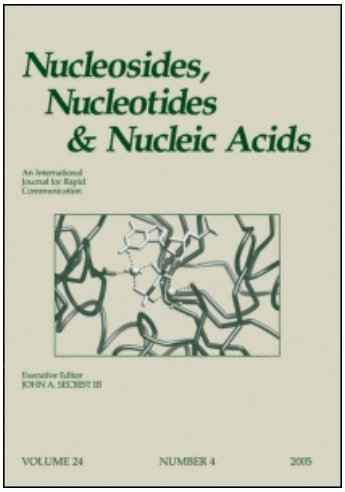

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Synthesis and IMP Dehydrogenase (Type I and Type II) Inhibitory Activity of Isosteric NAD Analogs Derived from Thiophenfurin and Furanfurin

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SYNTHESIS AND IMP DEHYDROGENASE (TYPE I AND TYPE II) INHIBITORY ACTIVITY OF ISOSTERIC NAD ANALOGS DERIVED FROM THIOPHENFURIN AND FURANFURIN

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ABSTRACT: Thiophene-3-carboxamide adenine dinucleotide (TFAD), and furan-3-carboxamide adenine dinucleotide (FFAD), two NAD analogs, were synthesized and evaluated as inhibitors of inosine monophosphate dehydrogenase type I and type II.

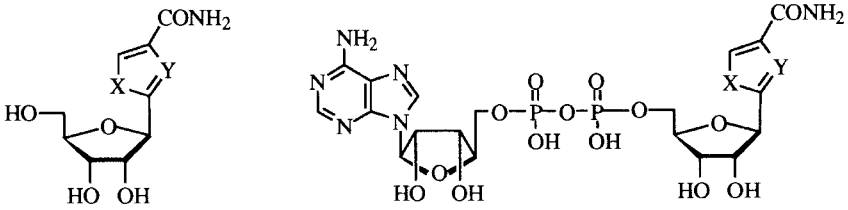
Thiophenfurin (5-β-D-ribofuranosylthiophene-3-carboxamide, **1**) and furanfurin (5-β-D-ribofuranosylfuran-3-carboxamide, **2**) are two isosters of tiazofurin, a C-glycosylthiazole nucleoside with potent antineoplastic activity in human tumor systems. While thiophenfurin was found to be active as an antitumor agent both *in vitro* and *in vivo*, furanfurin proved to be inactive.[1] The mechanism of action of both tiazofurin and thiophenfurin appears to be inhibition of inosine monophosphate dehydrogenase (IMPDH), the enzyme which catalyzes the nicotinamide adenine dinucleotide (NAD)-dependent oxidation of inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP).[2a-c] The resulting decrease in GTP and dGTP biosynthesis produces the inhibition of tumor cell proliferation. In sensitive cells, tiazofurin is metabolized to nicotinamide adenine dinucleotide (NAD) analog TAD, which is a strong inhibitor of IMPDH.[3a,b]

It has recently been discovered that IMPDH exists as two isoforms, type I and type II.[4a,b] Type I is expressed and is the prevalent species in normal cell, while type II isoform is up-regulated and predominates in neoplastic and replicating cells.[5-7] Thus, the selective inhibition of type II IMPDH may provide improved selectivity against target cells in anticancer chemotherapy.

In a recent paper, we reported that thiophenfurin was more easily converted to the NAD analog thiophene-3-carboxamide adenine dinucleotide (TFAD) than tiazofurin in

myelogenous leukemia K562 cells, whereas furanfurin was converted with difficulty to furan-3-carboxamide adenine dinucleotide, FFAD. Thus, the inactivity of furanfurin may be due to its inability to be converted to the dinucleotide in target cells or to the failure of the dinucleotide to inhibit the enzyme.

In order to check these hypotheses, we synthesized the NAD analogs of thiophenfurin and furanfurin and examined their inhibitory effects against IMP dehydrogenase type I and type II.



Thiophenfurin: X = S, Y = CH
Furanfurin: X = O, Y = CH
Tiazofurin: X = S, Y = N

TFAD: X = S, Y = CH
FFAD: X = O, Y = CH
TAD: X = S, Y = N

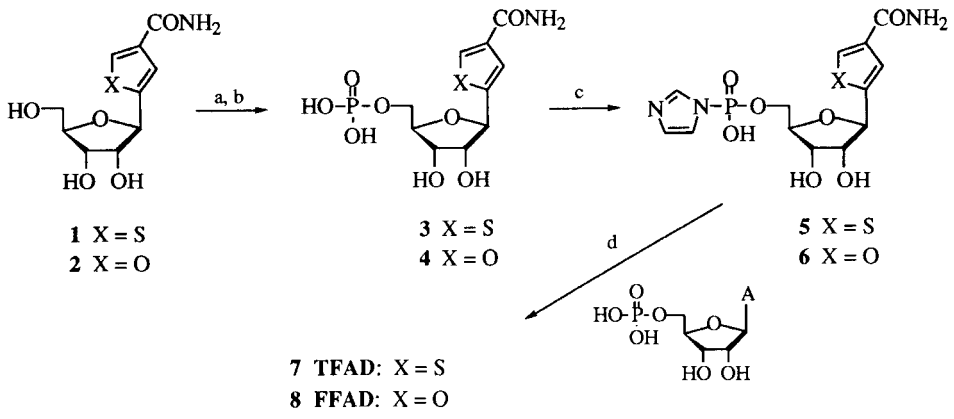
The NAD analogs of thiophenfurin (**7**, TFAD), and furanfurin (**8**, FFAD) were synthesized by the imidazole-catalyzed coupling of the corresponding 5'-monophosphates **3** and **4** with AMP, as shown in Scheme 1.

Compounds **3** and **4** were prepared by phosphorylation of the C-nucleosides **1** and **2** with $POCl_3$ in $(MeO)_3PO$, followed by addition of aqueous triethylammonium bicarbonate. The 5'-monophosphates were purified as triethylammonium salts by chromatography of the reaction mixture on a silica gel RP-18 column. Triethylammonium salts of **3** and **4** were converted into free acid by passing a water solution of the salts through a column of Dowex 50x8 (H^+ form). Activation of nucleotides **3** and **4** with carbonyldiimidazole, and reaction of the *in situ* formed imidazolidates **5** and **6** with AMP gave the desired dinucleotides TFAD and FFAD, which were purified as diammonium salts.

BIOLOGICAL RESULTS

The inhibitory effects of TFAD and FFAD against recombinant human IMP dehydrogenase type I and type II[8] were examined using TAD as reference compound. The K_i values of these compounds for each isoform of IMPDH were determined as described by Magasanik et al.[9]

Among the NAD analogs, TFAD was the most potent inhibitor of both enzymes' isoforms. TFAD inhibited human type I IMPDH (K_i towards IMP and NAD utilization of 0.37 μM and 0.47 μM, respectively), and type II IMPDH (K_i towards IMP and NAD



Reagents: (a) $POCl_3$, $(MeO)_3PO$; then 2 M TEAB; (b) Dowex 50/H^+; (c) carbonyldiimidazole; (d) tri-n-butylamine, DMF.

SCHEME 1

utilization of 0.43 μM and 0.44 μM, respectively). FFAD proved to be a weak inhibitor of type I IMPDH (K_i towards IMP and NAD utilization of 37.9 μM and 135.1 μM, respectively), and type II IMPDH (K_i towards IMP and NAD utilization of 57.9 μM and 99.1 μM, respectively). TAD was found to be slightly less potent than TFAD against both types of IMPDH (K_i towards IMP and NAD utilization of 0.91 μM and 0.47 μM, respectively) and type II IMPDH (K_i towards IMP and NAD utilization of 0.43 μM and 0.44 μM, respectively). These results demonstrate that the inactivity of furanfurin as antitumor agent is due, not only to its poor ability to be converted to the anabolite FFAD in target cells,[1] but also to the low potency of this anabolite as IMPDH inhibitor.

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