Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 71 (2006) 122–126
Antiviral Research
www.elsevier.com/locate/antiviral

Mini-review

# Ribonucleotide reductase inhibitors as anti-herpes agents

Stanislaw F. Wnuk [a], Morris J. Robins [b,*]

[a] *Department of Chemistry, Florida International University, University Park, Miami, FL 33199, United States*
[b] *Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602-5700, United States*

Received 8 February 2006; accepted 9 March 2006

Dedicated to Professor Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

**Abstract**

Ribonucleotide reductases (RNRs) supply the 2′-deoxyribonucleotide building blocks for DNA synthesis in mammalian cells and for herpes viruses. The viral-encoded RNRs have unique protein sequences that differ from mammalian enzyme primary structures. Selective inhibition of a viral RNR might provide an approach to new anti-herpes agents with minimal effects on the mammalian host RNRs. This review summarizes efforts to develop anti-herpes agents that selectively target viral-encoded RNRs.


*Keywords:* Ribonucleotide reductases (RNRs); Heterocyclic thiosemicarbazones; Oligopeptide/peptoid binding to RNR-R2 C-terminal sequences

## Contents

1. Introduction ..... 122
2. Heterocyclic thiosemicarbazone derivatives and acyclic phosphonylmethyl ethers ..... 123
3. Specific oligopeptide/peptoid binding with RNR-R2 C-terminal sequences ..... 123
4. Conclusions and future prospects ..... 125
Acknowledgments ..... 125
References ..... 125

## 1. Introduction

Antiviral drugs have been used for treatment of herpes simplex virus (HSV) infections for over 45 years (Field, 2001). Many anti-HSV agents are nucleoside/tide analogue prodrugs that inhibit viral DNA polymerases after in vivo conversion to the triphosphate level. Most HSV isolates are sensitive to acyclovir (Fig. 1), which is used extensively in clinical medicine. The herpes-encoded thymidine kinase phosphorylates acyclovir to its monophosphate. Cellular nucleotide kinases then convert this intermediate prodrug to the di- and triphosphate, which bind and inhibit HSV DNA polymerases quite selectively. Ribonucleotide reductases (RNRs) provide the only de novo route to the 2′-deoxynucleotide building blocks for DNA synthesis employed by mammalian host cells and for HSV genomes. Ator et al. (1986) demonstrated that reduction of nucleoside 5′-diphosphates (NDPs) to their 2′-deoxy counterparts by HSV-RNR followed the same mechanistic pathway elucidated for mammalian and other class 1 RNRs, and that mechanism-based inhibitors that inactivated the bacterial and mammalian RNRs also exerted time-dependent inactivation of the RNR encoded by HSV-1. Gemcitabine (2′-deoxy-2′,2′-difluorocytidine) is a potent mechanism-based inhibitor of RNRs used clinically in anticancer applications (Robins, 2003). However, no small molecules – including nucleoside analogues – that function primarily as RNR inhibitors have shown sufficient specificity to warrant use as anti-HSV drugs. Isolation, subunit composition, and characterization (Jordan and Reichard, 1998); elucidation of mechanisms for substrate reduction and mechanism-based inhibition (Stubbe and van der Donk, 1998); and alternative

* Corresponding author. Tel.: +1 801 422 6272; fax: +1 801 422 1053.
*E-mail address:* morris_robins@byu.edu (M.J. Robins).

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.03.002

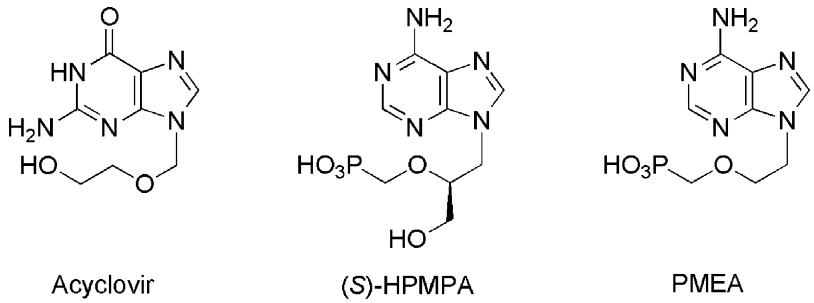



Fig. 1. Acyclovir and related acyclic inhibitors of HSV-encoded RNR.

mechanistic considerations and drug potential of inhibitors of class 1 RNRs (Robins, 2003) have been reviewed extensively. Briefly, class 1 RNRs are at least dimer–dimers with a double complement of components (α, α, β, β), and direct association of the R1 and R2 subunits is required for enzymatic activity.

## 2. Heterocyclic thiosemicarbazone derivatives and acyclic phosphonylmethyl ethers

Spector and Joens (1985) evaluated inhibitors of mammalian RNR for their activity against HSV-1 RNR. They found that 5-amino-1-formyl-4-methylisoquinoline thiosemicarbazone (MAIQ) (Fig. 2) caused significant inactivation of HSV-1 RNR, and concluded that the viral and mammalian enzymes had similar sensitivities to a wide variety of inhibitors. Substituted thiocarbonyl hydrazone derivatives of 2-acetylpyridine (A723U, A1110U, and 348U87) (Fig. 2) were found to be potent inactivators of RNRs encoded by HSV types 1 and 2 and also by varicella-zoster virus (Spector et al., 1985, 1989; Safrin et al., 1993). It was noteworthy that these agents inhibited the viral RNRs at lower doses than were required for the human enzyme, and that they potentiated the anti-herpetic activity of acyclovir markedly. Enlarged dGTP pools that resulted from treatment of infected cells with acyclovir were decreased by co-treatment with AU1110U, and concomitant major increases in the pool sizes of acyclovir triphosphate were noted. Inactivation of HSV-1 RNR with a maximum first order constant of 11–14 $h^{-1}$ at 1–2 μM of A1110U was observed (Spector et al., 1989), and selective, synergistic inactivation of viral RNR relative to that of the mammalian enzyme was found with the iron complex of A1110U (Porter et al., 1990). Certain acyclic phosphonylmethyl ethers including HPMPA and PMEA (Fig. 1) also were found to inhibit the HSV-1 encoded RNR (Cerný et al., 1990), but they did not exhibit adequate selectivity relative to the mammalian enzyme for development as anti-herpes drugs.

## 3. Specific oligopeptide/peptoid binding with RNR-R2 C-terminal sequences

Although limited selectivity for inhibition of viral RNRs (relative to mammalian enzymes) has been observed with small molecules, structure versus antiviral activity selectivity trends are lacking. Cohen et al. (1986) and Dutia et al. (1986) independently reported inhibition of HSV-RNR by inclusion of peptide fragments corresponding to the C-terminal sequence of the viral enzyme subunit R2 in enzyme preparations containing both of the requisite R1 and R2 subunits. Thus, association of native R1 with a C-terminal R2 peptide fragment interfered with formation of active $(R1–R2)_2$ holoenzyme complexes. This resulted in specific inhibition of the viral RNR (relative to mammalian enzymes, which have different R2 C-terminal sequences). Addition of a tyrosine residue to the N-terminal glycine of the heptapeptide ($H_2N$-Gly-Ala-Val-Val-Asn-Asp-Leu-COOH) at the C-terminal of HSV-RNR-R2 gave an octapeptide YGAVVNDL that inhibited HSV-RNR (Dutia et al., 1986). Inclusion of an alanine residue between the N-terminal tyrosine and glycine residues of the octapeptide provided nonapeptide Y*A*GAVVNDL (Fig. 3), which was a more potent inhibitor (apparent $K_I = 15$ μM) than the Ala-deleted natural sequence octapeptide. The nonapeptide was noncompetitive with respect to the substrate CDP (Cohen et al., 1986; Dutia et al., 1986).

Gaudreau et al. (1987, 1990, 1992) studied short peptides related to YAGAVVNDL, and concluded that: (1) the 9-mer length is important because increasing the length to a 12- or 15-mer had little effect on the inhibitory potency, but shortening it to an 8-mer resulted in an order of magnitude loss of potency,

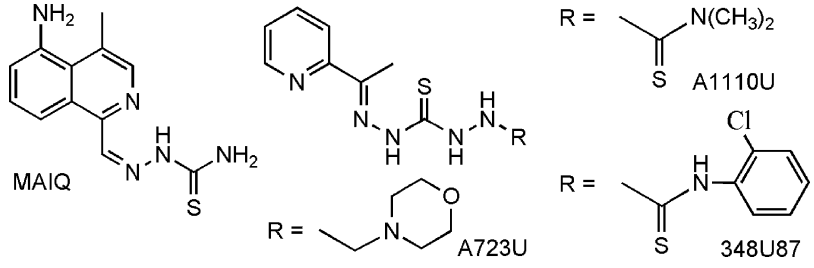
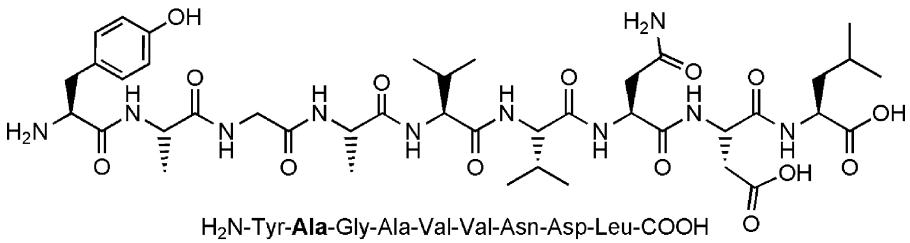
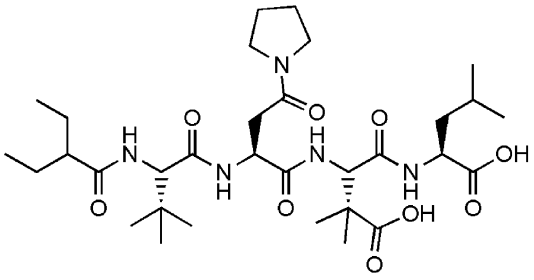



Fig. 2. Thiosemicarbazone derivatives that inhibit HSV-encoded RNR.

HSV-R2 C-Terminal Nonapeptide Y**A**GAVVNDL



$H_2N$-Tyr-**Ala**-Gly-Ala-Val-Val-Asn-Asp-Leu-COOH



An Early Analogue of the Pentapeptide VVNDL

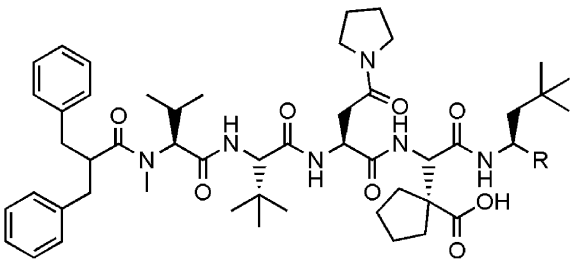


BILD 1257 R = $CO_2H$
BILD 1263 R = $CH_2OH$

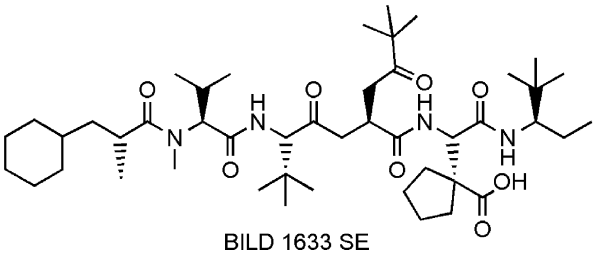


BILD 1633 SE

Fig. 3. Peptides and peptidomimetic inhibitors of HSV-RNR.

(2) abolition of the positive charge at the N-terminus by acetylation or removal of the amino group increased the binding affinity two- to three-fold, (3) an unmodified carboxyl group at the C-terminus is important for binding because replacement of the C-terminal Leu with its amide led to a four-fold decrease in potency, (4) the AGA fragment linked to the N-terminus of the peptide does not interact directly with the R1 subunit because its replacement with a 9-aminononanoic acid spacer [–$NH(CH_2)_8C(O)$–] had little effect on the inhibitory activity, (5) each of the VVNDL residues contributes significantly to binding with the R1 subunit with especially high specificity for the C-terminal Leu residue, and (6) the hydrophobic character of the N-terminus of the peptide is very important. Chang et al. (1992) confirmed that the five C-terminal residues were the most important regions for inhibitory potency because YVVNDL and YAGAVVNDL showed similarly potent inhibition of HSV-RNR. Structural modifications of the Tyr (e.g., $(Bn)_2CHCO$–VVNDL(γ-Me)), Asn, and Leu residues of YVVNDL produced peptides that were as much as two orders of magnitude more potent.

Moss et al. (1993) reported extended SAR studies of pentapeptide (VVNDL) analogues. Three orders of magnitude increases in potency ($IC_{50} = 0.18\ \mu M$) were achieved by modification of the N-terminal valine to a 2,2-diethylacetyl group, addition of a methyl group at the β-carbon of the penultimate valine, dialkylation of the asparagine nitrogen (i.e., replacement of $NH_2$ with pyrrolidine), and dimethylation of the β-carbon of the aspartic acid residue. Next, β-alkyl and β,β-dialkyl aspartic acid derivatives were studied, which led to the finding that introduction of a cyclopentyl ring on the aspartic acid residue resulted in a six-fold enhancement of binding to the R1 subunit (Moss et al., 1994, 1995). Methylation at the γ-carbon of leucine and the N-terminal amide nitrogen led to development of BILD 1257 (Fig. 3). Reduction of the carboxyl group of the C-terminal residue gave the hydroxymethyl analogue BILD 1263, which showed an increased cellular uptake with an accompanying magnitude increase in anti-HSV potency in cell culture. This was the first example of an RNR subunit-association inhibitor with "in vivo" antiviral activity. It suppressed replication of HSV-1, HSV-2, and acyclovir-resistant HSV strains in cell culture and strongly potentiated the antiviral activity of acyclovir (Liuzzi et al., 1994).

Further efforts were focused on improving activity against HSV in cell culture. Replacement of the C-terminal 2-(4-methylleucinol) side-chain in BILD 1263 with the more lipophilic 3-(2,2-dimethylpentyl) group led to the discovery of BILD 1357 (structure in: Moss et al., 1996b). Additional modifications included incorporation of: (1) a stereochemically defined 2,6-dimethylcyclohexylamino group at the N-terminus, (2) ketomethylene amide-bond isosters, and (3) a ureido group next to the N-terminus to produce BILD 1351 (Moss et al., 1996a,b). Lawetz and Liuzzi (1998) reported that BILD 1351 and 1357 were significantly more potent than BILD 1263. Duan et al. (1998) noted that the related BILD 1633 SE (Fig. 3), with a 2(*R*)-methyl-3-cyclohexylpropanoyl group at the N-terminus, was more potent than acyclovir against strains of wild-type HSV as well as against acyclovir-resistant mutants ($IC_{50} = 3$ nM, determined by a competitive binding assay). Efforts to extend such exciting results toward clinical development were frustrated by the finding that herpes viral replication is not linearly dependent on the HSV-encoded RNR. Thus, in contrast with selective inhibition of HSV-encoded polymerases, which prevents synthesis of the viral genome, selective inhibition of HSV-encoded RNRs does not stringently limit the pool of deoxynucleotides, which are available from cellular RNR activity.

Cooperman and co-workers have successfully employed the concept of blocking R1–R2 subunit-association with mammalian RNRs (Pender et al., 2001; Gao et al., 2005). They found that an N-terminal acetylated heptapeptide (AcFTLDADF) derivative of the mammalian R2 C-terminal sequence competed with R2 for binding with the mammalian R1 subunit, which inhibited the enzyme activity. Similar studies have been pursued with inhibition of R1–R2 subunit-association with peptide sequence analogues derived from RNRs of other species, and reviews are available (Cooperman, 2003; Coen and Schaffer, 2003; Loregian et al., 2002).

## 4. Conclusions and future prospects

Inhibition of herpes virus replication by sequence specific peptoid–protein interference with the normal protein–protein associations that produce active enzymes (i.e., interference with quaternary association of enzyme subunits) is clever and appealing. However, the finding that herpes viral replication is not linearly dependent on virus-encoded RNRs severely limits the potential for development of single-agent antiviral drugs based on this concept. Such specific peptoid inhibitors of RNR activities have joined the ranks of highly interesting bioresponse modifiers that are invaluable for elucidating detailed cellular processes. Indeed, Cooperman and co-workers have recently shown that dimer–dimer and heximer–dimer association of class 1 mammalian RNRs appear to be physiologically active clusters that produce 2′-deoxynucleoside 5′-diphosphates in mammalian cells (Gao et al., 2005). Subunit-association inhibitors of this type might find successful applications as anticancer agents.

## Acknowledgments

We thank NIH/NIGMS (S06GM08205) (S.F.W.) and pharmaceutical company unrestricted gift funds (M.J.R.) for support.

## References

Ator, M.A., Stubbe, J., Spector, T., 1986. Mechanism of ribonucleotide reductase from herpes simplex virus type 1. J. Biol. Chem. 261, 3595–3599.

Cerný, J., Votruba, I., Vonka, V., Rosenberg, I., Otmar, M., Holý, A., 1990. Phosphonylmethyl ethers of acyclic nucleoside analogues: inhibitors of HSV-1 induced ribonucleotide reductase. Antiviral Res. 13, 253–264.

Chang, L.L., Hannah, J., Ashton, W.T., Rasmusson, G.H., Ikeler, T.J., Patel, G.F., Garsky, V., Uncapher, C., Yamanaka, G., McClements, W.L., Tolman, R.T., 1992. Substituted penta- and hexapeptides as potent inhibitors of herpes simplex virus type 2 ribonucleotide reductase. Bioorg. Med. Chem. Lett. 2, 1207–1212.

Coen, D.M., Schaffer, P.A., 2003. Antiherpesvirus drugs: a promising spectrum of new drugs and drug targets. Nat. Rev. Drug Discov. 2, 278–288.

Cohen, E.A., Gaudreau, P., Brazeau, P., Langelier, Y., 1986. Specific inhibition of herpervirus ribonucleotide reductase by a nonapeptide derived from a carboxy terminus of subunit 2. Nature 321, 441–443.

Cooperman, B.S., 2003. Oligopeptide inhibition of class I ribonucleotide reductases. Biopolymers (Peptide Sci.) 71, 117–131.

Duan, J., Liuzzi, M., Paris, W., Lambert, M., Lawetz, C., Moss, N., Jaramillo, J., Gauthier, J., Deziel, R., Cordingley, M.G., 1998. Antiviral activity of a selective ribonucleotide reductase inhibitor against acyclovir-resistant herpes simplex virus type 1 in vivo. Antimicrob. Agents Chemother. 42, 1629–1635.

Dutia, B.M., Frame, M.C., Subak-Sharpe, J.H., Clark, W.N., Marsden, H.S., 1986. Specific inhibition of herpervirus ribonucleotide reductase by synthetic peptides. Nature 321, 439–441.

Field, H.J., 2001. Herpes simplex virus antiviral drug resistance—current trends and future prospects. J. Clin. Virol. 21, 261–269.

Gao, Y., Kashlan, O.B., Kaur, J., Tan, C., Cooperman, B.S., 2005. Mechanisms of action of peptide inhibitors of mammalian ribonucleotide reductase targeting quaternary structure. Biopolymers (Peptide Sci.) 80, 9–17.

Gaudreau, P., Michaud, J., Cohen, E.A., Langelier, Y., Brazeau, P., 1987. Structure–activity studies on synthetic peptides inhibiting herpes simplex virus ribonucleotide reductase. J. Biol. Chem. 261, 12413–12416.

Gaudreau, J., Paradis, H., Langelier, Y., Brazeau, P., 1990. Synthesis and inhibitory potency of peptides corresponding to the subunit 2 C-terminal

region of herpes virus ribonucleotide reductases. J. Med. Chem. 33, 723–730.

Gaudreau, J., Brazeau, P., Richer, M., Cormier, J., Langlois, D., Langelier, Y., 1992. Structure–function studies of peptides inhibiting the ribonucleotide reductase activity of herpes simplex virus type 1. J. Med. Chem. 35, 346–350.

Jordan, A., Reichard, P., 1998. Ribonucleotide reductases. Annu. Rev. Biochem. 67, 71–98.

Lawetz, C., Liuzzi, M., 1998. The antiviral activity of the ribonucleotide reductase inhibitor BILD 1351 SE in combination with acyclovir against HSV type-1 in cell culture. Antiviral Res., 35–46.

Liuzzi, M., Deziel, R., Moss, N., Beaulieu, P., Bonneau, A.-M., Bousquet, C., Chafouleas, J.G., Garneau, M., Jaramillo, J., Krogsrud, R.L., Lagacé, L., McCollum, R.S., Nawoot, S., Guindon, Y., 1994. A potent peptidomimetic inhibitor of HSV ribonucleotide reductase with antiviral activity in vivo. Nature 372, 695–698.

Loregian, A., Marsden, H.S., Palu, G., 2002. Protein–protein interactions as targets for antiviral chemotherapy. Rev. Med. Virol. 12, 239–262.

Moss, N., Deziel, R., Adams, J., Aubry, N., Bailey, M., Baillet, M., Beaulieu, P., DiMaio, J., Duceppe, J.-S., Ferland, J.-M., Gauthier, J., Ghiro, E., Goulet, S., Grenier, L., Lavallee, P., Lepine-Frenette, C., Plante, R., Rakhit, S., Soucy, F., Wernic, D., Guindon, Y., 1993. Inhibition of herpes simplex virus type 1 ribonucleotide reductase by substituted tetrapeptide derivatives. J. Med. Chem. 36, 3005–3009.

Moss, N., Déziel, R., Ferland, J.-M., Goulet, S., Jones, P.-J., Leonardo, S.F., Pitner, T.P., Plante, R., 1994. Herpes simplex virus ribonucleotide reductase subunit association inhibitors: the effect and conformation of β-alkylated aspartic acid derivatives. Bioorg. Med. Chem. 2, 959–970.

Moss, N., Beaulieu, P., Duceppe, J.-S., Ferland, J.-M., Gauthier, J., Ghiro, E., Goulet, S., Grenier, L., Llinas-Brunet, M., Plante, R., Wernic, D., Deziel, R., 1995. Peptidomimetic inhibitors of herpes simplex virus ribonucleotide reductase: a new class of antiviral agents. J. Med. Chem. 38, 3617–3623.

Moss, N., Beaulieu, P., Duceppe, J.-S., Ferland, J.-M., Gauthier, J., Ghiro, E., Goulet, S., Guse, I., Llinas-Brunet, M., Plante, R., Plamondon, L., Wernic, D., Déziel, R., 1996a. Ureido based peptidomimetic inhibitors of herpes simplex virus ribonucleotide reductase: an investigation of inhibitor bioactive conformation. J. Med. Chem. 39, 2178–2187.

Moss, N., Beaulieu, P., Duceppe, J.-S., Ferland, J.-M., Garneau, M., Gauthier, J., Ghiro, E., Goulet, S., Guse, I., Jaramillo, J., Llinas-Brunet, M., Malenfant, E., Plante, R., Poirier, M., Soucy, F., Wernic, D., Yoakim, C., Déziel, R., 1996b. Peptidomimetic inhibitors of herpes simplex virus ribonucleotide reductase with improved in vivo antiviral activity. J. Med. Chem. 39, 4173–4180.

Pender, B.A., Wu, X., Axelsen, P.H., Cooperman, B.A., 2001. Toward a rational design of peptide inhibitors of ribonucleotide reductase: structure–function and modeling studies. J. Med. Chem. 44, 36–46.

Porter, D.J., Harrington, J.A., Spector, T., 1990. Herpes simplex type 1 ribonucleotide reductase: selective and synergistic inactivation by A1110U and its iron complex. Biochem. Pharmacol. 39, 639–646.

Robins, M.J., 2003. Ribonucleotide reductases: radical chemistry and inhibition at the active site. Nucleosides Nucleotides Nucleic Acids 22, 519–534.

Safrin, S., Schacker, T., Delehanty, J., Hill, E., Corey, L., 1993. Topical treatment of infection with acyclovir-resistant mucocutaneous herpes simplex virus with the ribonucleotide reductase inhibitor 348U87 in combination with acyclovir. Antimicrob. Agents Chemother. 37, 975–979.

Spector, T., Joens, T.E., 1985. Herpes simplex type 1 ribonucleotide reductase. J. Biol. Chem. 260, 8694–8697.

Spector, T., Averett, D.R., Nelson, D.J., Lambe, C.U., Morrison Jr., R.W., St Clair, M.H., Furman, P.A., 1985. Potentiation of antiherpetic activity of acyclovir by ribonucleotide reductase inhibition. Proc. Natl. Acad. Sci. U.S.A. 82, 4254–4257.

Spector, T., Harrington, J.A., Morrison Jr., R.W., Lambe, C.U., Nelson, D.J., Averett, D.R., Biron, K., Furman, P.A., 1989. 2-Acetylpyridine 5-[(dimethylamino)thiocarbonyl]-thiocarbonohydrazone (A111OU), a potent inactivator of ribonucleotide reductases of herpes simplex and varicella-zoster viruses and a potentiator of acyclovir. Proc. Natl. Acad. Sci. U.S.A. 86, 1051–1055.

Stubbe, J., van der Donk, W.A., 1998. Protein radicals in enzyme catalysis. Chem. Rev. 98, 705–762.