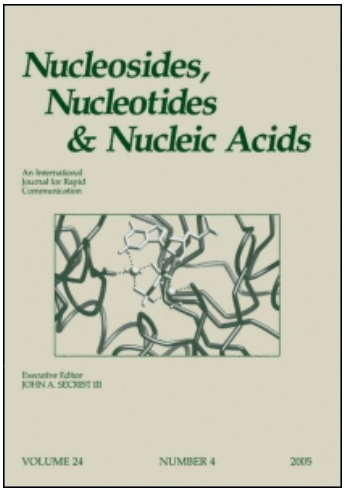

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Cappellacci, L. , Franchetti, P. , Grifantini, M. , Messini, L. , Sheikha, G. Abu , Nocentini, G. , Moraca, R. and Goldstein, B. M.(1995) 'Synthesis, Antitumor Activity and Crystallographic Studies of Analogues of Tiazofurin', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 637 — 640
To link to this Article: DOI: 10.1080/15257779508012440
URL: http://dx.doi.org/10.1080/15257779508012440

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 637-640 (1995)

SYNTHESIS, ANTITUMOR ACTIVITY AND CRYSTALLOGRAPHIC STUDIES OF ANALOGUES OF TIAZOFURIN

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Abstract. The syntheses and antitumor activity of 2-β-D-ribofuranosylfuran-4-carboxamide (furanfurin) and 2-β-D-ribofuranosylthiophene-4-carboxamide (thiophenfurin) are reported. The X-ray structure of ethyl 2-β-D-ribofuranosylthiophene-4-carboxylate, precursor of thiophenfurin, is also presented. Only thiophenfurin showed activity as an antitumor agent both *in vitro* and *in vivo*.

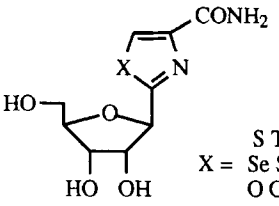
Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC-286193, **1**) and selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide, NSC-340847, **2**) are two widely studied C-nucleosides endowed with several biological effects. These include effective antitumor activity both *in vitro* and *in vivo*, the ability to induce differentiation in neoplastic cells, to inhibit G protein-mediated cellular signaling mechanism and to down-regulate oncogene activity.[1,2]

The biological effects of these nucleosides, which are structurally related to ribavirin, appear to be due to inhibition of inosine monophosphate dehydrogenase (IMPd) which induces the shutdown of guanine nucleotide synthesis.[1] In sensitive cells, tiazo- and selenazofurin are converted to the corresponding NAD analogues (TAD and SAD) which are excellent inhibitors of IMPd.

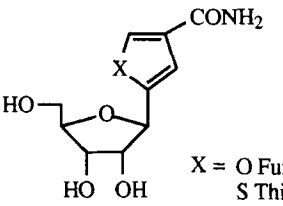
Crystallographic studies of tiazo- and selenazofurin showed close contacts between the thiazole S or selenazole Se heteroatoms and the furanose oxygen. These close contacts have been attributed to an electrostatic attractive interaction between the positively charged sulfur or selenium and the lone-pair of electrons on the furanose oxygen, as suggested by molecular orbital calculations.[2]

Recently we synthesized oxazofurin (**3**), an analogue of tiazo- and selenazofurin in which the S and Se heteroatoms are substituted by oxygen. This compound was found to be inactive as an antitumor and antiviral agent.[3a,b] The inactivity of oxazofurin might be due to the O---O1' repulsion which does not allow oxazole and furanose moieties to assume the right conformation to bind the enzyme. This hypothesis was supported by quantum-mechanical-based computations.[4]

As part of our studies dealing with the structure-activity relationships of such C-nucleosides, we now report on the synthesis of oxazo- and tiazofurin analogues in which the base is replaced by other five-membered ring heterocycles such as furan and thiophene (furanfurin **4**, and thiophenfurin **5**, respectively).



X = S Tiazofurin (**1**)
Se Selenazofurin (**2**)
O Oxazofurin (**3**)



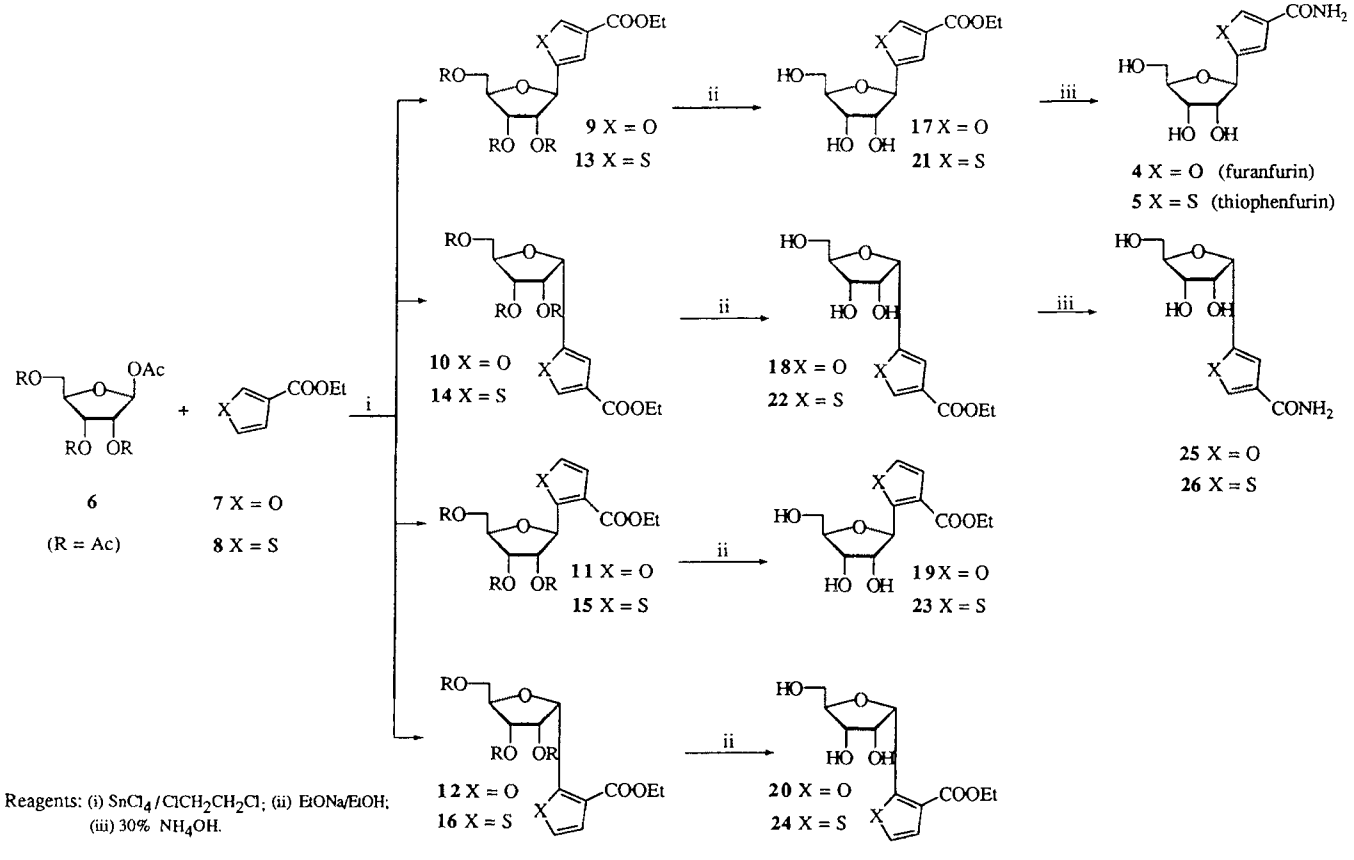
X = O Furanfurin (**4**)
S Thiophenfurin (**5**)

The synthesis of furanfurin (**4**) and thiophenfurin (**5**) were carried out as outlined in scheme 1. The reaction of tetra-*O*-acetyl-β-D-ribofuranose (**6**) with ethyl 3-furancarboxylate (**7**) or ethyl 3-thiophene-carboxylate (**8**) in 1,2-dichloroethane in the presence of stannic chloride affords 2- and 5-glycosylated regioisomers (5/2 = 2.5) as mixtures of α and β anomers (**9-16**) in 65-70% yields. The 2- and 5-regio-isomers were separated by flash chromatography and converted to deblocked ethyl esters **17-24** by treat-ment with sodium ethoxide in ethanol. The glycosylation position and the anomeric configuration were determined by ^{1}H-NMR, ^{13}C-NMR and proton-proton nuclear Overhauser effect (NOE) difference spectroscopy.

Finally furanfurin (**4**), its α-anomer **25**, thiophenfurin (**5**) and its α-anomer **26** were obtained by ammonolysis of compounds **17**, **18**, **21** and **22** with 30% ammonium hydroxide.

The structure of thiophenfurin was confirmed by determination of the structure of the precursor ethyl 2-β-D-ribofuranosylthiophene-4-carboxylate **21** by X-ray analysis (Fig. 1). The molecular structure of **21** shares several features observed in the structures of related thiazole and selenazole nucleosides.[2] The thiophene ring is planar, with the ethyl carboxylate substituent at C-4 coplanar to the heterocycle. The C-glycosidic torsion angle is 46.5°, somewhat higher than that observed in the thiazole nucleosides. However, the thiophene sulfur remains *cis* to the furanose oxygen, with a marginally close non-bonded S---O contact of 3.04 Å.

Furanfurin and thiophenfurin have been evaluated for their ability to inhibit the growth *in vitro* of P388 and L1210 murine lymphocytic leukaemia, K562 human erythroid leukaemia, HL-60 human promyelocytic leukaemia and LoVo human colon adenocarcinoma. Antitumor assay was based on cell viability, as monitored by the MTT method.[5] Furanfurin was inactive at the highest tested concentration of 2 x 10^{-4} M. On the contrary, thiophenfurin appeared to be a good antitumor agent with a potency comparable to that of tiazofurin. Thiophenfurin was superior to tiazofurin as inhibitor of the growth of K562 human erythroid leukaemia and LoVo human colon adenocarcinoma with IC_{50} values of respectively 31 and 102 μM (IC_{50} of tiazofurin >128 μM). Thiophenfurin exhibited substantial activity *in vivo* against L1210 leukaemia on mice with a T/C of 168 at 25 mg/kg/day for 9 days.



Scheme 1

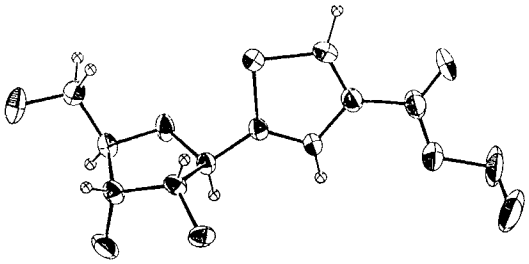


Fig. 1. Molecular structure of ethyl 2-β-D-ribofuranosylthiophene-4-carboxylate (**21**). Non-hydrogen atoms are represented by thermal ellipsoids at the 30% probability level.

In conclusion these results confirm the hypothesis that the presence of the sulfur in the five-membered ring heterocycle in position 2 with respect to the glycosidic bond is fundamental for the antitumor activity of this type of C-nucleosides, whereas the nitrogen atom contributes little to the activity of tiazofurin. The inactivity of furanfurin might be due, similarly to oxazofurin, to a repulsive 1,4 intramolecular interaction between the furan and furanose oxygens, which forces the molecule to assume a conformation unsuitable for the conversion to the corresponding NAD analogue and/or for the interaction with IMPd.

Acknowledgement. *This work was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (40% funds), and CNR, Italy.*

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