Review

Krzysztof W. Pankiewicz* and Krzysztof Felczak

From ribavirin to NAD analogues and back to ribavirin in search for anticancer agents

DOI 10.1515/hc-2015-0133
Received June 25, 2015; accepted August 17, 2015; previously published online September 17, 2015

Abstract: Ribavirin, a broad-spectrum antiviral agent is used in the clinic alone or in combination with other antivirals and/or interferons. Numerous structural analogues of ribavirin have been developed, among them tiazofurin, which is inactive against viruses but is a potent anticancer drug. Tiazofurin was found to inhibit nicotinamide adenine dinucleotide (NAD)-dependent inosine monophosphate dehydrogenase (IMPDH) after metabolic conversion into tiazofurin adenine dinucleotide (TAD), which binds well but could not serve as IMPDH cofactor. TAD showed high selectivity against human IMPDH vs. other cellular dehydrogenases. Mycophenolic acid (MPA) was even more specific, binding at the cofactor-binding domain of IMPDH. Ribavirin adenine dinucleotide, however, did not show any significant inhibition at the enzymatic level. We synthesized numerous NAD analogues in which natural nicotinamide riboside was replaced by tiazofurin, MPA moiety, or benzamide riboside, and the adenosine moiety as well as the pyrophosphate linker were broadly modified. Some of these compounds were found to be low nanomolar inhibitors of the enzyme and sub-micromolar inhibitors of cancer cell line proliferation. The best were as potent as tyrosine kinase inhibitor gleevec heralded as a 'magic bullet' against chronic myelogenous leukemia. In recent years, ribavirin was rediscovered as a potential anticancer agent against number of tumors including leukemia. It was clearly established that its antitumor activity is related to the inhibition of an oncogene, the eukaryotic translation initiation factor (eIF4E).

Keywords: acute myelogenous leukemia (AML); anticancer agents; chronic myelogenous leukemia (CML); inosine monophosphate dehydrogenase (IMPDH); mycophenolic acid; nicotinamide adenine dinucleotide (NAD) analogues; ribavirin; tiazofurin.

Introduction

In 1972, ribavirin (virazole) was synthesized by Robins and coworkers [1] and was found to be a broad-spectrum antiviral agent active against both DNA and RNA viruses [2]. Although its mechanism of action is not fully understood, even after more than 40 years of studies and more than 13 000 research articles,[1] ribavirin is still used in the clinic today and has recently played an important role in therapies against hepatitis C virus infection (in combination with other drugs and interferons). Ribavirin is a ribonucleoside, structurally similar to the natural 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICA riboside) (Figure 1). When phosphorylated in the cell to the monophosphate level, it mimics inosine monophosphate (IMP) and inhibits IMP dehydrogenase (IMPDH), a key enzyme in *de novo* synthesis of purine ribonucleotides. As a triphosphate, it is structurally similar to the 'cap' of mRNA and interferes with the translation processes. The ribavirin's nucleobase could also be considered as a fragment of adenine or guanine (depending on its conformation around the carboxamido bond), which is able to hydrogen bond with both uracil and cytosine. This leads to mutations often responsible for an 'error catastrophe' [3]. All the above activities severely influence replication of RNA viruses [4]. How ribavirin works against DNA viruses remains a mystery, especially because the 2′-deoxy-ribavirin does not show any biological activity.

In the subsequent years, numerous analogues of ribavirin have been synthesized as potential antiviral agents, among them tiazofurin [5] (Figure 1). Tiazofurin (in contrast to ribavirin) is a C-nucleoside, in which the natural glycosyl carbon-nitrogen (C-N) bond is replaced by carbon-carbon (C-C) linkage.

***Corresponding author: Krzysztof W. Pankiewicz,** University of Minnesota Center for Drug Design, MMC 204, 516 Delaware Street NE, Minneapolis, MN 55455, USA, e-mail: panki001@umn.edu
Krzysztof Felczak: University of Minnesota Center for Drug Design, MMC 204, 516 Delaware Street NE, Minneapolis, MN 55455, USA

1 A total of 13 005 publications are listed by PubMed as on June 10, 2015, and hundreds with a strictly chemical content and no biological data could be found in other sources.

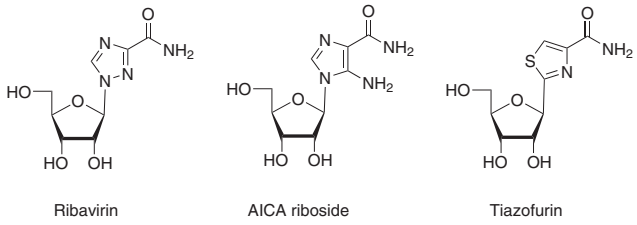

Figure 1 Structures of ribavirin, AICA riboside, and tiazofurin.

Contrary to expectations, this heterocyclic nucleoside did not show any antiviral activity. After some time on the shelf, tiazofurin was rediscovered as a potent inhibitor of proliferation of several cancer cell lines, including acute and chronic myelogenous leukemia (AML and CML, respectively) [6]. It was soon established that the anticancer activity of tiazofurin was related to its inhibition of IMPDH [7–9]. Tiazofurin was phosphorylated to the 5′-monophosphate but did not inhibit IMPDH as a substrate analogue. In contrast to ribavirin, it was instead coupled in the cell with ATP (by NMN adenylyl transferase) to form a nicotinamide adenine dinucleotide (NAD) analogue – tiazofurin adenine dinucleotide, called TAD [8, 10, 11] (Figure 2).

TAD mimics NAD but cannot participate in hydride transfer and selectively inhibits IMPDH. Two isoforms of the human enzyme are known [12, 13] (IMPDH1 and IMPDH2), and TAD inhibits both of them with equal potency (K_i=110 nM). Tiazofurin entered several clinical trials as a potential anticancer agent [14–19] and was approved by the Food and Drug Administration (FDA) as an orphan drug for patients in blast crisis of CML [20].

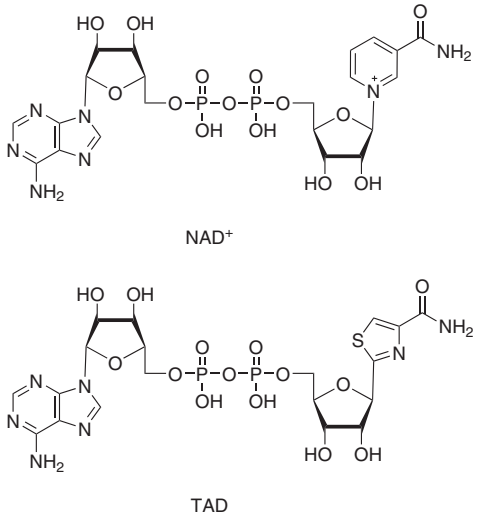

Figure 2 Structures of NAD and TAD.

However, its broad application is limited because of the development of resistance, mainly due to the expression of phosphodiesterase called TAD-ase, which cleaves the TAD pyrophosphate bond to the inactive tiazofurin and adenosine monophosphates [21, 22].

In the 1980s, at the Memorial Sloan-Kettering Cancer Center, classes of C-nucleosides (related to ψ-isocytidine) were developed as potential anticancer agents [23–25]. It was anticipated that some of these compounds might mimic well the natural nicotinamide riboside and possibly show a potent anticancer activity. In addition, if these C-nucleosides were synthesized as their corresponding NAD (TAD)-like analogues, their therapeutic potential might be significantly improved. Indeed, if they are resistant to the action of phosphodiesterases, enter the cell, cannot participate in hydride transfer, and selectively inhibit IMPDH, they might be of high therapeutic potential. Herein is a review describing our early enthusiasm, some first-generation inhibitors, and recently developed derivatives as well as offering some comments about the future of the field.

Early enthusiasm

The attachment of nicotinamide ring to the sugar moiety through a C-C glycosyl bond instead of the C-N bond leads to its C-nucleoside isosters (Figure 3), which more closely resemble the parent nicotinamide riboside than tiazofurin [26, 27]. Contrary to expectations, we found that these nucleosides did not show any anticancer activity. Possibly, they were not converted in the cell into their corresponding NAD analogues, or if converted, these dinucleotide analogues did not inhibit IMPDH.

When NAD analogues were prepared and submitted to biological evaluation against cellular dehydrogenases, C-NAD (but not C-PAD) was found to inhibit alcohol dehydrogenase (K_i=1 nM) about ~20 000-fold more potently than other cellular dehydrogenases, including IMPDH [28]. Meanwhile, the synthetic ribavirin adenine dinucleotide (**1**) was poor inhibitor of IMPDH (K_i=235 μM) and AICA analogue **2** was inactive [29] (Figure 4).

Interestingly, when the benzamide riboside was prepared [30], it proved to be as potent as tiazofurin in anticancer activity and was found to be metabolized in the cell to the corresponding NAD analogue [31] – benzamide adenine dinucleotide (BAD) [32] (Figure 5).

As mentioned above, tiazofurin and benzamide riboside are effectively converted in the cell into their corresponding NAD analogues, which is rather a unique

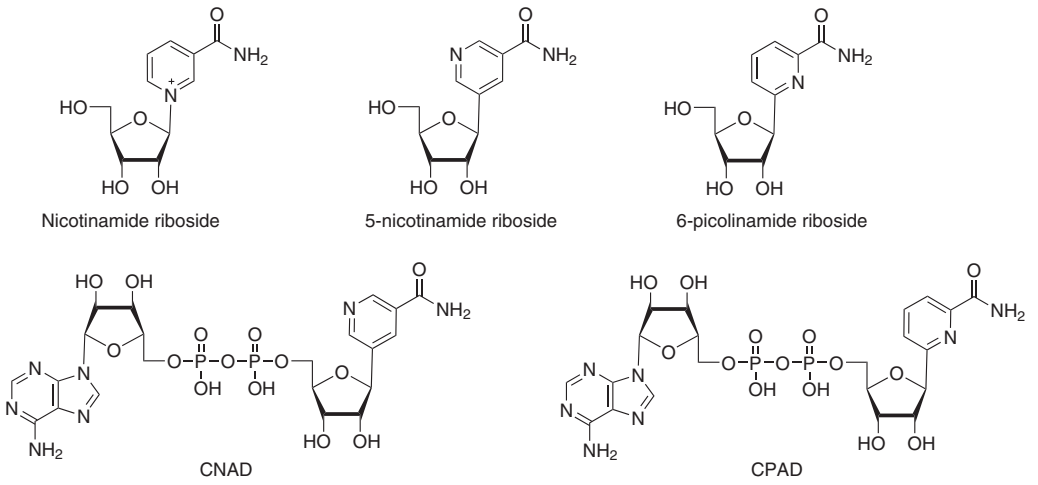

Figure 3 Structures of nicotinamide riboside and its C-nucleoside isosters, as well as NAD analogues – C-NAD and C-PAD.

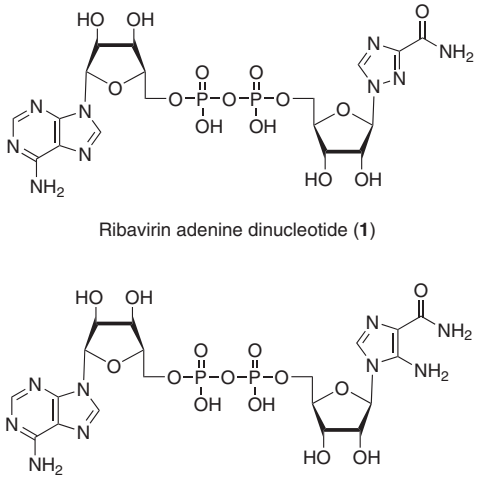

Figure 4 Structures of ribavirin and AICA adenine dinucleotides **1** and **2**.

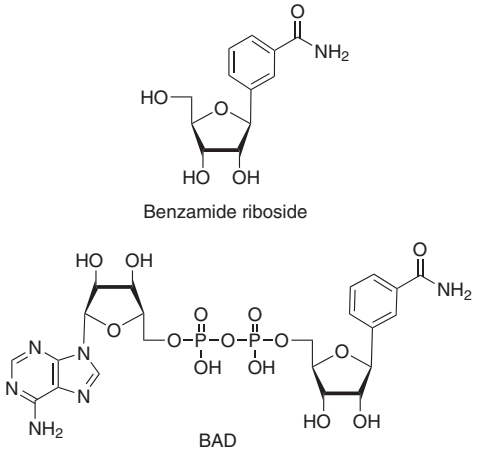

Figure 5 Structures of benzamide riboside and BAD.

metabolic activation. Grifantini and coworkers [33–35] prepared numerous analogues of tiazofurin, which followed this pathway as well, and in recent reviews, tiazofurin and some of its analogues containing heterocyclic sugar analogues were reported [36, 37]. All the above NAD analogues cannot be considered as potential drugs because, as pyrophosphates, they are not able to cross the cell membrane and do not enter the cell. Even when they were synthesized in the cell, they were metabolically unstable being cleaved by cellular phosphodiesterases. However, when their pyrophosphate oxygen is replaced by a carbon atom, these compounds become bis(phosphonate)s, known to enter cells and be resistant to phosphodiesterase cleavage. Thus, some nucleosides that were inactive at the nucleoside level, when chemically converted into NAD analogues, might show interesting biological activities. Consequently, synthetic bis(phosphonate) NAD analogues independent of metabolic activation and resistant to the action of cellular phosphodiesterases were expected to show improved therapeutic potential.

Mycophenolic adenine dinucleotides (MAD) analogues

Mycophenolic acid (MPA), a potent and selective inhibitor of IMPDH, is approved as an immunosuppressant [38]. Numerous attempts to use MPA as an anticancer agent failed, mainly due to its unfavorable metabolic profile. In the human body, MPA is extensively glucuronidated and removed in urine (a dose of 2–3 g a day is needed to maintain the therapeutic level of the drug) [39]. MPA binds to IMPDH at the nicotinamide mononucleotide

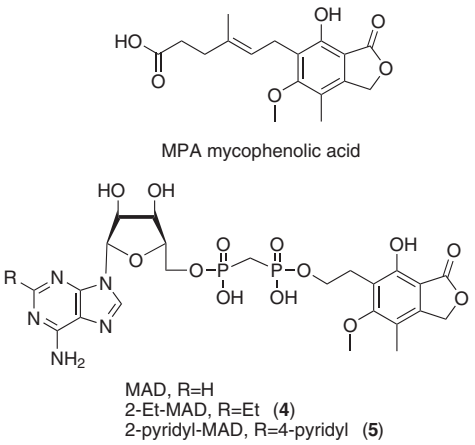

Figure 6 Structures of MPA and bis(phosphonate) MAD analogues **4** and **5**.

subdomain (N subsite) of the cofactor (NAD)-binding pocket. The adenosine subdomain (A subsite) is empty. The attachment of adenosine to the MPA moiety through bis(phosphonate) linker afforded the corresponding MAD derivatives (Figure 6). These compounds were able to enter cells, showed nanomolar inhibition of the enzyme, and, most importantly, were resistant to glucuronidation [40]. Thus, they severely inhibit proliferation of several cancer cell lines with IC_{50}s in low micromolar or even sub-micromolar range.

In general, the NAD(P)-binding domain is modular in nature [41–43]. In addition to N and A subsites, it also contains the pyrophosphate-binding subsite (P subsite or P-groove). Each subsite plays an important role in securing proper and tight binding of NAD-like inhibitors; however, each has its own requirements.

The N subsite is the most restricted, and we used tiazofurin, benzamide riboside, or MPA as the replacement for NMN. These compounds played a role of an anchor, which holds well only at the N subdomain of the cofactor-binding pocket of human IMPDH (Figure 7).

The A-subsite region of the NAD-binding domain of NAD-dependent enzymes is the most diversified. Even isoforms of IMPDH show differences. In IMPDH2, the adenine ring of NAD is sandwiched between the side chains of His 253 and Phe 282. Thr 45 is in close contact with the C2 and N3 of the adenine ring. In IMPDH1, these amino acids are replaced by Arg 253, Tyr 282, and Ile 45 [44]. Modification of the adenine system at C2 was therefore an attractive idea. Indeed, the substitution of TAD (K_i=110 nM) with an ethyl group afforded a 2-ethyl analogue **3** (K_i=1 nM), which proved to be 100-fold more potent against IMPDH1 than TAD (K_i=110 nM). Insertion of other substituents (such as iodo, phenyl, amino, aminophenyl, ethylene) at the C2 of adenine afforded generally more potent compounds, but no significant selectivity against IMPDH1 or IMPDH2 was observed.

The corresponding bis(phosphonate) MAD analogues substituted at the adenine moiety were then synthesized. The 2-ethyl analogue **4** was a little less potent against IMPDH1 (K_i=16 nM) and IMPDH2 (K_i=38 nM) but a new

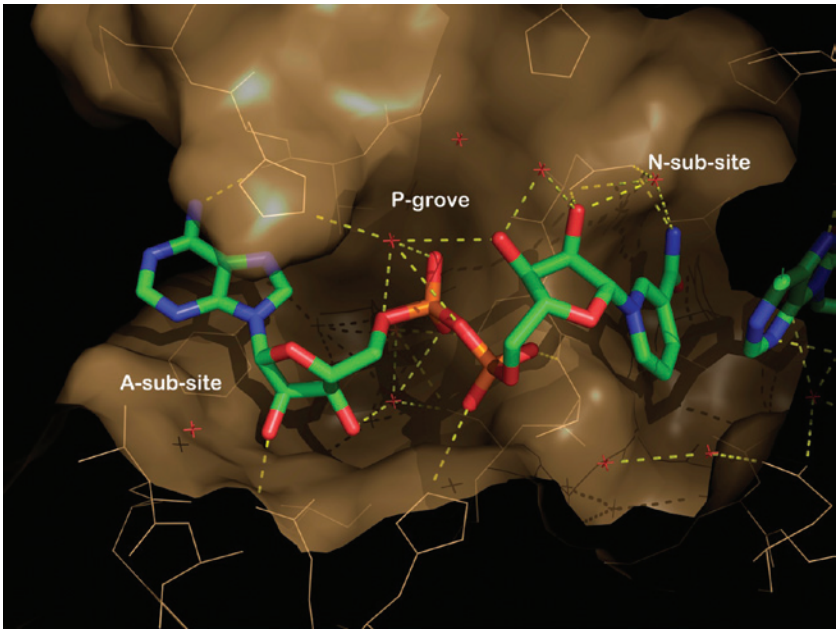

Figure 7 The modular nature of the cofactor-binding domain of NAD-dependent IMPDH (PDB entry 1NFb).

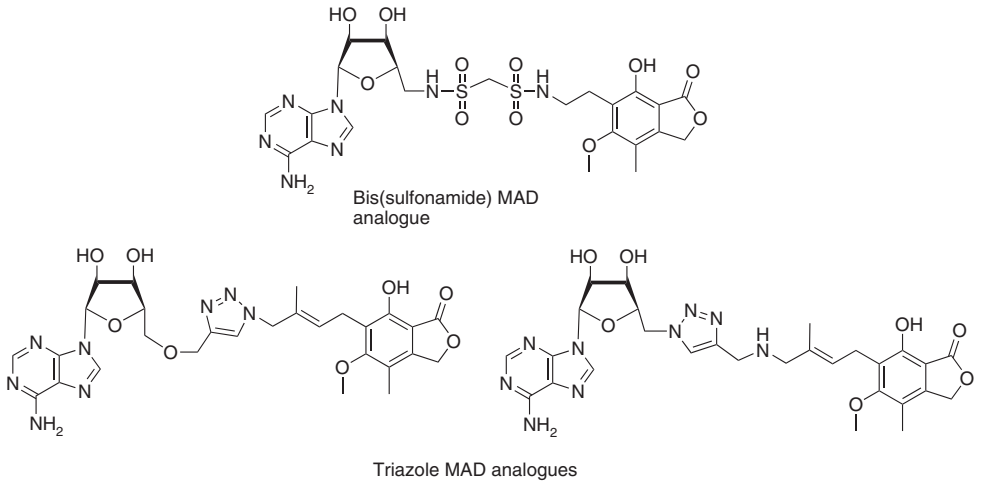

Figure 8 Structures of bis(sulfonamide) and triazole mycophenolic adenosine analogues.

4-pyridyl derivative **5** showed sub-nanomolar inhibition of the type 1 isoform (K_i=0.5 nM) with K_i=14 nM against IMPDH2. As expected, these compounds inhibited the proliferation of K562 cells of CML (**4**, IC_{50}=1 μM, and **5**, IC_{50}=0.4 μM). They were as potent as the tyrosine kinase inhibitor gleevec (imanitib, IC_{50}=0.6 μM), heralded as a 'magic bullet' against CML. It is likely that these two MAD analogues **4** and **5**, due to a different mechanism of action, might show synergistic effect with imanitib (or with second-generation drugs, i.e. dasatinib and nilotinib) [45].

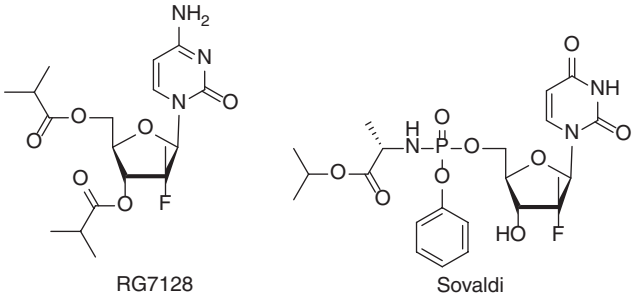

Figure 9 Structures of RG7128 (isobutyric prodrug of PSI 6130) and phosphoroamidate prodrug Sovaldi.

Mycophenolic adenosine analogues without dinucleotide connection

The P-groove subdomain is a large, shallow cavity where interactions of the enzyme with the phosphate groups of the cofactor analogue are weak, maintained mainly by water molecules (represented by red stars in Figure 7). It is likely, therefore, that modification of the natural pyrophosphate linkage or even replacement of its phosphorus atoms may be well tolerated. In fact, we synthesized a variety of NAD analogues in which the mycophenolic and adenosine moieties were connected by linkers of a different length and chemical character. Bis(sulfonamide) or triazole analogues (Figure 8) illustrate well such limitless possibilities.

No matter how drastic the modification – all non-dinucleotide analogues were found to be potent nanomolar inhibitors of IMPDH (range=34–167 nM for the isoform type 2). Unexpectedly, however, when the bis(phosphonate) group was replaced and the negative charge removed, all of the neutral analogues lost their ability to inhibit proliferation of cancer cell lines. It is possible that these neutral nicotinamide adenine analogues are not able to enter the cell.

Numerous carboxylic acids were used for esterification of poorly absorbed nucleosides to make prodrugs that helped transfer polar nucleosides across the cell membrane. A well-known example is isobutyric acid ester of 2′-deoxy-2′fluoro-2′-C-methylcytidine (RG7128) [46], a predecessor of sofosubuvir (sovaldi), the blockbuster anti-hepatitis C drug (Figure 9).

We reversed this idea and used a nucleoside (adenosine) to facilitate the transport of MPA into the cell. We synthesized mycophenolic adenosine ester **6** and found it, as expected, to be a potent inhibitor of IMPDH1 (K_i=163 nM), IMPDH2 (K_i=102 nM), as well as sub-micromolar inhibitor (IC_{50}=0.5 μM) of K562 cell proliferation. This adenosine prodrug, in contrast to morpholinoethyl ester of MPA (MMF, FDA approved; Figure 10) was found to be resistant to glucuronidation.

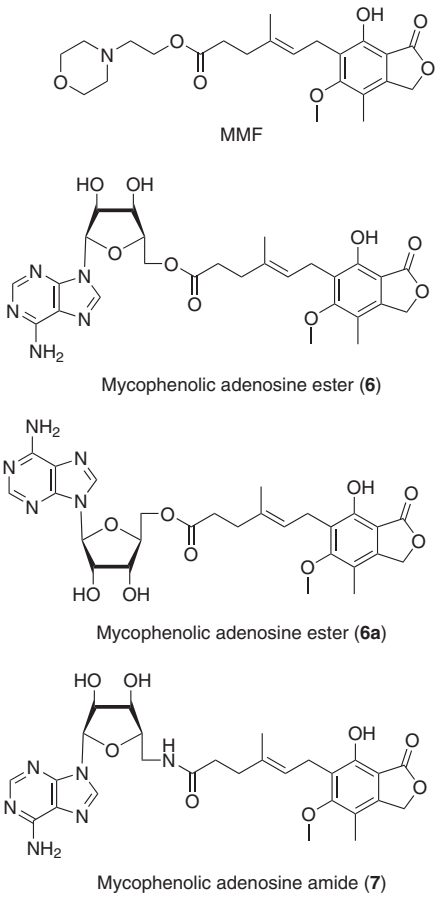

Figure 10 Structures of MMF and mycophenolic adenosine ester **6** and mycophenolic adenosine amide **7**.

In addition to its inhibitory activity against IMPDH, ester **6** is expected to serve as a depot form of MPA due to the action of cellular esterases. As cellular esterases work in the stereoselective manner, we also synthesized a mycophenolic adenosine ester **6a** containing L-adenosine. This isomer was found to be only 2-fold less potent (IMPDH2 K_i=0.23 μM) than its natural counterpart and approximately a 10-fold weaker inhibitor of the type 1 isoform (IMPDH1 K_i=2.5 μM). The stability of L- and D-isomers in human plasma was dramatically different. Surprisingly, unnatural L-isomer **6a** was hydrolyzed by esterases much faster (half-life $t_{1/2}$=~1 h) than natural D-isomer **6** ($t_{1/2}$=~24 h). Next we prepared the amide derivative **7**, which showed even more potent inhibition of IMPDH2 (K_i=84 nM) and, as expected, was neither glucuronidated nor hydrolyzed. However, it showed no activity *in vitro* against K562 cell proliferation.

To improve the metabolic stability of MPA-adenosine esters, compounds with more stable linkers could be prepared. Insertion of an amino acid between MPA moiety and adenosine would lead to new analogues with improved metabolic profile [47]. Earlier, we and others [48, 49] attached methyl esters of a number of amino acids to MPA, among them L- and D-valine methyl esters. Both compounds were found to be moderate inhibitors of IMPDH2 (K_i~1 μM). The hydrolysis of the methyl ester group and the attachment of 5′-amino adenosine afforded two new mycophenolic-valine-adenosine diamides **8** and **8a**, which showed a dramatic improvement of inhibitory activity against IMPDH (Figure 11).

The compound **8** containing D-valine inhibited IMPDH1 (K_i=3 nM) 30-fold more potently than IMPDH2 (K_i=88 nM). This example illustrates well the ability of the P-groove for accommodation of much larger structural elements then the natural pyrophosphate group. Actually, compounds **8** and **8a**, as well as most of other mycophenolic-amino acid-adenosine diamides (not discussed herein), showed potent enzymatic activity but no activity at the cellular level. Thus, our search for metabolically suitable linkers continues.

Back to the future

Recently, ribavirin has been reported as a promising drug against cancer, including breast, colon, lung, and AML [50–53]. The compound entered numerous clinical studies against variety of cancers and is still being evaluated in the clinic [54]. It was clearly established that the antitumor activity of ribavirin is related to the inhibition of an oncogene, the eukaryotic translation initiation factor eIF4E [55–57]. Interestingly, ribavirin has also anticancer

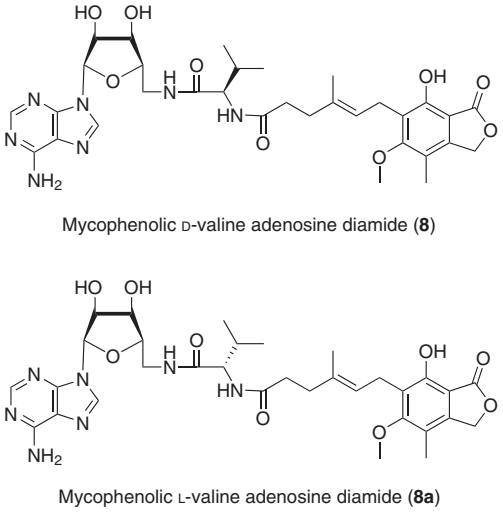

Figure 11 Mycophenolic-(D)- and (L)-valine-adenosine diamides **8** and **8a**.

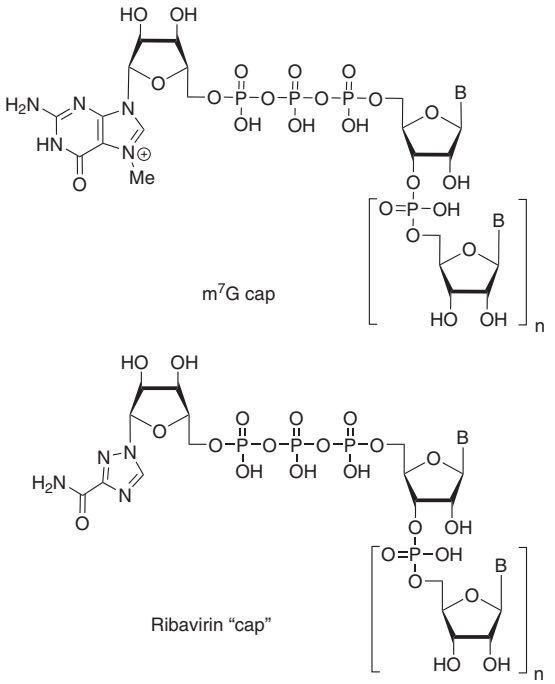

Figure 12 The m^{7}G cap and ribavirin 'cap' structures.

potential because its 5′-monophosphate acts as a mimic of IMP and inhibits IMPDH [58, 59] often overexpressed in cancer cells [60, 61]. Synergistic inhibition of cancer cell proliferation by ribavirin and tiazofurin has been reported [62]. Ribavirin 5′-triphosphate is structurally similar to the m^{7}G 'cap' structure (Figure 12), and its cap-related activity affecting translation of RNA has been studied and reported (also in relation to cancer) [63–65].

Finally, it was found that ribavirin inhibits histone methyl transferase, and it was declared as 'a tri-targeted antitumor repositioned drug' [66]. Consequently, it is possible that ribavirin effectiveness in patients with AML is because of the combination of its multitarget activity.

It is interesting to note that in the recent phase II clinical trial, the problem of resistance to ribavirin emerged. It was found that it is caused by an inducible glucuronidation, possibly at the amido group of the drug [67]. It has now been proposed to use ribavirin in combination with inhibitors of glucuronyl transferases.

In conclusion, both ribavirin and NAD analogues (distant relatives of ribavirin) are still under investigation as potential anticancer drugs. It remains to be seen if these compounds would emerge in the near future as agents potent and safe enough to be introduced to the clinic.

Acknowledgments: We appreciate the financial support of the Center for Drug Design, University of Minnesota.

References

[1] Witkowski, J. R.; Robins, R. K.; Sidwell, R. W.; Simon, L. N. Design, synthesis, and broad spectrum antiviral activity if 1-β-D-ribofuranosyl-1,2,4-triazole-3′-carboxamide and related nucleosides. *J. Med. Chem.* **1972**, *15*, 1150–1152.

[2] Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T. Broad-spectrum antiviral activity of Virazole: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* **1972**, *177*, 705–706.

[3] Crotty, S.; Cameron, C. E.; Andino, R. RNA virus error catastrophe: direct molecular test by using ribavirin. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 6895–6900.

[4] Graci, J. D.; Cameron, C. E. Mechanisms of action of ribavirin against distinct viruses. *Rev. Med. Virol.* **2006**, *16*, 37–48.

[5] Srivastava, P. C.; Pickering, M. V.; Allen, L. B.; Streeter, D. G.; Campbell, M. T.; Witkowski, J. T.; Sidwell, R. W.; Robins, R. K. Synthesis and antiviral activity of certain thiazole C-nucleosides. *J. Med. Chem.* **1977**, *20*, 256–262.

[6] Srivastava, P. C.; Revankar, G. R.; Robins, R. K. Synthesis and biological activity of nucleosides and nucleotides related to the antitumor agent 2-β-D-ribofuranosylthiazole-4-carboxamide. *J. Med. Chem.* **1984**, *27*, 266–269.

[7] Lui, M. S.; Faderan, M. A.; Liepnieks, J. J.; Natsumeda, Y.; Olah, E.; Jayaram, H. N.; Weber, G. Modulation of IMP dehydrogenase activity and guanylate metabolism by tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide). *J.* Biol.Chem. **1984**, *259*, 5078–5082.

[8] Gebeyehu, G.; Marquez, V. E.; Kelley, J. A.; Cooney, D. A.; Jayaram, H. N.; Johns, D. G. Synthesis of thiazole-4-carboxamide adenine dinucleotide. A powerful inhibitor of IMP dehydrogenase. *J. Med. Chem.* **1983**, *26*, 922–925.

[9] Jackson, R. C.; Weber, G.; Morris, H. P. IMP dehydrogenase, an enzyme linked with proliferation and malignancy. *Nature* **1975**, *256*, 331–333.

[10] Cooney, D. A.; Jayaram, H. N.; Glazer, R. I.; Kelley, J. A.; Marquez, V. E.; Gebeyehu, G.; Van Cott, A. C.; Zwelling, L. A.; Johns, D. G. Studies on the mechanism of action of tiazofurin metabolism to an analog of NAD with potent IMP dehydrogenase-inhibitory activity. *Adv. Enzyme Regul.* **1983**, *21*, 271–303.

[11] Cooney, D. A.; Jayaram, H. N.; Gebeyehu, G.; Betts, C. R.; Kelley, J. A.; Marquez, V. E.; Johns, D. G. The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase-inhibitory properties. *Biochem. Pharmacol.* **1982**, *31*, 2133–2136.

[12] Natsumeda, Y.; Ohno, S.; Kawasaki, H.; Konno, Y.; Weber, G.; Suzuki, K. Two distinct cDNAs for human IMP dehydrogenase. *J. Biol. Chem.* **1990**, *265*, 5292–5295.

[13] Natsumeda, Y.; Carr, S. F. Human type I and II IMP dehydrogenases as drug targets. *Ann. NY Acad. Sci.* **1993**, *696*, 88–93.

[14] Weber, G.; Yamaji, Y.; Olah, E.; Natsumeda, Y.; Jayaram, H. N.; Lapis, E.; Zhen, W. N.; Prajda, N.; Hoffman, R.; Tricot, G. J. Clinical and molecular impact of inhibition of IMP dehydrogenase activity by tiazofurin. *Adv. Enzyme Regul.* **1989**, *28*, 335–356.

[15] Weber, G.; Nagai, M.; Natsumeda, Y.; Eble, J. N.; Jayaram, H. N.; Paulik, E.; Zhen, W. N.; Hoffman, R.; Tricot, G. Tiazofurin down-regulates expression of c-Ki-ras oncogene in a leukemic patient. *Cancer Commun.* **1991**, *3*, 61–66.

[16] Tricot, G.; Weber, G. Biochemically targeted therapy of refractory leukemia and myeloid blast crisis of chronic granulocytic leukemia with Tiazofurin, a selective blocker of inosine 5′-phosphate dehydrogenase activity. *Anticancer Res.* **1996**, *16*, 3341–3347.

[17] Jayaram, H. N.; Lapis, E.; Tricot, G.; Kneebone, P.; Paulik, E.; Zhen, W.; Engeler, G. P.; Hoffman, R.; Weber, G. Clinical pharmacokinetic study of tiazofurin administered as a 1-hour infusion. *Int. J. Cancer* **1992**, *51*, 182–188.

[18] Takebe, N.; Cheng, X.; Wu, S.; Bauer, K.; Goloubeva, O. G.; Fenton, R. G.; Heyman, M.; Rapoport, A. P.; Badros, A.; Shaughnessy, J.; et al. Phase I clinical trial of the inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (cellcept) in advanced multiple myeloma patients. *Clin. Cancer Res.* **2004**, *10*, 8301–8308.

[19] Takebe, N.; Cheng, X.; Fandy, T. E.; Srivastava, R. K.; Wu, S.; Shankar, S.; Bauer, K.; Shaughnessy, J.; Tricot, G. IMP dehydrogenase inhibitor mycophenolate mofetil induces caspase-dependent apoptosis and cell cycle inhibition in multiple myeloma cells. *Mol. Cancer Ther.* **2006**, *5*, 457–466.

[20] Grifantini, M. Tiazofurine ICN Pharmaceuticals. *Curr. Opin. Invest. Drugs* **2000**, *1*, 257–262.

[21] Jayaram, H. N. Biochemical mechanisms of resistance to tiazofurin. *Adv. Enzyme Regul.* **1985**, *24*, 67–89.

[22] Jayaram, H. N.; Cooney, D. A.; Glazer, R. I.; Dion, R. L.; Johns, D. G. Mechanism of resistance to the oncolytic C-nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC-286193). *Biochem. Pharmacol.* **1982**, *31*, 2557–2560.

[23] Burchenal, J. H.; Ciovacco, K.; Kalaher, K.; O'Toole, T.; Kiefner, R.; Dowling, M. D.; Chu, C. K.; Watanabe, K. A.; Wempen, I.; Fox, J. J. Antileukemic effects of pseudoisocytidine, a new synthetic pyrimidine C-nucleoside. *Cancer Res.* **1976**, *36*, 1520–1523.

[24] Woodcock, T. M.; Chou, T. C.; Tan, C. T.; Sternberg, S. S.; Philips, F. S.; Young, C. W.; Burchenal, J. H. Biochemical, pharmacological, and phase I clinical evaluation of pseudoisocytidine. *Cancer Res.* **1980**, *40*, 4243–4249.

[25] Pankiewicz, K. W.; Hirota, K.; Matsuda, A.; Watanabe, K. A. A synthesis of psi-cytidine. *Carbohydr. Res.* **1984**, *127*, 227–233.

[26] Kabat, M. M.; Pankiewicz, K. W.; Watanabe, K. A. Synthesis of 5-beta-D-ribofuranosylnicotinamide and its N-methyl derivative. The isosteric and isoelectronic analogues of nicotinamide nucleoside. *J. Med. Chem.* **1987**, *30*, 924–927.

[27] Pankiewicz, K. W.; Zeidler, J.; Ciszewski, L. A.; Bell, J. E.; Goldstein, B. M.; Jayaram, H. N.; Watanabe, K. A. Synthesis of isosteric analogues of nicotinamide adenine dinucleotide containing C-nucleotide of nicotinamide or picolinamide. *J. Med. Chem.* **1993**, *36*, 1855–1859.

[28] Li, H.; Hallows, W. H.; Punzi, J. S.; Marquez, V. E.; Carrell, H. L.; Pankiewicz, K. W.; Watanabe, K. A.; Goldstein, B. M. Crystallographic studies of two alcohol dehydrogenase-bound analogues of thiazole-4-carboxamide adenine dinucleotide (TAD), the active anabolite of the antitumor agent tiazofurin. *Biochemistry* **1994**, *33*, 23–32.

[29] Gebeyehu, G.; Marquez, V. E.; Van Cott, A.; Cooney, D. A.; Kelley, J. A.; Jayaram, H. N.; Ahluwalia, G. S.; Dion, R. L.; Wilson, Y. A.; Johns, D. G. Ribavirin, tiazofurin, and selenazofurin: mononucleotides and nicotinamide adenine dinucleotide analogues. Synthesis, structure, and interactions with IMP dehydrogenase. *J. Med. Chem.* **1985**, *28*, 99–105.

[30] Krohn, K.; Heins, H.; Wielckens, K. Synthesis and cytotoxic activity of C-glycosidic nicotinamide riboside analogues. *J. Med. Chem.* **1992**, *35*, 511–517.

[31] Gharehbaghi, K.; Paull, K. D.; Kelley, J. A.; Barchi, J. J., Jr.; Marquez, V. E.; Cooney, D. A.; Monks, A.; Scudiero, D.; Krohn, K.; Jayaram, H. N. Cytotoxicity and characterization of an active metabolite of benzamide riboside, a novel inhibitor of IMP dehydrogenase. *Int. J. Cancer* **1994**, *56*, 892–899.

[32] Zatorski, A.; Watanabe, K. A.; Carr, S. F.; Goldstein, B. M.; Pankiewicz, K. W. Chemical synthesis of benzamide adenine dinucleotide: inhibition of inosine monophosphate dehydrogenase (types I and II). *J. Med. Chem.* **1996**, *39*, 2422–2426.

[33] Franchetti, P.; Cappellacci, L.; Perlini, P.; Jayaram, H. N.; Butler, A.; Schneider, B. P.; Collart, F. R.; Huberman, E.; Grifantini, M. Isosteric analogues of nicotinamide adenine dinucleotide derived from furanfurin, thiophenfurin, and selenophenfurin as mammalian inosine monophosphate dehydrogenase (type I and II) inhibitors. *J. Med. Chem.* **1998**, *41*, 1702–1707.

[34] Franchetti, P.; Grifantini, M. Nucleoside and non-nucleoside IMP dehydrogenase inhibitors as antitumor and antiviral agents. *Curr. Med. Chem.* **1999**, *6*, 599–614.

[35] Franchetti, P.; Marchetti, S.; Cappellacci, L.; Yalowitz, J. A.; Jayaram, H. N.; Goldstein, B. M.; Grifantini, M. A new C-nucleoside analogue of tiazofurin: synthesis and biological evaluation of 2-beta-D-ribofuranosylimidazole-4-carboxamide (imidazofurin). *Bioorg. Med. Chem. Lett.* **2001**, *11*, 67–69.

[36] Merino, P.; Ghirardello, M.; Tejero, T.; Delso, I.; Matute, R. Recent advances on the enantioselective synthesis of C-nucleosides inhibitors of inosine monophosphate dehydrogenase (IMPDH). *Curr. Top. Med. Chem.* **2014**, *14*, 1212–1224.

[37] Romeo, G.; Chiacchio, U.; Corsaro, A.; Merino, P. Chemical synthesis of heterocyclic-sugar nucleoside analogues. *Chem. Rev.* **2010**, *110*, 3337–3370.

[38] Bentley, R. Mycophenolic acid: a one hundred year odyssey from antibiotic to immunosuppressant. *Chem. Rev.* **2000**, *100*, 3801–3826.

[39] Franklin, T. J.; Jacobs, V.; Jones, G.; Ple, P.; Bruneau, P. Glucuronidation associated with intrinsic resistance to mycophenolic acid in human colorectal carcinoma cells. *Cancer Res.* **1996**, *56*, 984–987.

[40] Lesiak, K.; Watanabe, K. A.; Majumdar, A.; Powell, J.; Seidman, M.; Vanderveen, K.; Goldstein, B. M.; Pankiewicz, K. W. Synthesis of a methylenebis(phosphonate) analogue of mycophenolic adenine dinucleotide: a glucuronidation-resistant MAD analogue of NAD. *J. Med. Chem.* **1998**, *41*, 618–622.

[41] Felczak, K.; Chen, L.; Wilson, D.; Williams, J.; Vince, R.; Petrelli, R.; Jayaram, H. N.; Kusumanchi, P.; Kumar, M.; Pankiewicz, K. W. Cofactor-type inhibitors of inosine monophosphate dehydrogenase via modular approach: targeting the pyrophosphate binding sub-domain. *Bioorg. Med. Chem.* **2011**, *19*, 1594–1605.

[42] Felczak, K.; Pankiewicz, K. W. Rehab of NAD(P)-dependent enzymes with NAD(P)-based inhibitors. *Curr. Med. Chem.* **2011**, *18*, 1891–1908.

[43] Petrelli, R.; Vita, P.; Torquati, I.; Felczak, K.; Wilson, D. J.; Franchetti, P.; Cappellacci, L. Novel inhibitors of inosine monophosphate dehydrogenase in patent literature of the last decade. *Recent Pat. Anticancer Drug Discov.* **2013**, *8*, 103–125.

[44] Colby, T. D.; Vanderveen, K.; Strickler, M. D.; Markham, G. D.; Goldstein, B. M. Crystal structure of human type II inosine monophosphate dehydrogenase: implications for ligand binding and drug design. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3531–3536.

[45] Drullion, C.; Lagarde, V.; Gioia, R.; Legembre, P.; Priault, M.; Cardinaud, B.; Lippert, E.; Mahon, F. X.; Pasquet, J. M. Mycophenolic acid overcomes imatinib and nilotinib resistance of chronic myeloid leukemia cells by apoptosis or a senescent-like cell cycle arrest. *Leukemia Res. Treat.* **2012**, *2012*, 861301.

[46] Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; et al. Design, synthesis, and antiviral activity of 2′-deoxy-2′-fluoro-2′-C-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005**, *48*, 5504–5508.

[47] Lee, W. A.; Gu, L.; Miksztal, A. R.; Chu, N.; Leung, K.; Nelson, P. H. Bioavailability improvement of mycophenolic acid through amino ester derivatization. *Pharm. Res.* **1990**, *7*, 161–166.

[48] Iwaszkiewicz-Grzes, D.; Cholewinski, G.; Kot-Wasik, A.; Trzonkowski, P.; Dzierzbicka, K. Synthesis and biological activity of mycophenolic acid-amino acid derivatives. *Eur. J. Med. Chem.* **2013**, *69C*, 863–871.

[49] Felczak, K.; Vince, R.; Pankiewicz, K. W. NAD-based inhibitors with anticancer potential. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 332–336.

[50] Borden, K. L.; Culjkovic-Kraljacic, B. Ribavirin as an anticancer therapy: acute myeloid leukemia and beyond? *Leuk. Lymphoma* **2010**, *51*, 1805–1815.

[51] Assouline, S.; Culjkovic-Kraljacic, B.; Bergeron, J.; Caplan, S.; Cocolakis, E.; Lambert, C.; Lau, C. J.; Zahreddine, H. A.; Miller, W. H., Jr.; Borden, K. L. A phase I trial of ribavirin and low-dose cytarabine for the treatment of relapsed and refractory acute myeloid leukemia with elevated eIF4E. *Haematologica* **2015**, *100*, e7–9.

[52] Walsh, D.; Meleady, P.; Power, B.; Morley, S. J.; Clynes, M. Increased levels of the translation initiation factor eIF4E in differentiating epithelial lung tumor cell lines. *Differentiation* **2003**, *71*, 126–134.

[53] Pettersson, F.; Del Rincon, S. V.; Emond, A.; Huor, B.; Ngan, E.; Ng, J.; Dobocan, M. C.; Siegel, P. M.; Miller, W. H., Jr. Genetic and pharmacologic inhibition of eIF4E reduces breast cancer cell migration, invasion, and metastasis. *Cancer Res.* **2015**, *75*, 1102–1112.

[54] Assouline, S.; Culjkovic, B.; Cocolakis, E.; Rousseau, C.; Beslu, N.; Amri, A.; Caplan, S.; Leber, B.; Roy, D. C.; Miller, W. H., Jr.; et al. Molecular targeting of the oncogene eIF4E in acute myeloid leukemia (AML): a proof-of-principle clinical trial with ribavirin. *Blood* **2009**, *114*, 257–260.

[55] Pelletier, J.; Graff, J.; Ruggero, D.; Sonenberg, N. Targeting the eIF4F translation initiation complex: a critical nexus for cancer development. *Cancer Res.* **2015**, *75*, 250–263.

[56] Osborne, M. J.; Borden, K. L. The eukaryotic translation initiation factor eIF4E in the nucleus: taking the road less traveled. *Immunol.Rev.* **2015**, *263*, 210–223.

[57] Culjkovic, B.; Borden, K. L. Understanding and targeting the eukaryotic translation initiation factor eIF4E in head and neck cancer. *J. Oncol.* **2009**, *2009*, 981679.

[58] Hedstrom, L. IMP dehydrogenase: mechanism of action and inhibition. *Curr. Med. Chem.* **1999**, *6*, 545–560.

[59] Hedstrom, L. IMP dehydrogenase: structure, mechanism, and inhibition. *Chem.Rev.* **2009**, *109*, 2903–2928.

[60] Kokeny, S.; Papp, J.; Weber, G.; Vaszko, T.; Carmona-Saez, P.; Olah, E. Ribavirin acts via multiple pathways in inhibition of leukemic cell proliferation. *Anticancer Res.* **2009**, *29*, 1971–1980.

[61] Chen, L.; Pankiewicz, K. W. Recent development of IMP dehydrogenase inhibitors for the treatment of cancer. *Curr. Opin. Drug Discov. Dev.* **2007**, *10*, 403–412.

[62] Natsumeda, Y.; Yamada, Y.; Yamaji, Y.; Weber, G. Synergistic cytotoxic effect of tiazofurin and ribavirin in hepatoma cells. *Biochem. Biophys. Res. Commun.* **1988**, *153*, 321–327.

[63] Issur, M.; Bougie, I.; Despins, S.; Bisaillon, M. Enzymatic synthesis of RNAs capped with nucleotide analogues reveals the molecular basis for substrate selectivity of RNA capping enzyme: impacts on RNA metabolism. *PLoS One* **2013**, *8*, e75310.

[64] Volpon, L.; Osborne, M. J.; Zahreddine, H.; Romeo, A. A.; Borden, K. L. Conformational changes induced in the eukaryotic translation initiation factor eIF4E by a clinically relevant inhibitor, ribavirin triphosphate. *Biochem. Biophys. Res. Commun.* **2013**, *434*, 614–619.

[65] Kentsis, A.; Topisirovic, I.; Culjkovic, B.; Shao, L.; Borden, K. L. Ribavirin suppresses eIF4E-mediated oncogenic transformation by physical mimicry of the 7-methyl guanosine mRNA cap. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 18105–18110.

[66] De la Cruz-Hernandez, E.; Medina-Franco, J. L.; Trujillo, J.; Chavez-Blanco, A.; Dominguez-Gomez, G.; Perez-Cardenas, E.; Gonzalez-Fierro, A.; Taja-Chayeb, L.; Duenas-Gonzalez, A. Ribavirin as a tri-targeted antitumor repositioned drug. *Oncol. Rep.* **2015**, *33*, 2384–2392.

[67] Zahreddine, H. A.; Culjkovic-Kraljacic, B.; Assouline, S.; Gendron, P.; Romeo, A. A.; Morris, S. J.; Cormack, G.; Jaquith, J. B.; Cerchietti, L.; Cocolakis, E.; et al. The sonic hedgehog factor GLI1 imparts drug resistance through inducible glucuronidation. *Nature* **2014**, *511*, 90–93.