1**, c-fos is gradually being downregulated and its expression is completely absent following treatment with 200 μg/mL concentration. At the same time there is a slight upregulation in fra-1 expression as the concentration of **1** is increased. In the case of compound **2,** there is almost complete loss of c-fos expression at 10 μg/mL after which there is a slight increase in its expression in the range 25–100 μg/mL. At 200μg/mL there is again a downregulation of c-fos. There is an upregulation of fra-1 as the cells have been treated with increasing concentrations of the compound **2** (Figure 2a).

The level of c-fos gets gradually elevated as normal cells proceed towards the cancerous stage and at the same time fra-1 gets downregulated.[3] Both compounds **1** and **2** were found to decrease the expression of c-fos and increase the expression of fra-1 with their increasing concentrations. This happens when the cancerous cervical epithelial cells trasverse toward normalcy.[3] The c-fos gene is also considered as protooncogene[4] and its expression has been found to decrease at m-RNA as well as protein levels

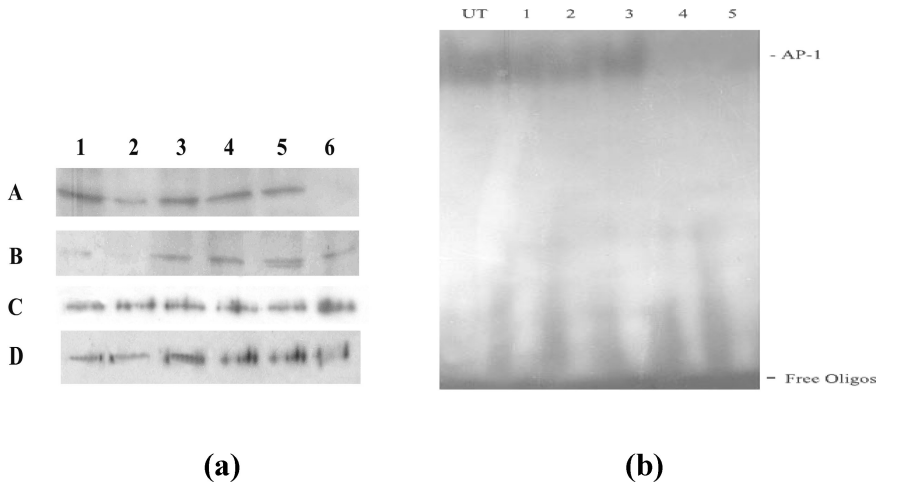

FIGURE 2 (a) Western Blot Experiment with SiHa cells: A & C. Effect of Ara-NDDP (**1**) on c-fos & fra-1 expression levels, respectively. B & D. Effect of SAH analog (**2**) on c-fos & fra-1 expression, respectively. Lanes 1-6 show the concentration of both the compounds: **1** = 0μg, **2** = 10μg, **3** = 25μg, **4** = 50μg and **5** = 100μg.(b). Gel Shift Assay: Effect of SAH analog (compound **2**) on the binding efficiency of HPV specific genome sequence. Lanes **1–5** are the concentrations of the compound **2** with which the SiHa cells were treated, **UT** = Untreated cells, **1** = 10 μg, **2** = 25 μg, **3** = 50 μg, **4** = 100 μg and **5** = 200 μg.

as cells senesce.[5,6] Senescence or aging, besides apoptosis, is a commonly known and also preferred antioncogenic mechanism of anticancer drugs.[7]

An Electrophoretic Mobility Shift Assay (Figure 2b) showed that there was a complete inhibition of AP-1 binding to the HPV specific gene sequence following treatment with 100 μg/mL concentration of compound **2**. The binding of the host cell transcription factor AP-1 to HPV genome is an essential prerequisite for the transcription of the tumorigenic genes E6 and E7 of HPV. Any molecule which interferes with this binding shall interfere with the tumorigenicity of HPV, as is being done by compound **2** at a concentration of 100 μg/mL.

Thus, on the basis of the above-mentioned observations, compounds **1** and **2** have shown potential as probable anticancer agents against cervical cancer caused by HPV.

EXPERIMENTAL

A concentration dependent treatment of HeLa and SiHa cells was done with compounds 1 and 2. Compound 1 was dissolved in DMSO while compound 2 was dissolved in H_2O. For a pilot experiment, a range of six concentrations was chosen, viz. 0 μg, 10 μg, 25 μg, 50 μg, 100 μg, and 200 μg. These concentrations were maintained for both nucleosides. Petridishes (60 mm) were used for the treatment of cells with the first one being taken as

untreated or control in which no nucleoside was added. Before starting treatment, cells were harvested (2.2×10^6 cells/mL DMEM/plate) and plated in petridishes with 5 mL DMEM. The cells were incubated for 24 h to get about 40–50% confluency and then treated with the nucleoside analogs. After 24-h incubation following drug treatment the cells were photographed to look for morphological changes in them before and after treatment with the nucleoside analogs.

For Western Blotting experiments nuclear extracts prepared by the method of Riol et al.[8] with certain modifications[4] and used for band shift analysis (30 μg of protein per lane) were separated on 10% polyacrylamide gel. The proteins were probed with rabbit polyclonal antibodies (Santa Cruz Biotech, USA) raised against c-fos and fra-1 at a dilution of 1:1000. The bands were visualized with goat-antirabbit immunoglobulin G antibody conjugated with horseradish peroxidase, using the Santa Cruz Luminol reagent detection kit.

For electrophoretic mobility shift assay (EMSA), an oligonucleotide with an AP-1 consensus sequence 5′-CGCTTG ATGACTCA GCCGGA A-3′, with central part as consensus binding site was used. Consensus binding site has been underlined was used. This above oligonucleotide was annealed and labeled with [$\gamma-^{32}$P] ATP (3,000 Ci/mmol, Jonaki, Hyderabad) by T4 polynucleotide kinase and gel purified on a 15% polyacrylamide gel.The binding reaction was performed in a 25 μL reaction volume containing 50% glycerol, 60mM HEPES, pH −7.9, 20mM Tris-HCl, pH–7.9, 300 mM KCl, 5 mM EDTA, 5 mM DTT, 100 μg of bovine serum albumin per mL, 2.5 μg of poly (dI-dC), and 10 μg of nuclear extract. After 5 min, 10,000 cpm of the [$\gamma-^{32}$P] ATP 5′-end labeled double stranded oligonucleotide probe was added, the incubation was continued for additional 25 min at room temperature. The DNA-protein complexes were resolved on 4.5% non-denaturing polyacrylamide gel (29:1 cross-linking ratio), dried and exposed overnight to KODAK X-Omat Films.

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