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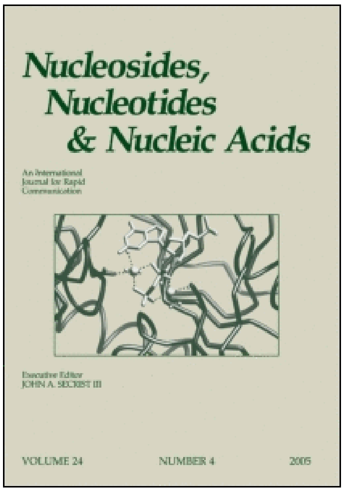

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A New Tiazofurin Pronucleotide: Synthesis and Biological Evaluation of *Cyclo*Saligenyl-Tiazofurin Monophosphate

L. Cappellacci[a]; G. Barboni[a]; P. Franchetti[a]; C. Martini[b]; H. N. Jayaram[c]; M. Grifantini[ad]
[a] Department of Chemical Sciences, University of Camerino, Camerino, Italy [b] Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, Pisa, Italy [c] Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA [d] Dipartimento di Scienze Chimiche, Camerino, MC, Italy

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A New Tiazofurin Pronucleotide: Synthesis and Biological Evaluation of *Cyclo*Saligenyl-Tiazofurin Monophosphate

**L. Cappellacci,[1] G. Barboni,[1] P. Franchetti,[1] C. Martini,[2]
H. N. Jayaram,[3] and M. Grifantini[1,*]**

[1]Department of Chemical Sciences, University of Camerino, Camerino, Italy
[2]Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology, University of Pisa, Pisa, Italy
[3]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA

ABSTRACT

Synthesis and biological activities of *cyclo*saligenyl-tiazofurin monophosphate (*Cyclo*Sal-TRMP), a new tiazofurin pronucleotide, are reported. *Cyclo*Sal-TRMP proved to be active in vitro against human myelogenous leukemia K562 cell line and as A_1 adenosine receptor agonist.

Key Words: Tiazofurin; *Cyclo*Sal-pronucleotide; Anticancer; IMPDH inhibitor.

Tiazofurin (2-*β*-D-ribofuranosylthiazole-4-carboxamide, NSC-286193), is a *C*-nucleoside with demonstrated clinical efficacy as an antitumor agent. The antitumor activity of this compound derives from a combination of cytotoxicity and maturation-inducing activities. Both effects result from inhibition of the target enzyme inosine monophosphate dehydrogenase (IMPDH), which induces the shutdown of guanine nucleotide synthesis.[1] In tumor cells, tiazofurin is converted to tiazofurin

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*Correspondence: M. Grifantini, Dipartimento di Scienze Chimiche, Via S. Agostino 1, 1-62032 Camerino, MC, Italy; Fax: +39 0737 637345; E-mail: mario.grifantini@unicam.it.

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adenine dinucleotide (TAD) that is a potent inhibitor of the enzyme. The resulting decrease in GTP and dGTP biosynthesis produces inhibition of tumor cell proliferation. Clinical studies in Phase I-II pointed out that repeated administration of this drug in patients often led to side effects on the central nervous system. It has been hypothesized that the thiazole ring of tiazofurin, which confers on the molecule a kind of purine-like property, allows the drug to interact with the CNS adenosine receptors (AR). We confirmed this hypothesis in binding studies that allowed us to establish that tiazofurin is able to bind selectively with A_1-type ARs. This affinity is not related with the IMPDH inhibitory activity since tiazofurin analogs that do not inhibit the enzyme proved able to interact with adenosine receptors.[2] It is well known that tiazofurin needs to be phosphorylated by cellular kinases to the 5′-monophosphate (TRMP) which is then transformed into the NAD analog, TAD, by NAD pyrophosphorylase. In tumor cells resistant to tiazofurin, a decrease was observed in this enzyme which did not allow the formation of sufficiently high levels of TAD. Also the scanty TRMP production in cells containing low kinase levels can be the cause of drug resistance development.

In order to overcome the first step of metabolic activation of tiazofurin and to avoid tiazofurin effects on the CNS, we planned the synthesis of 5′-phosphotriester prodrugs, able to cross the cell membranes by passive diffusion and to deliver the nucleotide intracellularly upon release of non-toxic masking group. Herein, we report on the synthesis and biological activity of the 5′-(*cyclo*saligenyl) phosphotriester derivative of tiazofurin (*cyclo*Sal-TRMP, **1**). On account of the lack of the free 5′-hydroxy group in the ribose moiety, derivative **1** should poorly interact with adenosine receptors, since it is well known that the 5′-hydroxy group contributes strongly to the potency of adenosine analogs such as AR agonists. The *cyclo*Sal approach was designed to release the monophosphate selectively by chemical activation, involving a highly selective coupled two-step cascade mechanism (Fig. 1).[3]

RESULTS AND DISCUSSION

The synthesis of *cyclo*Sal-TRMP (**1**) was performed as reported in Sch. 1 starting from salicylalcohol (**4**) and protected tiazofurin as 2′,3′-isopropylidene derivative (**6**). *Cyclo*Sal-TRMP was purified by preparative HPLC (H_2O/MeCN, 60:40) and obtained as a 1:1 diastereomeric mixture (47% yield) as determined by ^1H-, and ^{31}P-NMR spectrometry.

*Cyclo*Sal-TRMP was found to be 4-fold less active against K562 cells (IC_{50} 19 μM) relative to the free nucleoside (6.4 μM). Because the antitumor activity of *cyclo*-Sal like **1** reflects the release rate of the active 5′-monophosphorylated form, and knowing that the rate-determining step in the activating process shown in Fig. 1 is the chemical hydrolysis of the prodrug, we determined the stability of **1** in phosphate buffer, pH 7.3 at 37°C. It was found that *cyclo*Sal-TRMP was converted into monophosphate **3** with a half-life of 1.5 h, as determined by HPLC/MS and ^{31}P-NMR data.

The lower antitumor activity of *cyclo*Sal-TRMP compared to tiazofurin might be explained by the fact that compound **1** could serve only as TR depot form

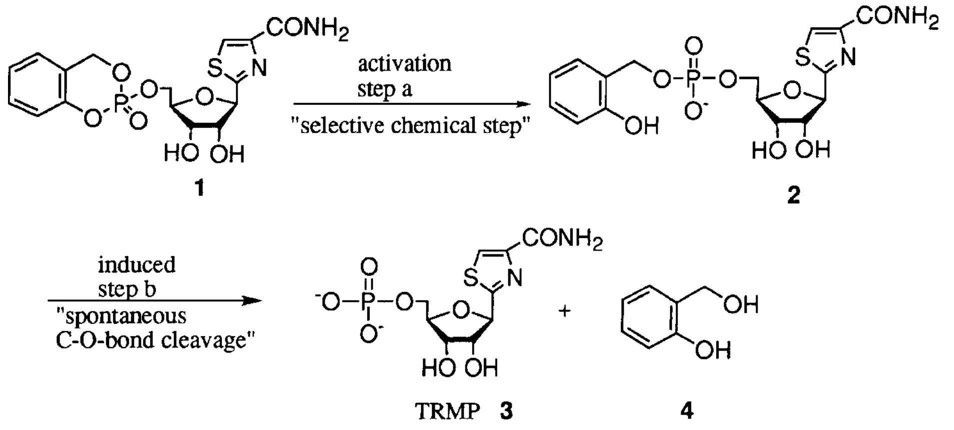
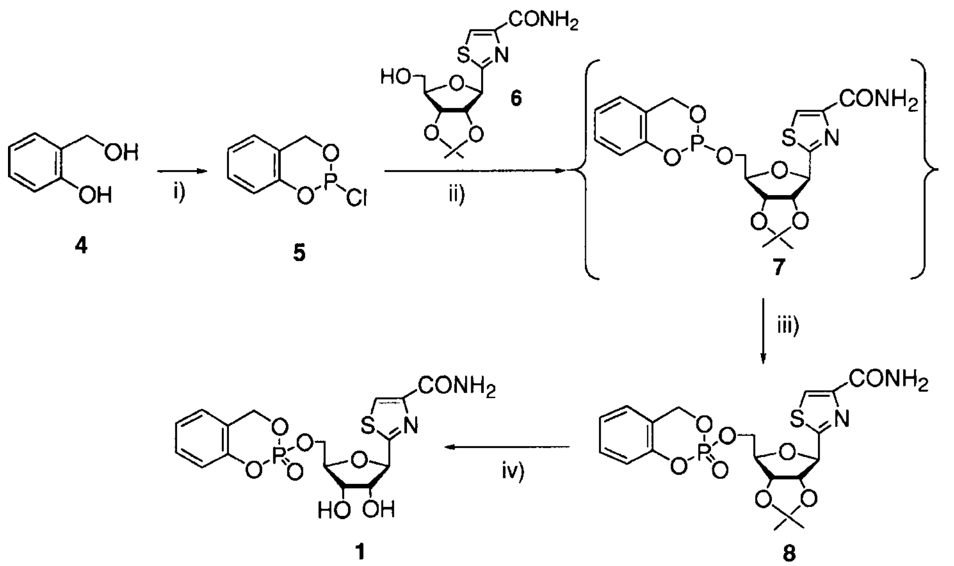


Figure 1. The hydrolysis pathway of *cyclo*Sal-TRMP.

and not as TRMP prodrug. Apparently, the pro-nucleotide concept which works with a lot of antiviral nucleosides does not do so with tiazofurin. This conclusion confirms what was found by other groups working on pro-nucleotide concepts applied to antitumor nucleosides such as 5-fluoro-2′-deoxyuridine and ara-C.[4]

Concerning the action on adenosine receptors, compound **1** was found to be a selective A_1 receptor agonist with an affinity similar to that of tiazofurin. Although the potentiality of **1** as tiazofurin prodrug should be better investigated through in vivo experiments, the results of in vitro studies led us to suppose that the side-



Scheme 1. 1) PCl_3, pyridine a., Et_2O a., −10°C, 2 h; 2) DIPEA, DMF a., 0°C, 20 min.; 3) TBHP, DMF a., 30 min.; 4) $HCOOH/H_2O$, 1 h.

effects of tiazofurin on the central nervous system, fundamentally due to its interaction with adenosine receptors, should occur also with *cyclo*Sal-TRMP.

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