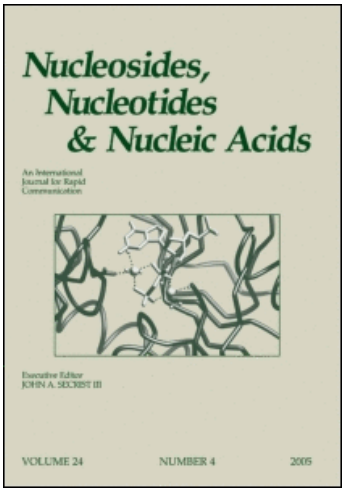

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NOVEL MYCOPHENOLIC ADENINE BIS(PHOSPHONATE)S AS POTENT ANTICANCER AGENTS AND INDUCERS OF CELLS DIFFERENTIATION

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ABSTRACT: An effective treatment of myeloid leukemias would rely on inducing myeloid cells to undergo differentiation. It has been demonstrated that inhibition of IMPDH with mycophenolic acid or tiazofurin resulted in inhibition of cell growth as well as induction of differentiation. We synthesized a number of bis(phosphonate) analogues of tiazofurin-, benzamide-, and mycophenolic-adenine dinucleotide which were found to be cytotoxic as well as effective inducers of cell differentiation.

Although lymphoid leukemias, particularly in children, are among malignant diseases the most amenable to effective therapies, treatments of myeloid leukemias are much less effective and these diseases are generally fatal.[1] Current chemotherapies for myeloid leukemias are hazardous due to high toxicity and low specificity of available drugs.[1] A more effective therapy would rely on inducing myeloid leukemic cells to undergo differentiation into mature myeloid cells.[2,3] It has been demonstrated that inhibition of inosine monophosphate dehydrogenase (IMPDH) resulted in such differentiation and maturation processes.[4] The differentiation-inducing action due to IMPDH inhibition has been observed in several human cancer cell lines, including T-lymphoid CEM-2 leukemia,[5] K-562 erythroleukemia,[6] and MCF-7 breast cancer.[7] It was also demonstrated that antisense oligomer based on the human IMPDH cDNA caused induction of differentiation in HL-60 and K562 human leukemia cell lines.[8]

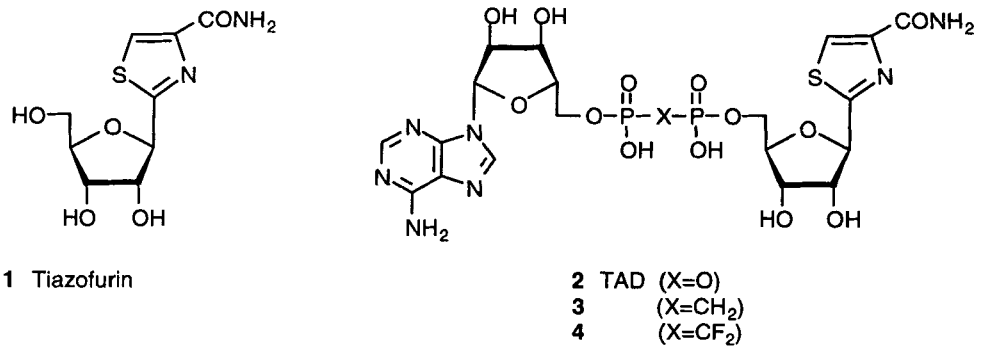
The level of IMPDH activity was found to be much higher in several tumors than in normal tissues. Human IMPDH exists as two isoforms, type I and type II, which are of

identical size (514 amino acids) sharing 84% of sequence identity. Type I is expressed in normal cells, while type II is up-regulated in neoplastic and replicating cells where it is a preponderant isoform. Interestingly, when neoplastic cells are induced to differentiate, the type II isoform is down-regulated to a level below that of type I.[9] Thus, inhibition of IMPDH (possibly type II) is expected to provide a useful tool for overcoming the differentiation block that exist in tumor cells.

Clinical studies of tiazofurin, a potent inhibitor of IMPDH, clearly indicated the tiazofurin-induced differentiation in humans.[4] Unfortunately, tiazofurin cytotoxicity precluded its broad application in chemotherapy of cancer. It is, therefore, an urgent need for the development of agents less toxic than tiazofurin but that induce differentiation. Herein we report the results of our recent studies on the design and development of such new inhibitors.

TIAZOFURIN AND RELATED NUCLEOSIDE ANALOGUES

More than a decade ago it was found that tiazofurin (**1**) requires a unique metabolic activation. It is phosphorylated by adenosine kinase to the 5'-mononucleotide which is then coupled with AMP by NAD-pyrophosphorylase to give a mimic of NAD, tiazofurin adenine dinucleotide, called TAD (**2**, **FIG. 1**). TAD was synthesized and found to be a potent and specific inhibitor of IMPDH (K_i = 0.1 μM).[10]



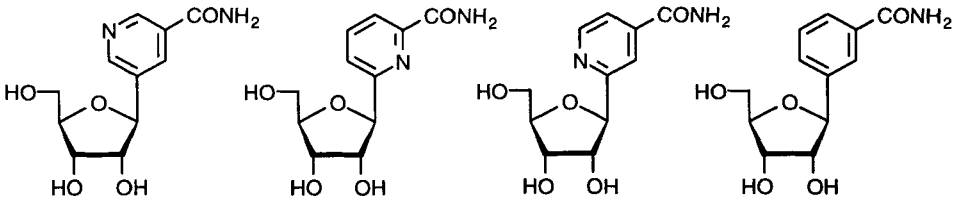
1 Tiazofurin

2 TAD (X=O)
3 (X=CH_2)
4 (X=CF_2)

FIG. 1. Tiazofurin, TAD, and its analogues.

These studies prompted us to synthesize of a number of nucleoside analogues related to tiazofurin or nicotinamide riboside in hope that such analogues could be similarly metabolized in cells to the corresponding NAD analogues and thereby inhibiting IMPDH. The closest mimics of nicotinamide riboside, C-nucleoside isosteres, such as C-nicotinamide[11] (**5**, **FIG. 2**), C-picolinamide[11] (**6**), and C-isonicotinamide riboside[12] (**7**), were prepared but they showed poor antitumor activity when compared with tiazofurin.

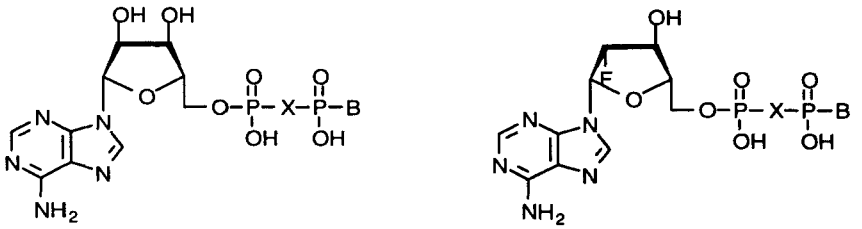
Surprisingly, benzamide riboside (**8**) showed a high toxicity at nanomolar concentration to 49.1 lymphoma cells.[13] It was found that, in contrast to benzamide riboside, C-nucleoside isosteres were not converted in cells to the corresponding NAD analogues. When,



5 C-Nicotinamide riboside | **6** C-Picolinamide riboside | **7** C-Isonicotinamide riboside | **8** Benzamide riboside

FIG. 2 C-Nucleoside analogues of nicotinamide riboside.

however, NAD analogues in the form of pyrophosphates C-NAD (**9**, **Fig. 3**), C-PAD (**10**), and BAD (**11**) were prepared from these nucleosides, all compounds showed a potent inhibitory activity against IMPDH.[11]



9 C-NAD (X=O; B=**5**)
10 C-PAD (X=O; B=**6**)
11 BAD (X=O; B=**8**)
12 ($X=CH_2$; B=**8**)

13 ($X=CH_2$; B=**1**)
14 ($X=CF_2$; B=**1**)
15 ($X=CH_2$; B=**8**)

FIG. 3. Analogues of NAD.

Studies of the relationship between metabolism, cytotoxicity, and resistance of tiazofurin revealed that TAD accumulation was the most important determinant of its anticancer activity. It was found that high level of a specific phosphodiesterase, called TADase, was responsible for TAD breakdown and development of the cell line resistant to tiazofurin.

In 1986, methylenebis(phosphonate) analogue of TAD (**3**, in which the pyrophosphate oxygen was replaced with methylene linkage) was synthesized by Marquez *et al.*[14] and, as expected, found to be resistant to TADase breakdown,. This compound was proved to be active in tiazofurin resistant cell line. In spite of this compound **3** has not been developed further as potential anticancer agent.

BIS(PHOSPHONATE) ANALOGUES OF NAD

Tiazofurin, its mononucleotide, and TAD are in dynamic equilibrium in cells where TAD is constantly synthesized from tiazofurin and simultaneously degraded by pyrophostatases, phosphodiesterases, and phosphatases to the tiazofurin mononucleotide and AMP. We found that in cell extracts and in serum these nucleotides are farther dephosphorylated to tiazofurin and adenosine and the latter is converted into inosine by the action of adenosine deaminase. This equilibrium is also affected by the possibility that TAD degradation products would participate in other biochemical pathways. Tiazofurin mononucleotide, for example, can be further phosphorylated to the level of di- and triphosphates which may inhibit other cellular enzymes or be incorporated into nucleic acids. Such activity is expected to result in general toxicity.

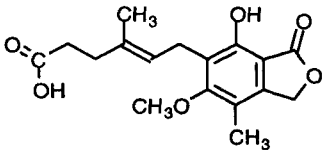
In contrast, methylenebis(phosphonate) (MBP) analogues as well as difluoromethylene-bis(phsophonate) (DMBP) analogues of such pyrophosphates as TAD and BAD should be stable. Recently, we prepared[15] a number of such analogues (**3**, **4**, **12-15**) and found that these bis(phosphonate)s were completely resistant to degradation by cellular enzymes. After 18 hours of incubation in cell extracts from K562 cell line which overexpresses phosphodiesterase, compounds **3**, **4**, **13**, and **14** were recovered unchanged (compounds **12** and **15** were not tested). In contrast, 80% of TAD was hydrolyzed to tiazofurin and inosine under the same conditions.

All these bis(phosphonate) analogues were assayed for both inhibitory activity against human IMPDH type II (K_i's or IC_{50}'s) and antiproliferative activity against K562 erytroleukemic cells (IC_{50}'s). In addition the ability to induce differentiation in K562 cells was estimated by determining the fraction of benzidine positive cells converted following incubation with each analogue.

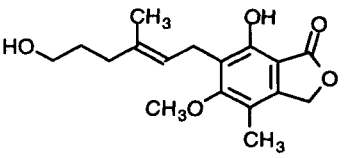
It was found that the activity of bis(phosphonate)s against the isolated IMPDH was often as good as that of the parent pyrophosphates, for example TAD (**2**, 0.1 μM), MPB analogue **3** (0.1 μM), DMBP analogue **4** (0.3 μM), or BAD (**11**, 0.8 μM) and MBP analogue **12** (0.8 μM). With an exception of **3** (18.0 μM), however, other MBP derivatives such as **12** (68.0 μM), **13** (70.0 μM), and **15** (no effect up to high millimolar level) did not effectively inhibit growth of K562 cells. Interestingly, DMBPs analogues **4** (11.0 μM) and **14** (13.0 μM) showed much better growth inhibition of these cells *in vitro* demonstrating their ability to enter cells. Indeed, in concentrations that is required to inhibit 50% of cell growth (appr. 15.0 μM) DMBP analogues **4** and **14** induced approximately 50% of differentiation of these K562 cells. Finally, some of the new analogues exhibited good specificity against IMPDH: the MBP analogue of BAD (**12**) was 50 fold less potent

than BAD (**11**) as an inhibitor of horse liver alcohol dehydrogenase (ADH, 6.3 μM *versus* 333 μM) and **14** was not active against ADH up to high millimolar level.

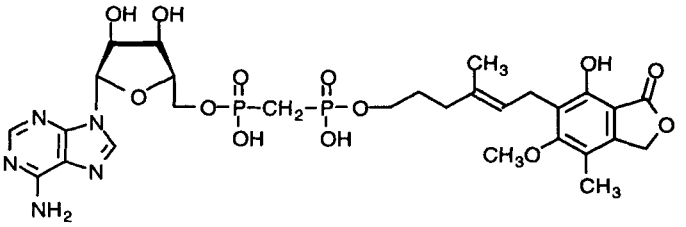
Recently, we synthesized[16] a new MBP analogue of NAD in which nicotinamide riboside was replaced with mycophenolic alcohol (**17**, **FIG. 4**). Since it has been



16 Mycophenolic acid



17 MPAlc



18

FIG. 4. Methylenebis(phosphonate) analogue of MAD.

revealed that mycophenolic acid (**16**), the most potent inhibitor of IMPDH, was bound to the enzyme in the cofactor binding domain in the place reserved for nicotinamide mononucleotide, we prepared the MBP analogue of mycophenolic adenine dinucleotide (MAD, **18**) by coupling the alcohol **17** with adenine 5'-MBP. As expected, the MBP analogue of MAD (**18**) was found to be a potent inhibitor of growth of K562 cells (IC_{50} = 1.5 μM). It was also found to be an effective inducer of cell differentiation (in concentration of 10 μM 91% of conversion was observed). These results clearly indicate that bis(phosphonate) analogues of NAD are of potential therapeutic interest. Further studies on the synthesis of other such analogues are in progress.

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REFERENCES

1. Wright, D.G.; Boosalis, M.S.; Waraska, K.; Oshry, L.J.; Weintraub, L.R.; Vosburgh, E. *Anticnancer Res.*, **1996**, *16*, 3349-3351.
2. Tricot, G. and Weber, G. *Anticancer Res.*, **1996**, *16*, 3341-3347.

3. Olah, E.; Csokay, B.; Prajda, N.; Kote-Jari, Z.; Yeh, Y.A.; Weber, G. *Anticancer Res.*, **1996**, *16*, 2469-2477.
4. Weber, G.; Prajda, N.; Abonyi, M.; Look, K.Y.; Tricot, G.*Anticancer Res.*, **1996**, *16*, 3313-3322
5. Kiguhi, K.; Collart, F.R.; Henning-Chubb, C.; Huberman. *Exp. Cell Res.*, **1990**, *187*, 47-53.
6. Olah, E.; Natsumeda, Y.; Ikegami, T.; Kote, Z.; Horanyi, M.; Szelenyi, J.; Paulik, E.; Kremmer, T.; Hollan, S.R.; Sugar, J.; Weber, G. *Proc. Natl. Acad. Sci.*, **1988**, *85*, 6533-6537.
7. Sidi, Y.; Panet, C.; Wasserman, L.; Cyjon, A.; Novogrodsky, A.; Nordenberg, J. *Eur. J. Cancer Clin. Oncol.*, **1989**, *25*, 883-889.
8. Tsutani, H.; Inai, K.; Imamura, S.; Ueda, T.; Nakamura, T. *Purine and Pyrimidine Metabolism in Man VIII*, Sahota, A. and Taylor, M., eds, Plenum Press, **1995**, 757-760.
9. Carr, S.F.; Papp, E.; Wu, J.C.; Natsumeda, Y. *J. Biol. Chem.*, **1933**, *268*, 27286-27290.
10. Gebeyehu, G.; Marquez, V.E.; Kelly, J.A.; Cooney, D.A.; Jayaram, H.N.; Johns, D.G. *J. Med. Chem.*, **1983**, *26*, 922-925.
11. Pankiewicz, K.W.; Zeidler, J.; Ciszewski, L.A.; Ellis Bell, J.; Goldstein, B.M.; Jayaram, H.N.; Watanabe, K.A. *J. Med. Chem.*, **1993**, *36*, 1855-1859.
12. Joos, P.E.; Esmans, E.L.; Dommisse, R.A.; Van Dongen, W.; Lepoivre, J.A.; Balzarini, J.; De Clerq, E. *Nucleosides & Nucleotides*, **1991**, *10*, 883-894.
13. Krohn, K.; Heins, H.; Wielckens, K. *J. Med. Chem.*, **1992**, *35*, 511-517.
14. Marquez, V.E.; Tseng, C.K.H.; Gebeyehu, G.; Cooney, D.A.; Ahluwalia, G.S.; Kelly, J.A.; Dalal, M.; Fuller, R.; Wilson, Y.A.; Johns, D.G. *J. Med. Chem.*, **1986**, *28*, 1726-1731.
15. Lesiak, K.; Watanabe, K.A.; Majumdar, A.; Seidman, M.; Vanderveen, K.; Goldstein, B.M.; Pankiewicz, K.W. *J. Med. Chem.*, **1997**, *40*, 2533-2538.
16. Lesiak, K.; Watanabe, K.A.; Majumdae, A.; Powell, J.; Seidman, M.; Vandeveen, K.; Goldstein, B.M.; Pankiewicz, K.W. *J. Med. Chem.*, **1998**, *41*, 618-622.