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Unsaturated Keto and Exomethylene Pyranonucleoside Analogues of Thymine and Uracil Exhibit Potent Antioxidant Properties

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ABSTRACT

Nucleoside analogues play an important role in the development of antitumor and antiviral agents. Specific sugar modified pyranonucleosides, like the keto and exocyclic methylene nucleosides, have been studied for their biological properties, but there is little information regarding their antioxidant activity. The present study reports the antioxidant activity of a series of α,β-unsaturated 2'- *or* 4'- *keto and exomethylene* 5'-*hydroxymethyl-lacking pyranonucleosides. The antioxidant activity was evaluated using an in vitro assay which is based on the capacity to protect DNA strand scission induced by peroxyl radicals* (*ROO*•). *The majority of the tested nucleoside analogues exhibit potent antioxidant properties against ROO*• *radicals. We conclude that the presence of a carbon-carbon double bond at α,β-disposition to exomethylene group at position* 2 *of the sugar moiety and the substitution of thymine with uracil improves the antioxidant capacity of these analogues.*

Keywords*: *D-Lyxopyranonucleoside Derivatives*, *D-Arabinonucleoside Derivatives*, *DNA Damage*, *Peroxyl Radicals.

1. Introduction

Nucleosides are structural modules of nucleic acids with fundamental importance in all living systems [1]. They constitute the basis for development of antitumor and antiviral agents because they act as selective inhibitors of key enzymes involved in cancer or viral replication [2], or as nucleic acid chain terminators which interrupt cellular replication [3-6]. Sugar modified pyranonucleosides are recognized as an important class of biologically active molecules [7-12]. Among them, unsaturated keto [13-15], as well as exomethylene pyranonucleoside analogues [16-19], exhibit interesting antitumor and antiviral properties, while early studies demonstrated that the presence of a primary hydroxyl and hydroxymethyl group in the sugar moiety does not seem to be critical for biological activity [18,20-21].

In order to investigate the antioxidant properties of nucleoside analogues we have previously synthesized a new class of unsaturated 3'-fluoro-4'-ketonucleosides, that of N^4-benzoyl cytosine and N^6-benzoyl adenine, respectively [19]. Most of the aforementioned compounds showed significant ability to protect DNA from the strand breaking activity of ROO• radicals. Furthermore, those nucleoside analogues containing an *α,β*-unsaturated keto system were the most potent against the activity of ROO• radicals.

In extending these studies the antioxidant activity of a series of *α,β*-unsaturated 2'- or 4'- keto and exomethylene 5'-hydroxymethyl-lacking pyranonucleoside analogues was investigated. Specifically, the present study is the first attempt to correlate structural modifications of the aforementioned nucleoside analogues with the ability to inhibit ROO• radicals-induced DNA damage.

2. Results

Five out of the eight tested nucleoside analogues inhibited the DNA damage induced by ROO• radicals (**Table 1**). Compound 4 was the most potent as it exhibited 22% inhibition of radical-induced DNA damage at the concentration of 20 μM. It should be noted that all compounds had no effect on plasmid conformation when they were tested alone at the highest concentration.

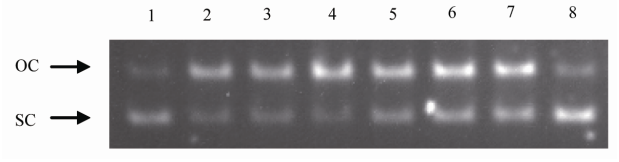


Figure 1. Effect of nucleoside analogue 4 on peroxyl radical-induced plasmid DNA strand scission. Bluescript-SK + plasmid DNA (1 μg/10 μL) was incubated in the presence of 2.5 mM AAPH for 45 min in the dark and the reaction products were analyzed in 0.8% agarose gel. Lane 1: negative control. Lane 2: 2.5 mM AAPH. Lanes 3-7: AAPH plus 5, 10, 20, 50, 100 μM of the nucleoside analogue respectively. Lane 8: plasmid DNA plus 100 µM of the nucleoside analogue. OC: open circular; SC: supercoiled.

The presence of an exomethylene group at 2' position compared to the 4' position of the sugar moiety seems to be important for the antioxidant properties of the nucleoside analogues. Specifically, compounds 1 and 3 which contain the exomethylene group at 4' position of the sugar moiety had no activity, while their corresponding compounds 2 and 4 which contain the exomethylene group at 2' position exhibited potent antioxidant activity (**Table 1**). Compound 2 inhibited ROO$^{\bullet}$ radicals to 16% and 36% at 50 and 100 μM respectively and compound **4** was a potent inhibitor even at 20 μM (**Table 1** and **Figure 1**). The differences in the potency observed between compounds 2 and 4 can be attributed to the different nucleobase (thymine and uracil respectively).

Similar to the exomethylene group the effect of the position of an unsaturated keto system in these nucleoside analogues was examined. Compound **5** had no inhibitory activity at any concentration, while compound **6** had antioxidant activity only at the highest tested concentration (100 μM). Both compounds have thymine as nucleobase but differ at the position of the unsaturated keto system. It is possible that the translocation of the keto group at position 2' favors the antioxidant activity of the compounds. However, this is not the case when thymine is replaced by uracil, as exhibited by the similar antioxidant capacities of compounds 7 and 8 (22% and 19% inhibition at 100 μM respectively).

3. Discussions

The present study reports the antioxidant properties of α,β-unsaturated keto and exomethylene D-arabino- and D-lyxo-pyranonucleoside analogues with thymine and uracil as heterocyclic base. Specifically, these nucleoside analogues were evaluated for the ability to inhibit ROO$^{\bullet}$ radicals-induced DNA damage.

Our results demonstrate that the α,β-unsaturated 2'-exomethylene nucleosides exhibit potent antioxidant activities. This property is further reinforced when the nucleobase thymine is replaced by uracil. The potent antioxidant properties of these compounds can be explained by a radical stabilization resonance effect, which can be attributed to their structural properties. It should be mentioned that the α,β-unsaturated 4'-exomethylene nucleosides had no antioxidant properties.

In contrast to the exomethylene group the influence of the keto group in the antioxidant properties of these compounds is less efficient. The α,β-unsaturated 2'-keto nucleosides exhibit antioxidant properties only at concentrations of 100 μM. The 2'-keto and the 4'-keto uracil nucleoside analogues showed similar antioxidant properties which does not apply to the 2'-keto and the 4'-keto thymine analogues. These results point to a nucleobase preference since the substitution of thymine with the smaller uracil leads to compounds with increased antioxidant abilities. It seems that uracil might be beneficial for the interaction of these compounds with the specific radicals.

4. Conclusions

The results of this study demonstrate that the presence of a carbon-carbon double bond at α,β-disposition to exomethylene group at 2'-position of the sugar moiety and uracil as nucleobase improves the antioxidant capacity of the nucleoside analogues. These might be necessary structural modifications that favor the interaction of these nucleosides with the radicals. ROO$^{\bullet}$ radicals are involved as a major initiating factor in lipid peroxidation chain reactions [22]. Thus, the ability of the tested compounds to protect DNA strand breakage by scavenging peroxyl radicals could suggest that these compounds may also prevent lipid peroxidation. Based on the above findings it would be interesting to further investigate the potential effectiveness of these nucleoside analogues in the prevention and probably the treatment of diseases caused by overproduction of free radicals. Further *in vitro* studies are required to elucidate the exact mechanisms involved in the antioxidant activity of these compounds.

5. Experimental

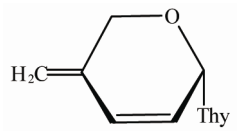
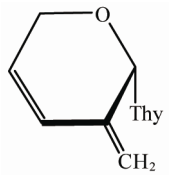
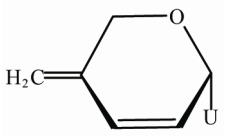
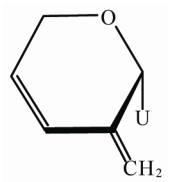
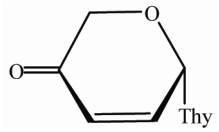
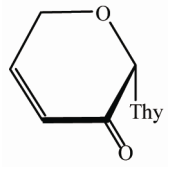
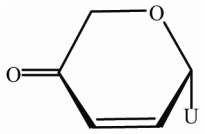
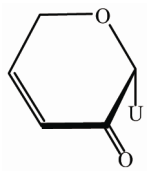
5.1. General

2.2'-azo-bis-2-amidinopropane dihydrochloride (AAPH) was purchased from Sigma-Aldrich (St Louis MO, USA). Bluescript-SK + plasmid DNA was isolated from a large scale bacterial culture. All chemicals and solvents used were of the highest quality commercially available.

5.2. Nucleoside analogues

Nucleoside analogues 1, 3, 5 and 7 were previously syn-

Table 1. Antioxidant properties of nucleosides analogues against ROO• radical induced DNA damage.

Compounds		% Inhibition				
		5 μM	10 μM	20 μM	50 μM	100 μM
1		NI‡	NI	NI	NI	NI
2		NI	NI	NI	16 ± 1†*	36 ± 3*
3		NI	NI	NI	NI	NI
4		NI	NI	22 ± 1*	21 ± 3*	25 ± 2*
5		NI	NI	NI	NI	NI
6		NI	NI	NI	NI	9 ± 1*
7		NI	NI	NI	NI	22 ± 3*
8		NI	NI	NI	NI	19 ± 2*

‡NI: no significant inhibition. †Values are the means ± SE of the percent inhibition from three independent experiments. *$p < 0.05$ when compared with control (plasmid DNA plus AAPH). Thy: Thymine, U: Uracil.

thesized [18] and analogues 2, 4, 6 and 8 were also previously synthesized [23]. All analogues were freshly prepared in DMSO.

5.3. Peroxyl Radical-Induced DNA Strand Scission Assay

The assay was performed using the method described by Chang *et al*. [24]. Peroxyl radicals were generated from thermal decomposition of AAPH. The reaction mixture (10 μL) containing 1 μg Bluescript-SK + plasmid DNA, 2.5 mM AAPH in phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4) and the tested product at different concentrations (5, 10, 20, 50, 100 μM) was incubated in darkness for 45 min at 37˚C. AAPH was added last right before incubation. The reaction was terminated by the addition of 3 μL loading buffer (0.25% bromophenol blue and 30% glycerol) and analyzed in 0.8% agarose gel electrophoresis at 70 V for 1 h. The gels were stained with ethidium bromide (0.5 μg/mL), destained with water, photographed by UV translumination using the Vilber Lourmat photodocumentation system (DP-001.FDC) (Torcy, France) and analyzed with Gel-Pro Analyzer version 3.0 (MediaCybernetics, Silver Spring, USA). Each experiment was carried out in triplicate. The use of DMSO at the tested concentrations did not affect the results of the assay.

5.4. Inhibition of Free Radical-Induced DNA Damage

The induction of DNA strand breaks by peroxyl ($ROO^{\bullet}$) was measured by the conversion of supercoiled Bluescript-SK + plasmid double stranded DNA to the open circular conformation analyzed in agarose gel electrophoresis. Preventive activity of the tested samples was assessed by the inhibition of conversion of supercoiled (unnicked) conformation to open circular (nicked). The percentage inhibition of radical-induced DNA strand cleavage by the tested compounds was calculated using the following equation:

$$\%\ inhibition = \frac{S_p - S}{S_p - S_o} \times 100 \qquad (1)$$

where S_o is the percentage of supercoiled conforma- tion in the negative control sample (plasmid DNA alone), S_p is the percentage of supercoiled conformation in the positive control sample (plasmid DNA with the radical initiating factor) and S is the percentage of supercoiled conformation in the sample containing plasmid DNA, the tested compound and the radical initiating factor. It should be noted that prior to treatment Bluescript-SK + plasmid DNA contained approximately 10% - 20% open circular DNA.

5.5. Statistical Analysis

All results are expressed as mean ±SD (n = 3). Statistical computations were carried out using the SPSS 13.0 software. For statistical analysis, one-way ANOVA was applied followed by Dunnett's test for multiple pair-wise comparisons. Dose response relationships were examined by Spearman's correlation analysis. Differences were considered significant at $p < 0.05$.

6. Acknowledgements

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