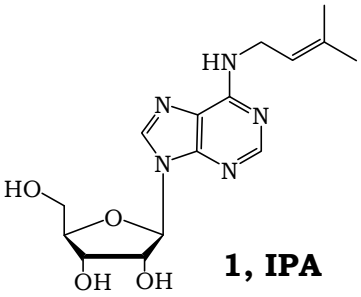
# Studies on the synthesis and the biological activity of nucleosides analogues related to isopentenyladenosine

Dott.ssa Roberta Ottria

*Docente guida Prof. Enzo Santaniello – Tutor Prof.ssa Pierangela Ciuffreda*

## Introduction

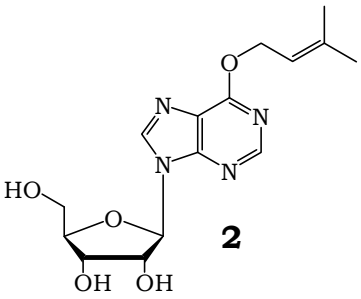
$N^6$-($\Delta^2$-Isopentenyl)adenosine (**1**, IPA), a modified nucleoside, is the only known cytokinin existing in animal cells. IPA has been detected in the cytosol of many eukaryotic and prokaryotic cells as a free compound or bound to tRNA. At the moment, however, the biological role of IPA in mammalians cells and its mechanism of action are not fully understood [1-3]. Spinola et al. [4] have recently demonstrated that IPA exerts a potent *in vitro* antitumoral activity on human epithelial cancer cell lines but has slight effect on tumor growth in rodents. This lack of *in vivo* activity could be related to the short plasma half-life of IPA, as for other nucleosides.



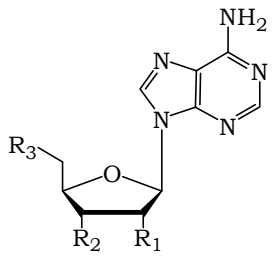
**1, IPA**

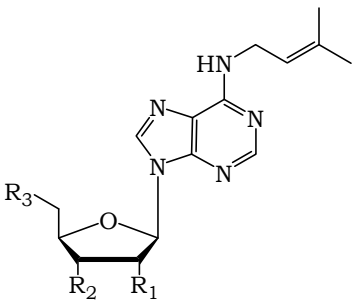
## Aim of the project/Experimental approach

In order to identify compounds endowed with *in vitro* and *in vivo* antiproliferative activity, we investigated structural modifications of IPA. In the first year of our PhD project, we demonstrated that a minor modification of IPA side chain (i.e. hydrogenation of the double bond) yielded a compound showing only 50% inhibition of cell growth and clone formation. Acyclonucleoside analogues of IPA were inactive. In the second year, we synthesized the *O*-isopentenylinosine (**2**) by reaction with 3,3-dimethylallyl bromide to investigate the effects of the change of the base on the antitumoral activity. We then synthesized deoxy-nucleosides derivatives (**7-10**) in order to study the importance of each hydroxyl group of the furanosidic moiety for the activity of IPA. For this purpose, only 2′-deoxyadenosine (**3**) was commercially available, so we synthesized the 3′-deoxy, 5′-deoxy and 2′,3′-dideoxy adenosine (compounds **4**, **5** e **6**). Compounds **7-10** were obtained in satisfactory yield by reaction of nucleosides **3-6** with 3,3-dimethylallyl bromide. All synthesized isopentenyl adenosine analogues (compounds **2**, **7-10**) have been tested in biological systems *in vitro*. Proliferation, clonogenity and migration assays were performed on the human



**2**





**3** : $R_1$=H $R_2$=$R_3$=OH

**4** : $R_2$=H $R_1$=$R_3$=OH

**5** : $R_3$=H $R_1$=$R_2$=OH

**6** : $R_1$=$R_2$=H $R_3$=OH

**7** : $R_1$=H $R_2$=$R_3$=OH

**8** : $R_2$=H $R_1$=$R_3$=OH

**9** : $R_3$=H $R_1$=$R_2$=OH

**10** : $R_1$=$R_2$=H $R_3$=OH

neoplastic cell line T24 (bladder carcinoma) on proliferating and quiescent cell cultures (10 μM). These preliminary experiments showed that all molecules were not active on proliferating cells, confirming that for the antitumoral activity of IPA the ribose moiety has to be kept intact.

An interesting observation is related to the activity of compound **9** that was the only analogue of IPA that was able to cause cell death of quiescent cultures. In order to verify that 3′-deoxyisopentenyladenosine (**9**) has effect on different types of cell line we also tested **9** on proliferating and quiescent cell cultures (10 μM) of different human and murine carcinoma cell lines colon (caco2), bladder (J82), breast (MDAMB231) and rodent lung carcinoma (LLC). These trials indicated that the activity of compound **9** is comparable in all cell lines.

Differently from compound **9**, 3′-deoxyadenosine (**5**) was found to be inactive with quiescent cells and this result demonstrates the primary importance of the isopentenyl chain for the activity of compound **9**. Additional studies are required in order to clarify the biological significance of the selective activity of **9** on quiescent neoplastic cells.

## References


[1] Laezza C., Migliaro A., Cerbone R., Tedesco I., Santillo M., Garbi C., Bifulco M. *Exp. Cell. Res.* 1997, **234**, 178.

[2] H. Kersten, *Prog. Nucleic Acid Res. Mol. Biol.* 1984, **31**, 59.

[3] H. M. Laten, S. Zahareas-Doktor, S. *Proc. Natl. Acad. Sci. USA* 1985, **82**,1113.

[4] Spinola M., Colombo F., Falvella S., Dragani T. A. *Int. J. Cancer* 2007; **120**, 2744.