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# BIOCHEMICAL AND ANTITUMOR ACTIVITY OF TIAZOFURIN AND ITS SELENIUM ANALOG (2-β-D-RIBOFURANOSYL-4-SELENAZOLECARBOXAMIDE)

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**Abstract**—2-β-D-Ribofuranosyl-4-selenazolecarboxamide (selenazofurin, CI-935), the selenium analog of tiazofurin (CI-909), was 3- to 10-fold more cytotoxic to murine or human tumor cells *in vitro* than tiazofurin and was also more active against P388 mouse leukemia *in vivo*. *In vitro* cytotoxicity could be reversed by guanosine or guanine but not by other purine nucleosides or bases. Three human tumor cell lines selected for selenazofurin or tiazofurin resistance showed cross resistance between selenazofurin and tiazofurin. Treatment with tiazofurin, selenazofurin, or mycophenolic acid decreased guanylate pools and caused an accumulation of IMP in WIL2 human lymphoma cells. The decrease in guanylate pools was accompanied by inhibition of RNA and DNA synthesis. The NAD analogs of tiazofurin and selenazofurin were inhibitors of L1210 IMP dehydrogenase (IMP:NAD oxidoreductase, EC 1.2.1.14), and both showed uncompetitive inhibition with respect to NAD having $K_{ii}$ values of $5.7 \times 10^{-8}$ M and $3.3 \times 10^{-8}$ M respectively.

2-β-D-Ribofuranosyl-4-thiazolecarboxamide (tiazofurin, CI-909†) was originally synthesized and tested as an antiviral agent [1]. When tested for antitumor activity, tiazofurin showed activity in L1210 and P388 mouse leukemias and in Lewis lung carcinoma *in vivo* [1, 2] and against human lymphoid tumors *in vitro* [3]. Recently, the selenium analog of tiazofurin has been synthesized [4]. The selenium analog, selenazofurin, also exhibited *in vivo* Lewis lung activity and was about 10-fold more cytotoxic than tiazofurin in murine tumors *in vitro* [4, 5].

Investigations into the biochemical modes of action of tiazofurin and selenazofurin showed that both drugs were metabolized into ribonucleoside monophosphates and subsequently into analogs of NAD in which the nicotinamide portion of the molecule was replaced by either tiazofurin or selenazofurin [6, 7]. These NAD analogs were potent inhibitors of IMP dehydrogenase (IMPDH) [7], the rate-limiting enzyme in guanylate synthesis [8], and the cytostatic effects of tiazofurin and selenazofurin correlated with the depletion of guanylate pools that occurred due to inhibition of this enzyme.

These studies extend the comparison of the *in vitro* and *in vivo* antitumor effects of tiazofurin and selenazofurin. Biochemical data support the earlier evidence that suggests that tiazofurin and selenazofurin function by a similar mechanism and provide a kinetic analysis of the inhibition of IMPDH by the NAD analogs of tiazofurin and selenazofurin.

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† Abbreviations: CI-909, tiazofurin, TF (2-β-D-ribofuranosl-4-thiazolecarboxamide); CI-935, selenazofurin, SZ (2-β-D-ribofuranosyl-4-selenazolecarboxamide); ROS, Ridgway osteogenic sarcoma; MPA, mycophenolic acid; and IMPDH, IMP dehydrogenase.

## MATERIALS AND METHODS

*Chemicals.* Purine and pyrimidine nucleotides, nucleosides and bases, poly d(A-T), and DNA (calf thymus) were purchased from the Sigma Chemical Co., St. Louis, MO. *Micrococcus luteus* DNA polymerase was obtained from Miles Laboratories, Elkhart, IN. [*Methyl*-$^3$H]Thymidine, [5-$^3$H]uridine, L-[4,5-$^3$H]leucine, deoxy-[8-$^3$H]adenosine 5′-triphosphate, [*methyl*-$^3$H]thymidine 5′-triphosphate, and [8-$^{14}$C]inosine 5′-monophosphate were purchased from Amersham, Arlington Heights, ILL. Tiazofurin was synthesized by the method of Srivastava *et al.* [1] and selenazofurin was synthesized by the method of Srivastava and Robins [4]. The NAD analog of tiazofurin was synthesized by the method of Michelson [9]. The NAD analog of selenazofurin was synthesized by the method of Furusawa *et al.* [10].

*Cell culture.* L1210 mouse leukemia cells were grown in RPMI 1640 supplemented with 50 μg/ml gentamycin sulfate and 5% fetal bovine serum. WIL2 human lymphoblasts and HCT-8 human adenocarcinoma cells were grown in the same medium with 10% fetal bovine serum.

*Growth inhibition studies.* Aliquots (20 μl) of drug solutions were distributed into 24-well Linbro plates. Two milliliters of cells ($3 \times 10^4$/ml for L1210 or $5 \times 10^4$/ml for WIL2) in RPMI medium was added to each well. Cells were incubated at 37° in 5% $CO_2$ in air for 3 days and then counted on a Coulter counter.

One milliliter of HCT-8 cells ($5 \times 10^3$/ml) was distributed into 24-well Linbro plates and then incubated for 2 days at 37° in 5% $CO_2$ in air. Aliquots (10 μl) of drug solutions were added, and the cells were incubated for an additional 4 days. Cells were lysed using lysing reagent (Scientific Products,

McGaw Park, ILL), and nuclei were counted on a Coulter counter.

*Chemotherapy studies.* The $CD2F_1$ mice used for P388 leukemia studies were purchased from Charles River Breeding Laboratories Inc., Portage, MI. AKR mice used for Ridgway osteogenic sarcoma (ROS) experiments were from Jackson Laboratories, Bar Harbor, ME. Tumor passages and drug treatments were performed as described in detail elsewhere [11]. In P388 studies, tiazofurin and selenazofurin were dissolved in 0.9% NaCl. In ROS studies, tiazofurin and selenazofurin were dissolved in water.

*Ribonucleotide analysis.* Samples for ribonucleotide analysis were prepared by adding 0.5 ml of ice-cold 0.7 M perchloric acid to the pellet of approximately $10^7$ WIL2 cells. Samples were mixed and then spun in a Beckman Microfuge B for 1 min to remove the precipitate. The supernatant fractions were neutralized with solid $KH_2CO_3$, chilled on ice for 10 min, and spun in the microfuge for 1 min. Supernatant fractions were assayed for ribonucleotides using a Perkin Elmer series 4 Liquid Chromatograph with a Kratos spectroflow 773 variable wavelength detector set at 254 nm. Columns and elution conditions were as described by Lui *et al.* [12]. Peaks were integrated using a Perkin Elmer Sigma 15 integrator and quantified by comparing peak area to that of known amounts of standards chromatographed under identical conditions.

*Deoxyribonucleotide analysis.* Deoxyribonucleotides were extracted from the pellet of approximately $10^8$ WIL2 cells in 60% methanol as described by Tyrsted [13]. dCTP and dGTP were measured by the DNA polymerase method using calf thymus DNA as template/primer [14]. TTP and dATP analysis was performed using a modified procedure which employs poly (dA-dT) as template/primer [15]. Standard curves were run for each dNTP in each assay.

*Incorporation of radioactive precursors into macromolecules.* Incorporation of rabiolabeled precursors into DNA, RNA, and protein was monitored by exposing logarithmically growing cells to either [*methyl*-$^3$H]thymidine, [5-$^3$H]uridine, or L-[4,5-$^3$H]-leucine, respectively, at a concentration of 1 $\mu$M and a specific activity of 1 $\mu$Ci/nmole. At regular intervals the cells from a 1-ml aliquot were injected into 2 vol. of ice-cold 15% trichloroacetic acid (TCA), and the precipitate was collected on glass fiber filters. The filters were washed five times with 2-ml aliquots of ice-cold 5% TCA, dried and placed in scintillation vials along with 10 ml of Ready-Solv (Beckman, Irvine, CA). Radioactivity was determined in a Beckman LS 6800 scintillation counter.

*IMP Dehydrogenase assay.* On day 7 the ascites fluid from twenty DBA/2 mice inoculated with $10^7$ L1210 cells was added to 10 vol. of 0.9% NaCl and centrifuged at 200 *g* for 10 min. The cell pellet was rinsed in saline and suspended in 2 vol. of 100 mM $KH_2PO_4$ (pH 7.2), 1 mM EDTA, 0.5 mM 2-mercaptoethanol (buffer A). The suspension was freeze-thawed × 3 and then homogenized with 15 strokes of a Dounce homogenizer with a tight-fitting pestle. The homogenate was centrifuged at $10^5$ *g* for 30 min at 4°. The $10^5$ *g* supernatant fraction was taken to 45% saturation by the slow addition of solid ammonium sulfate, stirred on ice for 10 min, and then centrifuged at $2 \times 10^4$ *g* for 15 min at 4°. The pellet was suspended in 2 ml of buffer A and dialyzed for 2–4 hr against 1 liter of buffer A at 0°. Assays were performed in 400 $\mu$l microfuge tubes containing 30 $\mu$l of enzyme dialysate, 30 $\mu$l of 0.567 mM IMP [8-$^{14}$C] (80 $\mu$Ci/ml) in buffer A, 20 $\mu$l of NAD solution (0–1 mM), and 20 $\mu$l of inhibitor solution. Reactions were started by addition of enzyme followed by a 10-sec spin in the microfuge and terminated by boiling for 2 min. Routine assays were incubated for 20 min at 37°. After boiling, samples were centrifuged to remove precipitated protein and assayed for products by thin-layer chromatography as described previously [16]. Kinetic parameters were determined by the method of Cleland [17].

The NAD analogs of tiazofurin and selenazofurin were assayed for purity by high performance liquid chromatography (HPLC) before use. Standard samples were quantified spectrophotometrically using $E_{254} = 17{,}800$ for the NAD analog of tiazofurin and $E_{259} = 17{,}800$ for the NAD analog of selenazofurin at pH 4.5 in 50 mM $NH_4H_2PO_4$.

## RESULTS

The growth inhibitory effects of tiazofurin and selenazofurin *in vitro* are summarized in Table 1. When cells were exposed continuously, the $IC_{50}$ values for tiazofurin were 10-fold higher than for selenazofurin in HCT8 and WIL2 and 3-fold higher in L1210 cells. When exposure time was reduced to 6 hr, the $IC_{50}$ values of tiazofurin and selenazofurin were then about equal and were higher by 20 to 200-fold over those for continuous exposure.

*In vivo* experiments showed selenazofurin and tiazofurin to be markedly active against P388 leukemia (Table 2). Selenazofurin was approximately 8-fold more potent than tiazofurin in this test. Tiazofurin was moderately active against Ridgway osteogenic sarcoma at 375 mg/kg/inj and toxicity was not reached at the highest dose tested (750 mg/kg/inj).

Table 1. *In vitro* growth inhibition by selenazofurin and tiazofurin*

| | | $IC_{50}$ (M) | |
|---|---|---|---|
| Cell line | Exposure time | Selenazofurin | Tiazofurin |
| L1210 | Continuous | $2.6 \times 10^{-7}$ | $8.3 \times 10^{-7}$ |
| WIL2 | Continuous | $3.3 \times 10^{-7}$ | $4.5 \times 10^{-6}$ |
| HCT8 | Continuous | $6.2 \times 10^{-7}$ | $5.5 \times 10^{-6}$ |
| HCT8 | 18 hr | $6.5 \times 10^{-7}$ | $6.9 \times 10^{-6}$ |
| HCT8 | 12 hr | $3.2 \times 10^{-7}$ | $1.8 \times 10^{-5}$ |
| HCT8 | 6 hr | $3.2 \times 10^{-5}$ | $3.8 \times 10^{-5}$ |

* L1210 and WIL2 cells were grown in the continuous presence of various drug concentrations and then counted on a Coulter counter after 72 hr. HCT 8 cells were plated and then grown in the presence of various drug concentrations for the indicated times. Drugs were removed and the cells were incubated for the remainder of 96 hr. Cells were lysed and their nuclei were counted on a Coulter counter.

Table 2. *In vivo* activity of tiazofurin and selenazofurin against P388 and ridgway osteogenic sarcoma

| Tumor | Drug | Dose (mg/kg/inj) | Schedule | %T/C* or T/C | Log cell kill | | Rating† |
|---|---|---|---|---|---|---|---|
| | | | | | Gross | Net | |
| P388 | Tiazofurin | 800 | D 3–7 | 181 | 7.7 | 4.7 | C |
| P388 | Tiazofurin | 400 | D 3–7 | 174 | 6.8 | 4.0 | C |
| P388 | Tiazofurin | 200 | D 3–7 | 158 | 5.3 | 2.5 | C |
| P388 | Selenazofurin | 200 | D 3–7 | 83 | | | TX |
| P388 | Selenazofurin | 100 | D 3–7 | 185 | 7.8 | 5.0 | B |
| P388 | Selenazofurin | 50 | D 3–7 | 174 | 6.7 | 4.0 | C |
| P388 | Selenazofurin | 25 | D 3–7 | 154 | 5.0 | 2.2 | C |
| P388 | Selenazofurin | 12 | D 3–7 | 142 | 3.9 | 1.1 | C |
| ROS | Tiazofurin | 750 | QD 3–7 | 34 | 0.7 | 0.2 | C |
| ROS | Tiazofurin | 375 | QD 3–7 | 38 | 0.8 | 0.3 | C |
| ROS | Tiazofurin | 188 | QD 3–7 | 85 | 0.2 | 0.3 | N |
| ROS | Tiazofurin | 94 | QD 3–7 | 104 | | −0.3 | N |
| ROS | Selenazofurin | 200 | QD 3–7 | 37 | 0.6 | 0.1 | TX |
| ROS | Selenazofurin | 100 | QD 3–7 | 32 | 0.7 | 0.2 | TX |
| ROS | Selenazofurin | 50 | QD 3–7 | 80 | 0.1 | −0.3 | N |

* Percent T/C (for P388) was calculated as 100 × [median lifespan (treated mice)/median lifespan (control mice)]. Results of ROS tests are expressed as %T/C where % T/C equals 100 × [median size (treated tumors)/median size (control tumors)]. Determination of tumor cell kill was carried out as described by Schabel *et al.* [18] and Corbett *et al.* [19].

† Rating: B = marked activity, C = moderate activity, N = inactive, and TX = toxic.

Table 3. *In vitro* growth inhibition by tiazofurin and selenazofurin in normal and resistant human tumor cells*

| Cell line | $IC_{50}$ (μM) | |
|---|---|---|
| | Tiazofurin | Selenazofurin |
| WIL2 | 4.6 | 0.38 |
| WIL2/TF$^r$ | 118 | 24 |
| WIL2/SZ$^r$ | 315 | 55 |
| HCT8 | 4.8 | 0.44 |
| HCT8/SZ$^r$ | 100 | 10 |

* WIL2 cells were grown in the continuous presence of various drug doses for 72 hr and then counted on a Coulter counter. HCT 8 cells were plated and then grown for 96 hr in the continuous presence of drug. Cells were lysed, and their nuclei were counted on a Coulter counter.

Selenazofurin was inactive against ROS at the highest nontoxic dose (50 mg/kg/inj).

Cells in tissue culture can be made resistant to tiazofurin or selenazofurin by growing them for several generations in the continuous presence of gradually increasing drug concentrations. The data in Table 3 show the $IC_{50}$ values for selenazofurin and tiazofurin obtained with human tumor cells made resistant to one or the other drug. All three cell lines, regardless of which drug was used for selection, showed decreased sensitivity to tiazofurin and selenazofurin. The WIL2/TF$^r$ cells, selected for tiazofurin resistance, showed even less sensitivity to selenazofurin than to tiazofurin, indicating a common mechanism of resistance to both drugs in these cells.

To compare further the modes of action of tiazofurin and selenazofurin, the ribonucleotide pools were measured in WIL2 and WIL2/SZ$^r$ cells exposed to 10 μM tiazofurin or selenazofurin or 10 μM mycophenolic acid (MPA), a known IMPDH inhibitor (Table 4) [20]. Both tiazofurin and selenazofurin showed the same pattern of alterations as those caused by MPA. Pyrimidine triphosphates increased, IMP accumulated, and guanylate pools (GMP, GDP, GTP) were decreased. In WIL2, 10 μM selenazofurin treatment resulted in the greatest depletion of guanine nucleotides. The data indicate that both tiazofurin and selenazofurin inhibited guanylate production at IMPDH and that selenazofurin was the more potent of the two drugs.

Table 4. Tiazofurin, selenazofurin, and MPA effects on WIL2 and WIL2/SZ$^r$ ribonucleotides*

| Cells | Treatment | IMP | GMP | UDP | ADP | GDP | UTP | CTP | ATP | GTP |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (nmoles/$10^9$ cells) | | | | | | | | |
| WIL2 | Control | 17 | 77 | 170 | 699 | 204 | 514 | 185 | 2237 | 627 |
| WIL2 | Tiazofurin | 265 | 18 | 213 | 664 | 100 | 761 | 285 | 2752 | 301 |
| WIL2 | Selenazofurin | 354 | 15 | 211 | 559 | 69 | 812 | 267 | 2243 | 201 |
| WIL2 | MPA | 359 | 16 | 192 | 503 | 75 | 971 | 339 | 2640 | 226 |
| WIL2/SZ$^r$ | Control | 261 | 71 | 274 | 1295 | 222 | 876 | 329 | 3945 | 476 |
| WIL2/SZ$^r$ | Tiazofurin | 125 | 51 | 208 | 842 | 140 | 947 | 342 | 4221 | 485 |
| WIL2/SZ$^r$ | Selenazofurin | 190 | 58 | 348 | 997 | 167 | 867 | 309 | 3787 | 428 |
| WIL2/SZ$^r$ | MPA | 381 | 24 | 411 | 829 | 107 | 1156 | 408 | 3511 | 243 |

* Early log WIL2 and WIL2/SZ$^r$ cells (<3 × $10^5$ cells/ml) were incubated with 10 μM drug for 2 hr at 37°. Ribonucleotides were assayed as described in the Experimental section.

Table 5. Tiazofurin and selenazofurin effects on WIL2 deoxyribonucleotides*

| Treatment | dATP | dGTP | dCTP | TTP |
|---|---|---|---|---|
| | (pmoles/$10^6$ cells) | | | |
| Control | 16 ± 6 | 5 ± 2 | 8 ± 2 | 13 ± 4 |
| Tiazofurin | 9 ± 5 | 2 ± 1 | 7 ± 1 | 34 ± 6 |
| Selenazofurin | 8 ± 4 | 3 ± 1 | 7 ± 1 | 32 ± 3 |

* Early log WIL2 cells (<3 × $10^5$ cells/ml) were incubated with 10 μM drugs for 2 hr at 37°. Deoxyribonucleotides were assayed as described in the Experimental section.

Values are means ± S.E.

Compared to the parent line, the sensitivity of WIL2/SZ$^r$ to MPA was maintained while selenazofurin and tiazofurin showed lesser effects on GTP pools. Since MPA acts through inhibition of IMPDH, the mode of resistance of WIL2/SZ$^r$ cannot be due to increased levels of that enzyme.

Deoxyribonucleotide analysis showed that both tiazofurin and selenazofurin depleted dGTP and dATP while TTP accumulated (Table 5). Reduction of purine ribonucleotides via ribonucleotide reductase was therefore inhibited [21], probably due to depletion of the GDP substrate.

Figure 1 shows the effects of tiazofurin and selenazofurin on nucleic acid synthesis. Both tiazofurin and selenazofurin inhibited both RNA and DNA synthesis, reaching maximum inhibition by about 90 min. Neither drug showed signs of affecting protein synthesis. The inhibition of RNA and DNA synthesis was probably due to depletion of nucleic acid precursors and is consistent with the proposed mechanism for tiazofurin action.

Salvaged guanine is converted directly to GMP, thus bypassing IMPDH. Hypoxanthine, though salvaged by the same enzyme [hypoxanthine (guanine) phosphoribosyltransferase (EC 2.4.2.8)], must be converted to guanylate via IMPDH. *In vitro* protection experiments showed that only guanine or guanosine protected cells from the effects of tiazofurin or selenazofurin (Table 6). Adenine, hypoxanthine, xanthine, xanthosine, inosine, and thymidine could not reverse tiazofurin or selenazofurin toxicity. This indicates that the critical event leading to tiazofurin or selenazofurin toxicity was inhibition of guanylate formation at IMPDH.

Previous reports demonstrated that cells metabolize tiazofurin and selenazofurin first to ribo-

Table 6. Protection of HCT8 from tiazofurin and selenazofurin *in vitro* cytoxicity by guanine and guanosine*

| Treatment | Percent of untreated controls (96 hr cell count) |
|---|---|
| Selenazofurin (0.8 μM) | 31 |
| Tiazofurin (7.7 μM) | 27 |
| Guanine (100 μM) | 97 |
| Selenazofurin (0.8 μM) + guanine (100 μM) | 86 |
| Tiazofurin (7.7 μM) + guanine (100 μM) | 91 |
| Guanosine (100 μM) | 101 |
| Selenazofurin (0.8 μM) + guanosine (100 μM) | 91 |
| Tiazofurin (7.7 μM) + guanosine (100 μM) | 74 |
| Adenine (100 μM) | 80 |
| Selenazofurin (0.8 μM) + adenine (100 μM) | 11 |
| Tiazofurin (7.7 μM) + adenine (100 μM) | 16 |
| Adenosine (100 μM) | 90 |
| Selenazofurin (0.8 μM) + adenosine (100 μM) | 35 |
| Tiazofurin (7.7 μM) + adenosine (100 μM) | 25 |
| Hypoxanthine (100 μM) | 94 |
| Selenazofurin (0.8 μM) + hypoxanthine (100 μM) | 38 |
| Tiazofurin (7.7 μM) + hypoxanthine (100 μM) | 26 |
| Inosine (100 μM) | 93 |
| Selenazofurin (0.8 μM) + inosine (100 μM) | 34 |
| Tiazofurin (7.7 μM) + inosine (100 μM) | 25 |
| Xanthine (100 μM) | 99 |
| Selenazofurin (0.8 μM) + xanthine (100 μM) | 38 |
| Tiazofurin (7.7 μM) + xanthine (100 μM) | 36 |
| Xanthosine (100 μM) | 99 |
| Selenazofurin (0.8 μM) + xanthosine (100 μM) | 25 |
| Tiazofurin (7.7 μM) + xanthosine (100 μM) | 24 |
| Deoxyguanosine (10 μM) | 95 |
| Selenazofurin (0.8 μM) + deoxyguanosine (10 μM) | 45 |
| Tiazofurin (7.7 μM) + deoxyguanosine (10 μM) | 20 |
| Thymidine (100 μM) | 81 |
| Selenazofurin (0.8 μM) + thymidine (100 μM) | 7 |
| Tiazofurin (7.7 μM) + thymidine (100 μM) | 39 |

* HCT8 cells were plated and then grown in the continuous presence of drug and protectant for 96 hr. Cells were lysed and their nuclei were counted on a Coulter counter.

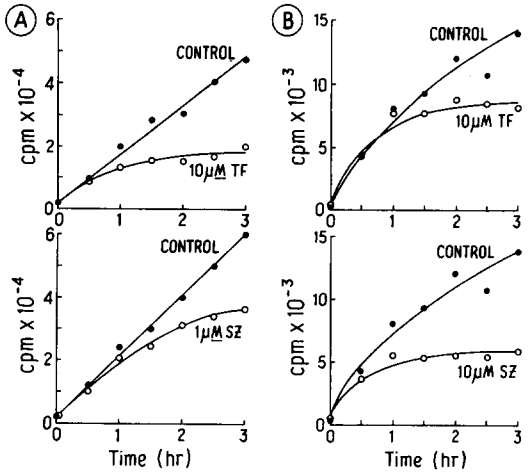


Fig. 1. Effects of tiazofurin (TF) and selenazofurin (SZ) on (A) thymidine incorporation into DNA and (B) uridine incorporation into RNA of L1210 cells. Key: (●) control, and (○) cells exposed to tiazofurin (TF) or selenazofurin (SZ).

nucleoside monophosphates and then to analogs of NAD that are potent inhibitors of IMPDH [7]. The double-reciprocal plots of the inhibition of IMPDH by the NAD analogs of tiazofurin and selenazofurin using a partially purified enzyme prepared from L1210 cells is shown in Fig. 2. The partial purification of IMPDH was necessary to remove interfering enzyme activities, and TLC analysis of the reaction products showed that no detectable nucleosides, GMP, AMP, or nucleoside di- and triphosphates were formed during a 20-min incubation. The NAD analogs of tiazofurin and selenazofurin were uncompetitive inhibitors of IMPDH with respect to NAD, having $K_{ii}$ values of $5.7 \times 10^{-8}$ M and $3.3 \times 10^{-8}$ M respectively. The $K_m$ for NAD was $6.5 \times 10^{-5}$ M.

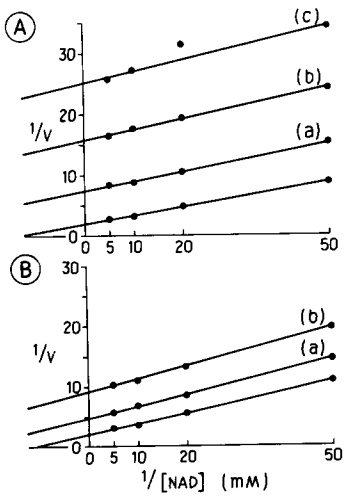


Fig. 2. Inhibition of IMPDH from L1210 cells by the NAD analogs of (A) tiazofurin and (B) selenazofurin with NAD as the variable substrate. Key: (a) 0.1 μM, (b) 0.2 μM, and (c) 0.3 μM inhibitor.

## DISCUSSION

It has been suggested that compounds which inhibit *de novo* purine synthesis produce their cytostatic effects mainly through depletion of guanylate pools [22]. *In vitro* and *in vivo* tests show that a number of inhibitors of guanylate synthesis show some efficacy and indeed some specificity as antineoplastic agents [23, 24].

IMPDH occupies a position immediately after a branch point in purine synthesis and has the rate-limiting activity in that branch. IMPDH is also a proliferation linked enzyme [25], meaning that the amount of IMPDH is generally higher in more rapidly dividing cells. The key regulatory role of this enzyme and its link to tissue proliferation make specific IMPDH inhibitors attractive candidates as new antitumor drugs.

IMPDH follows an ordered sequential mechanism with IMP binding first and with XMP released last [26]. The present kinetic data suggest that the NAD analogs of tiazofurin and selenazofurin bind at the NADH site of IMPDH. It is noteworthy that the NAD analogs of tiazofurin and selenazofurin more closely resemble NADH than NAD in that they lack the positive charge associated with the quarternary nitrogen of NAD. There was less than a 2-fold difference in the $K_{ii}$ values for the tiazofurin and selenazofurin NAD analogs. It is unlikely that this small difference in $K_{ii}$ can explain a 10-fold difference in cytotoxicity for tiazofurin and selenazofurin in L1210 cells (Table 1). Therefore, the rates at which tiazofurin and selenazofurin are metabolized may contribute to the higher potency of selenazofurin.

In comparison with other known inhibitors of IMPDH, tiazofurin and selenazofurin may provide certain advantages. For example, MPA is a very potent inhibitor of IMPDH and has good *in vitro* and *in vivo* antitumor activity. However, MPA is rapidly metabolized rendering it ineffective against human tumors [20]. Bredinin, a nucleoside analog of tiazofurin, shows some antitumor effects *in vitro*. However, since bredinin monophosphate shows competitive inhibition with respect to IMP for IMPDH ($K_i = 2 \times 10^{-8}$ M) [27], the IMP that accumulates in cells treated with bredinin [28] may possibly overcome the inhibition of IMPDH. The NAD analogs of tiazofurin and selenazofurin are also potent inhibitors of IMPDH, but these are uncompetitive inhibitors and therefore should not be displaced if IMP or NAD levels rise.

This study demonstrates that selenazofurin acts similarly to tiazofurin, by the depletion of guanylate pools due to the inhibition of IMPDH. Selenazofurin was more potent *in vitro* and *in vivo* than tiazofurin. This increased potency may have been, at least in part, due to the higher affinity of the NAD analog of selenazofurin for IMPDH compared to that for tiazofurin, but the importance of uptake and metabolism of tiazofurin and selenazofurin to relative drug efficacy awaits further investigation. Tiazofurin had good antitumor activity but, often, only at very high doses. Selenazofurin, acting by the same mechanism as tiazofurin but with greater potency, may be useful in treating tumors where tiazofurin is ineffective due to dose limitation.

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