

**Tubulin Polymerization Inhibition Assays.** Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.<sup>42</sup> Determination of  $IC_{50}$  values for the polymerization of purified tubulin was performed as described in detail elsewhere.<sup>6</sup> In brief, tubulin was preincubated at 37 °C with varying compound concentrations, reaction mixtures were chilled on ice, GTP (required for the polymerization reaction) was added, and polymerization was followed at 37 °C by turbidimetry at 350 nm in Gilford recording spectrophotometers equipped with electronic temperature controllers. Four instruments were used, and two control reaction mixtures were present in each experiment. The extent of polymerization after a 20-min incubation was determined (the values for the two controls were usually within 5% of each other).  $IC_{50}$  values were determined graphically. Active compounds were examined in at least three independent assays, while inactive compounds (defined as  $IC_{50}$  value > 40  $\mu$ M) were examined in at least two independent experiments.

**Acknowledgment.** This research was made possible by grant CA47476 and contract NO1-CM-17512, both awarded by the National Cancer Institute, DHHS. We are grateful for the services of Vicky Croy, who performed the

cytotoxicity testing in the Purdue Cell Culture Laboratory.

**Registry No.** 3, 134-96-3; 4a, 10031-82-0; 4b, 5736-85-6; 4c, 3446-89-7; 4d, 104-87-0; 4e, 4748-78-1; 4f, 122-03-2; 4g, 939-97-9; 4h, 120-14-9; 4i, 7311-34-4; 4j, 6527-32-8; 4k, 106852-80-6; 5a, 61240-20-8; 5b, 1530-38-7; 6a, 141172-07-8; 6b, 141172-05-6; 6c, 141172-06-7; 6d, 141172-08-9; 6e, 141172-09-0; 6f, 141172-10-3; 6g, 141172-11-4; 6h, 106053-26-3; 6i, 94608-23-8; 6j, 141172-12-5; 6k, 141172-13-6; 6l, 141172-14-7; 7a, 141172-15-8; 7b, 141172-16-9; 7c, 141172-17-0; 7d, 141172-18-1; 7e, 141172-19-2; 7f, 141172-20-5; 7g, 141172-21-6; 7h, 18513-95-6; 7i, 22255-22-7; 7j, 141172-22-7; 7k, 141172-23-8; 7l, 141172-24-9; 8a, 141197-76-4; 8b, 141172-25-0; 8c, 141172-26-1; 8d, 141172-27-2; 8e, 141172-28-3; 8f, 141172-29-4; 8g, 119041-22-4; 8h, 141172-30-7; 8i, 141172-31-8; 8j, 141172-32-9; 9, 39499-95-1; 10a, 4584-46-7; 10b, 869-24-9; 11a, 13338-63-1; 11b, 104-47-2; 12a, 2746-25-0; 12b, 21852-50-6; 13a, 951-82-6; 13b, 104-01-8; 14a, 141172-33-0; 14b, 141172-34-1; 14c, 141172-35-2; 15a, 141172-36-3; 15b, 141197-64-0; 15c, 141172-37-4; 15d, 141172-38-5; 15e, 141172-39-6; 15f, 141172-40-9; 16, 4521-61-3; 18, 109091-08-9; 19, 141172-41-0; 20, 101747-36-8; 21a, 97399-87-6; 21b, 134029-50-8; 22a, 97399-88-7; 22b, 134029-62-2; 22c, 134029-63-3; 23a, 99257-48-4; 23b, 141172-42-1; 23c, 97399-69-4; 23d, 141172-43-2; 24, 141172-44-3; 25, 141172-45-4; 26, 141172-46-5; 27, 47439-73-6; 28, 4668-06-8; 29, 141172-47-6; 30, 84716-73-4; 31, 141172-48-7; 32, 4668-07-9; 33, 3937-16-4; 34, 107485-77-8; 35, 107485-76-7; 36, 17918-14-8; 37, 141172-49-8; 38, 141172-50-1; 39, 141172-51-2; syringaldehyde, 134-96-3; *tert*-butyldimethylsilyl chloride, 18162-48-6; anisole, 100-66-3; acetophenone, 98-86-2.

- (42) Hamel, E.; Lin, C. M. Separation of Active Tubulin and Microtubule-Associated Proteins by Ultracentrifugation and Isolation of a Component Causing the Formation of Microtubule Bundles. *Biochemistry* 1984, 23, 4173-4184.

## 2-Acetylpyridine Thiocarbonohydrazones. Potent Inactivators of Herpes Simplex Virus Ribonucleotide Reductase

Todd A. Blumenkopf,\*† Joan A. Harrington,‡ Cecilia S. Koble,† Donald D. Bankston,† Robert W. Morrison, Jr.,† Eric C. Bigham,† Virgil L. Styles,† and Thomas Spector†

*Divisions of Organic Chemistry and Experimental Therapy, Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709. Received November 20, 1991*

A series of 2-acetylpyridine thiocarbonohydrazones was synthesized for evaluation as potential antiherpetic agents. The compounds were prepared by the condensation of 2-acetylpyridine with thiocarbonohydrazide followed by treatment with isocyanates or isothiocyanates. Many were found that were potent inactivators of ribonucleotide reductase encoded by HSV-1 and weaker inactivators of human enzyme. Several thiocarbonohydrazones (e.g. 38 and 39) inactivated HSV-1 ribonucleotide reductase at rate constants as much as seven times that of lead compound 2. In general, those substituted with weak electron-attracting groups offered the best combination of potency and apparent selective activity against the HSV-1 enzyme. Seven new thiocarbonohydrazones (21, 25, 31, 36, 38, 39, and 40) were apparently greater than 50-fold more selective than 2 against HSV-1 ribonucleotide reductase versus human enzyme. The results indicated new compounds worthy of further study as potentiators of acyclovir in combination topical treatment of herpes virus infections.

### Introduction

Recurrent labial and perioral herpes simplex virus type 1 infections (HSV-1), the common cold sore or fever blister, are the most frequent cutaneous virus infections encountered in immunocompetent patients.<sup>1</sup> HSV-1 encodes a unique ribonucleotide reductase (EC 1.17.4.1) in infected cells<sup>2,3</sup> that catalyzes the reduction of all four ribonucleoside diphosphates to 2'-deoxynucleoside diphosphates.<sup>4,5</sup> In marked contrast to the mammalian enzyme, which is highly regulated by nucleoside triphosphates,<sup>6,7</sup> the viral enzyme is insensitive to allosteric control.<sup>8-10</sup> Indeed, HSV-1 is able to replicate in the presence of thymidine at concentrations that are inhibitory to host cell DNA synthesis.<sup>4</sup> This insensitivity permits unrestricted synthesis of 2'-deoxynucleotides in HSV-1

infected cells, and thereby suggests that the reductase may have significance as a potential antiviral target.<sup>10</sup>

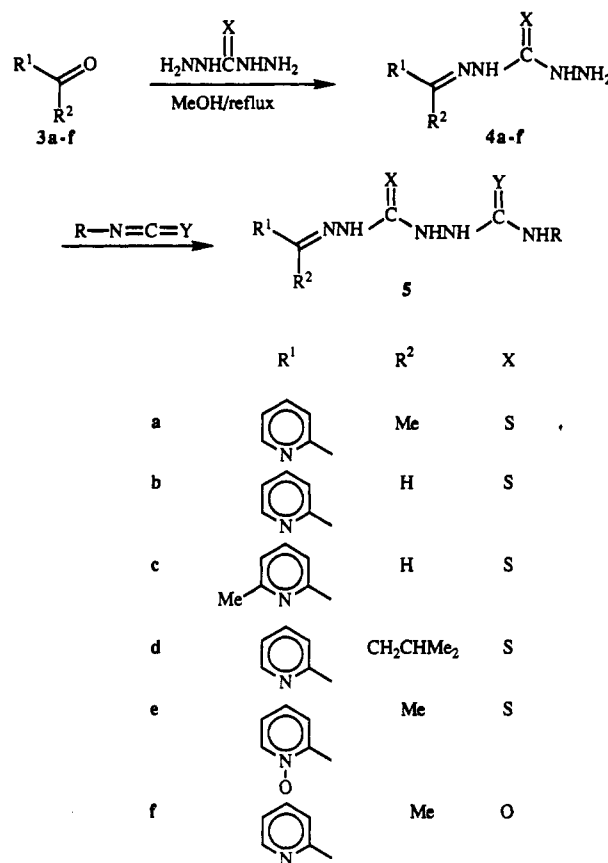
- (1) Spruance, S. L.; Freeman, D. J. Topical Treatment of Cutaneous Herpes Simplex Virus Infections. *Antiviral Res.* 1990, 14, 305-321.
- (2) Dutia, B. M. Ribonucleotide Reductase Induced by Herpes Simplex Virus has a Virus-Specified Constituent. *J. Gen. Virol.* 1983, 64, 513-521.
- (3) Swain, M. A.; Galloway, D. A. Herpes Simplex Virus Specifies Two Subunits of Ribonucleotide Reductase Encoded by 3'-Coterminal Transcripts. *J. Virol.* 1986, 57, 802-808.
- (4) Averett, D. R.; Lubbers, C.; Elion, G. B.; Spector, T. Ribonucleotide Reductase Induced by Herpes Simplex Type 1 Virus: Characterization of a Distinct Enzyme. *J. Biol. Chem.* 1983, 258, 9831-9838.
- (5) Averett, D. R.; Furman, P. A.; Spector, T. Ribonucleotide Reductase of Herpes Simplex Virus Type 2 Resembles That of Herpes Simplex Virus Type 1. *J. Virol.* 1984, 52, 981-983.
- (6) Thelander, L.; Reichard, P. Reduction of Ribonucleotides. *Annu. Rev. Biochem.* 1979, 48, 133-158.

\* Division of Organic Chemistry.

† Division of Experimental Therapy.

The value of herpes virus ribonucleotide reductase as a target for antiviral drugs used as single-agent therapies, however, is controversial.<sup>11-19</sup> Nevertheless, herpes virus infected cells treated with the antiviral drug acyclovir (ACV)<sup>20-23</sup> develop increased pools of deoxynucleotide dGTP, which can compete with the binding of acyclovir

Scheme I. Synthesis of Carbonohydrazones and Thiocarbonohydrazones (Y = O, S)



- (7) Hunting, D.; Henderson, J. F. Models of the Regulation of Ribonucleotide Reductase and their Evaluation in Intact Mammalian Cells. *CRC Crit. Rev. Biochem.* 1982, 13, 325-348.
- (8) Cohen, G. H. Ribonucleotide Reductase Activity of Synchronized KB Cells Infected with Herpes Simplex Virus. *J. Virol.* 1972, 9, 408-418.
- (9) Spector, T. Inhibition of Ribonucleotide Reductases Encoded by Herpes Simplex Viruses. *Pharmacol. Ther.* 1985, 31, 295-302.
- (10) Spector, T. Ribonucleotide Reductases Encoded by Herpes Viruses: Inhibitors and Chemotherapeutic Considerations. In *Inhibitors of Ribonucleoside Diphosphate Reductase Activity*; Cory, J. G., Cory, A. H., Eds.; Pergamon Press: New York, 1989; pp 235-243.
- (11) Leary, K.; Bratton, J.; Francke, B. Replication of Herpes Simplex Virus Type 1 in Hydroxyurea-Resistant Baby Hamster Kidney Cells. *J. Virol.* 1983, 47, 224-226.
- (12) Preston, V. G.; Palfreyman, J. W.; Dutia, B. M. Identification of a Herpes Simplex Virus Type 1 Polypeptide Which is a Component of the Virus-Induced Ribonucleotide Reductase. *J. Gen. Virol.* 1984, 65, 1457-1466.
- (13) Nutter, L. M.; Grill, S. P.; Cheng, Y.-C. Can Ribonucleotide Reductase be Considered as an Effective Target for Developing Antiherpes Simplex Virus Type II (HSV-2) Compounds? *Biochem. Pharmacol.* 1985, 34, 777-780.
- (14) Goldstein, D. J.; Weller, S. K. Herpes Simplex Virus Type 1-Induced Ribonucleotide Reductase Activity is Dispensable for Virus Growth and DNA Synthesis: Isolation and Characterization of an ICP6 lacZ Insertion Mutant. *J. Virol.* 1988, 62, 196-205.
- (15) Goldstein, D. J.; Weller, S. K. Factor(s) Present in Herpes Simplex Virus Type 1-Infected Cells can Compensate for the Loss of the Large Subunit of the Viral Ribonucleotide Reductase: Characterization of an ICP6 Deletion Mutant. *Virology* 1988, 166, 41-51.
- (16) Cameron, J. M.; McDougall, I.; Marsden, H. S.; Preston, V. G.; Ryan, D. M.; Subak-Sharpe, J. H. Ribonucleotide Reductase Encoded by Herpes Simplex Virus Is a Determinant of the Pathogenicity of the Virus in Mice and a Valid Antiviral Target. *J. Gen. Virol.* 1988, 69, 2607-2612.
- (17) Knipe, D. M. The Role of Viral and Cellular Nuclear Proteins in Herpes Simplex Virus Replication. *Adv. Virus Res.* 1989, 37, 85-123.
- (18) Turk, S. R.; Kik, N. A.; Birch, G. M.; Chiego, D. J., Jr.; Shipman, C., Jr. Herpes Simplex Virus Type 1 Ribonucleotide Reductase Null Mutants Induce Lesions in Guinea Pigs. *Virology* 1989, 173, 733-735.
- (19) Jacobson, J. G.; Leib, D. A.; Goldstein, D. J.; Bogard, C. L.; Schaffer, P. A.; Weller, S. K.; Coen, D. M. A Herpes Simplex Virus Ribonucleotide Reductase Deletion Mutant is Defective for Productive Acute and Reactivable Latent Infections of Mice and for Replication in Mouse Cells. *Virology* 1989, 173, 276-283.
- (20) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. Selectivity of Action of an Antiherpetic Agent, 9-(2-Hydroxyethoxymethyl)guanine. *Proc. Natl. Acad. Sci. U.S.A.* 1977, 74, 5716-5720.
- (21) Furman, P. A.; St. Clair, M. H.; Fyfe, J. A.; Rideout, J. L.; Keller, P. M.; Elion, G. B. Inhibition of Herpes Simplex Virus-Induced DNA Polymerase Activity and Viral DNA Replication by 9-(2-Hydroxyethoxymethyl)guanine and Its Triphosphate. *J. Virol.* 1979, 32, 72-77.
- (22) Furman, P. A.; Spector, T.; Fyfe, J. A. Acyclovir: Mechanism of Action. In *Human Herpesvirus Infections: Pathogenesis, Diagnosis, and Treatment*, Lopez, C., Roizman, B., Eds.; Raven Press: New York, 1986; pp 129-140.
- (23) Hopkins, S.; Furman, P. Molecular Basis for the Antiviral Activity of Acyclovir. In *Acyclovir Therapy for Herpesvirus Infections*; Baker, D. A., Ed.; Marcel Dekker: New York, 1990; pp 1-13.

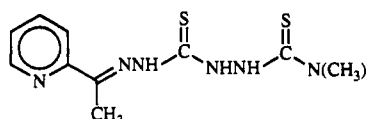
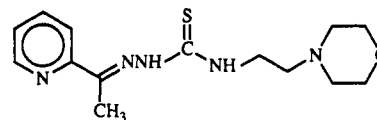
triphosphate (ACVTP) to herpes DNA polymerases.<sup>24-27</sup> Therefore, herpes virus ribonucleotide reductase inactivators in combination with ACV have been studied in these laboratories to prevent the build-up of dGTP.<sup>10,28</sup> Recent findings that cells infected with virus that encodes ribonucleotide reductase null mutants are hypersensitive to ACV support the use of herpes virus ribonucleotide reductase inactivators as potentiators of ACV.<sup>29</sup> While ACV is a potent and selective antiherpetic agent that is clinically effective against systemic and local infections caused by herpes simplex viruses,<sup>30</sup> treatment of herpes labialis in-

- (24) Furman, P. A.; Lambe, C. U.; Nelson, D. J. Effect of Acyclovir on the Deoxyribonucleoside Triphosphate Pool Levels in Vero Cells Infected with HSV-1. *Am. J. Med.* 1982, 73 (1A), 14-17.
- (25) This compound is identified as A723U in earlier publications; see: Spector, T.; Averett, D. R.; Nelson, D. J.; Lambe, C. U.; Morrison, R. W., Jr.; St. Clair, M. H.; Furman, P. A. Potentiation of Antiherpetic Activity of Acyclovir by Ribonucleotide Reductase Inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 1985, 82, 4254-4257.
- (26) Spector, T.; Harrington, J. A.; Morrison, R. W., Jr.; Lambe, C. U.; Nelson, D. J.; Averett, D. R.; Biron, K.; Furman, P. A. 2-Acetylpyridine 5-[(Dimethylamino)thiocarbonyl]thiocarbonohydrazone (A1110U), 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.* 1989, 86, 1051-1055.
- (27) Reardon, J. E.; Spector, T. Herpes Simplex Virus Type 1 DNA Polymerase: Mechanism of Inhibition by Acyclovir Triphosphate. *J. Biol. Chem.* 1989, 264, 7405-7411.
- (28) Reardon, J. E.; Spector, T. Acyclovir: Mechanism of Antiviral Action and Potentiation by Ribonucleotide Reductase Inhibitors. *Adv. Pharmacol.* 1991, 22, 1-27.
- (29) Coen, D. M.; Goldstein, D. J.; Weller, S. K. Herpes Simplex Virus Ribonucleotide Reductase Mutants Are Hypersensitive to Acyclovir. *Antimicrob. Agents Chemother.* 1989, 33, 1395-1399.

fections is one area where its effectiveness could be improved.<sup>31-35</sup> Furthermore, antiviral drug resistance has been observed with increasing frequency in patients with profound immune deficiency.<sup>36-39</sup> Clearly, improved therapies are needed for immunocompromised patients.

Recently, derivatives of 2-acetylpyridine thiosemicarbazone have been reported that inhibit HSV-1 ribonucleotide reductase<sup>40,41</sup> and demonstrate antiviral activity in vitro.<sup>42-44</sup> Earlier work in our laboratories identified 2-acetylpyridine thiosemicarbazone 1 as an inactivator of herpes virus ribonucleotide reductases that potentiates the antiviral activity of ACV in vitro.<sup>25,45</sup> Subsequently, thiocarbonohydrazone 2, a more potent inactivator,<sup>46</sup> was found to potentiate ACV both in vitro<sup>26</sup> and as topical

treatment in vivo.<sup>47,48</sup> Both compounds were found to decrease the dGTP pools in herpes virus infected cells and to markedly increase the pools of ACVTP.<sup>25,26</sup> Further studies, however, indicated hematological toxicity in rats dosed orally with 60 mg/kg of 2 (details of these studies will be published elsewhere<sup>49</sup>); although topical application would not be expected to produce comparable systemic levels of 2, toxicity resulting from oral dosing was nonetheless undesirable. Therefore, we continued to search for ribonucleotide reductase inactivators with similar or better efficacy and improved safety profiles.



We now report a series of thiocarbonohydrazones derived from 2-acetylpyridine that were studied as inactivators of HSV-1 ribonucleotide reductase. Several compounds are more potent against the HSV-1 enzyme, and are relatively selective against the viral enzyme versus the human enzyme, than those inactivators reported previously.

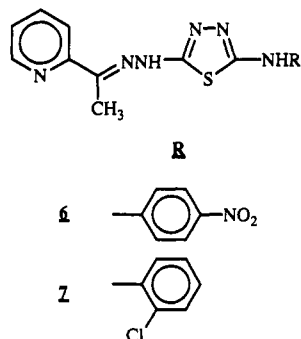
## Chemistry

Thiocarbonohydrazones of general structure 5, listed in Table I, were prepared via intermediates 4a-f upon treatment with the appropriate isocyanates or isothiocyanates (see Scheme I).<sup>50-53</sup> Intermediates 4a-f can be readily prepared in large quantity, without contamination from possible bis-adducts,<sup>54</sup> by treating the corresponding aldehydes and ketones with carbohydrazide (X = O) or thiocarbonylhydrazide (X = S) in methanol at reflux.

- (30) Dorsky, D. I.; Crumpacker, C. S. *Drugs Five Years Later: Acyclovir*. *Ann. Intern. Med.* 1987, 107, 859-874.
- (31) Fiddian, A. P.; Yeo, J. M.; Stubbings, R.; Dean, D. Successful Treatment of Herpes Labialis with Topical Acyclovir. *Br. Med. J.* 1983, 286, 1699-1701.
- (32) Shaw, M.; King, M.; Best, J. M.; Banatvala, J. E.; Gibson, J. R.; Klaber, M. R. Failure of Acyclovir Cream in Treatment of Recurrent Herpes Labialis. *Br. Med. J.* 1985, 291, 7-9.
- (33) Raborn, G. W.; McGaw, W. T.; Grace, M.; Tyrrell, L. D.; Samuels, S. M. Oral Acyclovir and Herpes Labialis: a Randomized, Double-Blind, Placebo-Controlled Study. *J. Am. Dent. Assoc.* 1987, 115, 38-42.
- (34) Raborn, G. W.; McGaw, W. T.; Grace, M.; Percy, J.; Samuels, S. Herpes Labialis Treatment with Acyclovir 5% Modified Aqueous Cream: A Double-Blind, Randomized Trial. *Oral Surg. Oral Med. Oral Pathol.* 1989, 67, 676-679.
- (35) Spruance, S. L.; Stewart, J. C. B.; Rowe, N. H.; McKeough, M. B.; Wenerstrom, G.; Freeman, D. J. Treatment of Recurrent Herpes Simplex Labialis with Oral Acyclovir. *J. Infect. Dis.* 1990, 161, 185-190.
- (36) Sibrack, C. D.; Gutman, L. T.; Wilfert, C. M.; McLaren, C.; St. Clair, M. H.; Keller, P. M.; Barry, D. W. Pathogenicity of Acyclovir-Resistant Herpes Simplex Virus Type 1 from an Immunodeficient Child. *J. Infect. Dis.* 1982, 146, 673-682.
- (37) Gray, J. J.; Wreghitt, T. G.; Baglin, T. P. Susceptibility to Acyclovir of Herpes Simplex Virus: Emergence of Resistance in Patients with Lymphoid and Myeloid Neoplasia. *J. Infect.* 1989, 19, 31-40.
- (38) Arbesfeld, D. M.; Thomas, I. Cutaneous Herpes Simplex Virus Infections. *Am. Fam. Physician* 1991, 43, 1655-1664.
- (39) Freifeld, A. G.; Ostrove, J. M. Resistance of Viruses to Antiviral Drugs. *Annu. Rev. Med.* 1991, 42, 247-259.
- (40) Turk, S. R.; Shipman, C., Jr.; Drach, J. C. Selective Inhibition of Herpes Simplex Virus Ribonucleoside Diphosphate Reductase by Derivatives of 2-Acetylpyridine Thiosemicarbazone. *Biochem. Pharmacol.* 1986, 35, 1539-1545.
- (41) Turk, S. R.; Shipman, C., Jr.; Drach, J. C. Structure-Activity Relationships among  $\alpha$ -(N)-Heterocyclic Acyl Thiosemicarbazones and Related Compounds as Inhibitors of Herpes Simplex Virus Type 1-specified Ribonucleoside Diphosphate Reductase. *J. Gen. Virol.* 1986, 67, 1625-1632.
- (42) Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. Antiviral Activity of 2-Acetylpyridine Thiosemicarbazones Against Herpes Simplex Virus. *Antimicrob. Agents Chemother.* 1981, 19, 682-685.
- (43) Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. Thiosemicarbazones of 2-Acetylpyridine, 2-Acetylquinoline, 1-Acetylisoquinoline, and Related Compounds as Inhibitors of Herpes Simplex Virus in vitro and in Cutaneous Herpes Guinea Pig Model. *Antiviral Res.* 1986, 6, 197-222.
- (44) Field, H. J.; Reading, M. J. The Inhibition of Bovine Herpesvirus-1 by Methyl 2-Pyridyl Ketone Thiosemicarbazone and its Effects on Bovine Cells. *Antiviral Res.* 1987, 7, 245-256.
- (45) Karlsson, A.; Harmenberg, J. Effects of Ribonucleotide Reductase Inhibition on Pyrimidine Deoxynucleotide Metabolism in Acyclovir-Treated Cells Infected with Herpes Simplex Virus Type 1. *Antimicrob. Agents Chemother.* 1988, 32, 1100-1102.
- (46) This compound is identified as A1110U or BW 1110U81 in earlier publications; see: Porter, D. J. T.; Harrington, J. A.; Spector, T. Herpes Simplex Virus Type 1 Ribonucleotide Reductase: Selective and Synergistic Inactivation by A1110U and its Iron Complex. *Biochem. Pharmacol.* 1990, 39, 639-646.
- (47) Ellis, M. N.; Lobe, D. C.; Spector, T. Synergistic Therapy by Acyclovir and A1110U for Mice Orofacially Infected with Herpes Simplex Viruses. *Antimicrob. Agents Chemother.* 1989, 33, 1691-1696.
- (48) Lobe, D. C.; Spector, T.; Ellis, M. N. Synergistic Topical Therapy by Acyclovir and A1110U for Herpes Simplex Virus Induced Zosteriform Rash in Mice. *Antiviral Res.* 1991, 15, 87-100.
- (49) Spector, T.; Lobe, D. C.; Ellis, M. N.; Blumenkopf, T. A.; Szczech, G. M. Inactivators of Herpes Simplex Virus Ribonucleotide Reductase: In Vivo Potentiation of the Antiviral Activity of Acyclovir and Hematological Profiles. *Antimicrob. Agents Chemother.* 1992, in press.
- (50) McElhinney, R. S. Derivatives of Thiocarbamic Acid. Part II. The Formation of 1,2,4-Triazole Salts from Amines and Methyl Dithiocarbamate. Some Attempts to prepare Substituted 1-Thiocarbamoylthiocarbonylhydrazides. *J. Chem. Soc. C* 1966, 1256-1260.
- (51) Kurzer, F.; Wilkinson, M. Heterocyclic Compounds from Urea Derivatives. Part XV. The Hydrazinolysis of 4-Substituted 1-Dithiomethoxycarbonyl-3-thiosemicarbazides. *J. Chem. Soc. C* 1968, 2108-2111.
- (52) Esmail, R.; Kurzer, F. Chemistry of Biureas. II. Synthesis and Cyclisation of Thiobiureas from (Thio)carbonylhydrazides and Ethoxycarbonyl Isothiocyanate. *Tetrahedron* 1977, 33, 2007-2012, and references cited therein.
- (53) Indukumari, P. V.; Joshua, C. P.; Rajan, V. P. Oxidation and Eliminative Cyclisation of S-Alkylisodithiobiureas. *Indian J. Chem.* 1981, 20B, 384-387.
- (54) Rajendran, G.; Jain, S. R. Synthesis and Characterization of Bis-thiocarbonohydrazones. *Indian J. Chem.* 1985, 24B, 680-682.

Reaction of intermediates 4a-f with isothiocyanates initially were conducted in ethanol at reflux (method A).<sup>53</sup> We later found that changing the solvent to DMF facilitated reaction at room temperature (method B).<sup>50-52</sup> This discovery was useful for the preparation of analogues of 5 where R was a phenyl group with a strongly electron-attracting substituent. For example, treatment of intermediate 4a with 4-nitrophenyl isothiocyanate in ethanol at reflux provided the desired product 20, but in poor yield. Thiadiazole 6, generated in situ from 20 upon loss of H<sub>2</sub>S, was a major byproduct. In contrast, compound 21 was prepared in 97% yield from 4a by reaction with the requisite isothiocyanate in DMF at room temperature. Similarly, compound 27 was prepared via method B in quantitative yield. The methods of synthesis and chemical data for thiocarbonohydrazone target compounds, along with chemical data for synthetic intermediates, are summarized in Table II.

Thiocarbonohydrazones of type 5 could be cyclized to the corresponding thiadiazoles upon reaction with oxidizing agents<sup>53</sup> and mild base. For example, treatment of 28 with *tert*-butyl hydroperoxide and triethylamine afforded thiadiazole 7 in excellent yield.



Acidity ( $pK_a$ ) and distribution constants ( $\log P^{55}$  and/or  $\log k'w^{56,57}$ ) were measured for selected compounds (see Table III). All new compounds are monosubstituted at the terminal nitrogen of the thiocarbonohydrazone chain, in contrast to lead compound 2, which is disubstituted in this position. While the  $pK_a$  values for protonation of the new compounds are comparable to that for 2,  $pK_a$  values for anion generation are approximately one unit more acidic than 2. All new analogues are more lipophilic than 2.

### Biological Methods

First-order rate constants for enzyme inactivation,  $k_{inact}$ , and initial velocities,  $v_0$ , of decelerating time courses of product formation were calculated by iterative nonlinear least squares analysis<sup>58</sup> as described previously.<sup>46,59</sup> The

$k_{inact}$  values for inactivators are reported as the net increase over the  $k_{inact}$  (inherent instability) of the uninhibited controls.

The relative potency of ribonucleotide reductase inhibitors is expressed by the inhibition index described in eq 1. This index accounts for both the rate constant for enzyme inactivation ( $k_{inact}$ ) and the percent inhibition of the initial velocity ( $v_0$ ) produced by an inhibitor. The latter is represented by  $f$ , which is defined in eq 2.

$$\text{inhibition index} = \left[ \frac{1 + k_{inact}}{f + 0.01} \right] - 0.99 \quad (1)$$

$$f = 1 - \frac{\% \text{ inhibition}}{100} \quad (2)$$

The value of 1.0 was added to the numerator to prevent zero values for non-inactivating inhibitors and 0.01 was added to the denominator to prevent zero values for compounds that produce 100% initial inhibition. The 1.0 and 0.01 factors were countered by subtracting 0.99 from the calculated ratio. Thus, a non-inhibiting, non-inactivating compound would have an inhibition index of zero.

All compounds were tested against both HSV-1 and human ribonucleotide reductase. The  $k_{inact}$ , percent inhibition, and inhibition index values determined from reactions  $\pm 5 \mu\text{M}$  inhibitor (unless otherwise indicated) are presented in Table I. Data determined at other concentrations are reported in the supplementary material.

For comparative purposes, an inhibitor's apparent selectivity toward herpes virus ribonucleotide reductase versus human enzyme was expressed as the ratio of the inhibition indices determined against the two enzymes.

The mode of inactivation of HSV-1 and human ribonucleotide reductase by 2 and 28 have been studied in detail and were shown to be quite complex.<sup>46,59</sup> Furthermore, the HSV-1 and human enzymes were assayed under different conditions (*vide infra*), as ATP is required for activation of the human but not the viral enzyme. Therefore, the apparent selectivity of compounds for inhibiting the HSV-1 versus the human ribonucleotide reductase is relative to the assay conditions reported herein, and was used as a basis for comparing inhibitors.

### Results and Discussion

New thiocarbonohydrazones listed in Table I (data for lead compound 2 is provided for reference) are divided into four sets. Three of these sets are based on the R group at the terminus of the thiocarbonohydrazone side chain, where R is (1) an alkyl group, compounds 8-12, (2) an acyl group, compounds 13 and 14, and (3) an aryl group, compounds 15-48. The fourth set examines structure/activity relationships where the side-chain terminating group (R) remains constant, but other parts of the molecules are varied.

Compounds in the first set (R is an alkyl group) inactivated HSV-1 ribonucleotide reductase at rate constants ( $k_{inact}$ ) comparable to lead compound 2. Inhibitors containing a simple alkyl group demonstrated an increase in potency against the viral enzyme as the lipophilicity of the inhibitor increased (HSV-1 enzyme inhibition index for  $10 > 9 > 8$ ). Compound 10 was roughly 3.5 times as active as 8 against the HSV-1 enzyme and 6-fold more relatively selective against the viral enzyme versus the human enzyme. Thiocarbonohydrazones with a morpholine ring attached to an alkyl side chain (11 and 12), as in the previously reported thiosemicarbazone 1,<sup>25,45</sup> showed no improvement in enzyme activity.

Of the compounds in set 2, where R is an acyl group, benzoyl analogue 13 offered modest enhancement in both

- (55) Minick, D. J.; Sabatka, J. J.; Hurlbert, B. S.; Brent, D. A. Automated Methods to Measure Lipophilicity and Acidity Constants. In *Automation of Pharmaceutical Operations—Supplement*; Fraade, D. J., Ed.; Aster: Springfield, OR, 1985; pp 31-36.
- (56) Minick, D. J.; Frenz, J. H.; Patrick, M. A.; Brent, D. A. A Comprehensive Method for Determining Hydrophobicity Constants by Reversed-Phase High-Performance Liquid Chromatography. *J. Med. Chem.* 1988, 31, 1923-1933.
- (57) Minick, D. J.; Brent, D. A. Modelling Octanol-Water Partition Coefficients by Reversed-Phase Liquid Chromatography. *J. Chromatog.* 1989, 461, 177-191.
- (58) Bevington, P. R. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw Hill: New York, 1969; pp 204-206.
- (59) Spector, T.; Harrington, J. A.; Porter, D. J. T. Herpes and Human Ribonucleotide Reductases: Inhibition by 2-Acetylpyridine 5-[(2-chloroanilino)thiocarbonyl]thiocarbonohydrazone (348U87). *Biochem. Pharmacol.* 1991, 42, 91-96.

**Table I.** Inhibition and Inactivation of HSV-1 and Human Ribonucleotide Reductases by 2-Acetylpyridine Thiocarbonohydrazones




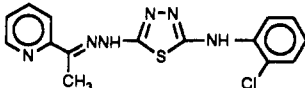
compound		HSV-1 enzyme			human enzyme			apparent enzyme selectivity <sup>c</sup>
no.	R	$k_{\text{inact}}^a$	% inhib <sup>a</sup>	inhib index <sup>b</sup>	$k_{\text{inact}}^a$	% inhib <sup>a</sup>	inhib index <sup>b</sup>	

<b>Set 1. R = alkyl group</b>								
2 <sup>d</sup>		8	39	14	4	8	4.4	3
8	Me	4	17	5	0.1	19	0.35	14
9	cyclopentyl	12	7	13	3	64	9.8	1.3
10	1-adamantanyl	14	47	27	0	53	1.1	24
11	CH <sub>2</sub> CH <sub>2</sub> -N<img alt="morpholine ring" style="vertical-align: middle;"/>	11	24	15	0.4	31	1.0	15
12	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -N<img alt="morpholine ring" style="vertical-align: middle;"/>	6.1	12	7.0	0	23	0.29	24
<b>Set 2. R = acyl group</b>								
13	COPh	15 <sup>e</sup>	38 <sup>e</sup>	24 <sup>e</sup>	2	54	5.4	4.5/
14	CO <sub>2</sub> Et	6	14	7	2	40	3.9	1.8
<b>Set 3. R = aryl group</b>								
15	Ph	20	7	21	3	59	8.5	2.5
16	2-MeC <sub>6</sub> H <sub>4</sub>	10	27	14	1	51	3	4.6
17	3-MeC <sub>6</sub> H <sub>4</sub>	19	28	26	0	54	1.1	23
18	4-MeC <sub>6</sub> H <sub>4</sub>	16	25	21	2	64	7	3.0
19	4-t-Bu-C <sub>6</sub> H <sub>4</sub>	16 <sup>g</sup>	10 <sup>g</sup>	18 <sup>g</sup>	ND <sup>h</sup>	ND	ND	ND
20	4-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	33 <sup>i</sup>	14 <sup>i</sup>	38 <sup>i</sup>	1.3	56	4.2	9.1/
21	3-NO <sub>2</sub> -4-ClC <sub>6</sub> H <sub>3</sub>	45	7	48	0	20	0.24	200
22	2-MeO-5-NO <sub>2</sub> -C <sub>6</sub> H <sub>3</sub>	40	44	71	3	11	3.5	21
23	2-CF <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	10	6	11	3	43	5.9	1.8
24	3-CF <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	34	15	40	1	31	1.9	21
25	4-CF <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	33	59	80	0	19	0.23	350
26	3-CNC <sub>6</sub> H <sub>4</sub>	32	0	32	0.6	80	6.6	4.8
27	4-CNC <sub>6</sub> H <sub>4</sub>	29	0	29	0	64	1.7	17
28	2-ClC <sub>6</sub> H <sub>4</sub>	27	0	27	0	57	1.3	21
29	3-ClC <sub>6</sub> H <sub>4</sub>	36	50	72	0	48	0.9	80
30	4-ClC <sub>6</sub> H <sub>4</sub>	39	61	99	0	73	2.6	38
31	2-BrC <sub>6</sub> H <sub>4</sub>	21	38	34	0	14	0.16	210
32	3-BrC <sub>6</sub> H <sub>4</sub>	40	15	47	2	21	2.8	17
33	4-BrC <sub>6</sub> H <sub>4</sub>	44	23	57	2	29	3.2	18
34	2-IC <sub>6</sub> H <sub>4</sub>	29	18	35	4	23	5.4	6.5
35	3-IC <sub>6</sub> H <sub>4</sub>	25	27	34	3	7	3.3	10
36	4-IC <sub>6</sub> H <sub>4</sub>	22	24	29	0	5	0.05	560
37	2-Me-4-ClC <sub>6</sub> H <sub>3</sub>	29	31	42	1	55	3.4	12
38	2,3-Cl <sub>2</sub> -C <sub>6</sub> H <sub>3</sub>	49	29	68	0	30	0.42	160
39	2,5-Cl <sub>2</sub> -C <sub>6</sub> H <sub>3</sub>	55	8	59	0	20	0.24	240
40	2,4,5-Cl <sub>3</sub> -C <sub>6</sub> H <sub>2</sub>	16	22	21	0	3	0.03	680
41	2-MeOC <sub>6</sub> H <sub>4</sub>	23	0	23	2.1	44	4.5	5.1
42	3-MeOC <sub>6</sub> H <sub>4</sub>	21	6.7	22	7 <sup>j</sup>	58 <sup>j</sup>	1.7 <sup>j</sup>	1.3 <sup>j</sup>
43	4-MeOC <sub>6</sub> H <sub>4</sub>	16	45	29	0	52	1.1	28
44	2-MeO-3-ClC <sub>6</sub> H <sub>3</sub>	25	0	25	0	50	1.0	26
45	2-MeO-5-ClC <sub>6</sub> H <sub>3</sub>	41	48	78	1	9	1.2	66
46	2-pyridyl	28	57	65	0	67	2.0	33
47	3-pyridyl	9	18	11	0	43	0.73	15
48	4-pyridyl	16	8	17	0	83	4.6	3.8

compound				HSV-1 enzyme			human enzyme			apparent enzyme selectivity <sup>c</sup>
no.	R <sup>1</sup>	R <sup>2</sup>	X Y	$k_{\text{inact}}^a$	% inhib <sup>a</sup>	inhib index <sup>b</sup>	$k_{\text{inact}}^a$	% inhib <sup>a</sup>	inhib index <sup>b</sup>	

<b>Set 4. Analogues of 28</b>										
49		H	S S	12	51	25	0	45	0.80	31
50		H	S S	16	41	27	0	65	1.8	15
51		CH <sub>2</sub> CHMe <sub>2</sub>	S S	14	38	23	0	25	0.33	70
52		H	S O	23	14	27	0	66	1.9	14

Table I (Continued)

no.	compound				HSV-1 enzyme			human enzyme			apparent enzyme selectivity <sup>c</sup>
	R <sup>1</sup>	R <sup>2</sup>	X	Y	$k_{\text{inact}}^a$	% inhib <sup>a</sup>	inhib index <sup>b</sup>	$k_{\text{inact}}^a$	% inhib <sup>a</sup>	inhib index <sup>b</sup>	
53		Me	O	S	5	30	7.5	ND	ND	ND	ND
54		Me	O	O	0	0	0	ND	ND	ND	ND
55		Me	S	S	1	11	1.2	ND	ND	ND	ND
7					15	53	32	ND	ND	ND	ND

<sup>a</sup>Data at 5  $\mu\text{M}$  compound concentration, unless otherwise indicated; inactivation rates per hour. <sup>b</sup>As defined in eq 1. <sup>c</sup>Ratio of HSV-1: human enzyme inhibition indices. <sup>d</sup>For compound structure, see text. <sup>e</sup>At 4.3  $\mu\text{M}$ . <sup>f</sup>Ratio determined using inhibition index values calculated at indicated concentrations for each enzyme. <sup>g</sup>At 3.6  $\mu\text{M}$ . <sup>h</sup>ND = not determined. <sup>i</sup>At 0.5  $\mu\text{M}$ . <sup>j</sup>At 6.5  $\mu\text{M}$ .

potency and apparent selectivity over lead compound 2.

In the third set of compounds (15–48), where the terminal R group on the thiocarbonohydrazone side chain is a phenyl or pyridyl ring, all inactivated HSV-1 ribonucleotide reductase at rate constants ( $k_{\text{inact}}$ ) comparable to or greater than that observed for 2. In contrast to the first set, no enhancement in potency was observed by increasing the lipophilicity of the terminating aryl ring (cf. 15–19).

In general, those compounds containing electron-attracting substituents (20–40, 44, 45) were more potent against the herpes virus enzyme than those substituted only with electron-donating functional groups (16–19, 41–43). Certain compounds (e.g. 38, 39) inactivated HSV-1 ribonucleotide reductase at rate constants ( $k_{\text{inact}}$ ) 6 to 7 times that of lead compound 2. Furthermore, the apparent enzyme selectivity of compounds with electron-attracting groups was, typically, superior to that observed in compounds with electron-donating substituents. Seven compounds in this set (21, 25, 31, 36, 38, 39, and 40) were apparently greater than 50-fold more selective than 2 for HSV-1 ribonucleotide reductase versus human enzyme. However, no clear relationship between the relative potency against either the herpes virus or human enzymes (as measured by  $k_{\text{inact}}$ , percent inhibition, or inhibition index values), or apparent enzyme selectivity, and the position of substituents on the phenyl ring in this set was observed.

Two compounds with strongly electron-attracting groups (21 and 25) were both potent and relatively selective against the viral enzyme; no inactivation of the human enzyme was observed. Others, such as 26 and 27, were potent inhibitors (see percent inhibition in Table I) of the human enzyme at concentrations close to those required for inactivation ( $k_{\text{inact}}$ ) of the herpes enzyme under the assay conditions. However, thiocarbonohydrazones with strongly electron-attracting substituents were most prone to cyclize in solution to their corresponding thiadiazoles (e.g. 6), suggesting that these compounds might be less suitable for long-term storage in a topical formulation.

Compounds with weakly electron-deficient halogen-substituted phenyl rings (28–40) seemed to offer the attractive combination of potency and apparent selectivity against the HSV-1 enzyme. In this series, rates of inactivation ( $k_{\text{inact}}$ ) of HSV-1 ribonucleotide reductase ranged from 16 to 55 per hour, a 2–7-fold improvement over lead

compound 2. Analogue 30 showed the highest inhibition index against the herpes enzyme of any compound we have studied. All but two of these halogenated compounds showed at least 10-fold relative selectivity between the viral and human enzymes. Several di- and trichlorinated analogues (38–40) showed enhanced apparent selectivity against the viral enzyme, in comparison to monochlorinated compounds 28–30.

Where R is a pyridyl group, 2-pyridyl analogue 46 was 4–6-fold more potent against the herpes virus enzyme than isomers 47 and 48. In addition, compound 46 was apparently 10-fold more selective against the HSV-1 versus the human enzyme than lead compound 2.

Eleven compounds were selected for further studies in vivo models as potentiators of ACV; details of these studies will be reported elsewhere.<sup>49</sup> From these studies, compound 28, which also inactivates VZV and HSV-2 ribonucleotide reductase,<sup>59</sup> was selected for development as combination topical therapy with ACV for cutaneous herpes virus infections.<sup>60</sup>

A study of the mode of inactivation of HSV-1 and human ribonucleotide reductases by 28 was recently published.<sup>59</sup> We now report what structural features in 28 were required to maintain activity against the HSV-1 enzyme (see Table I, set 4).

Four analogues (49–52) in set 4 had inhibition indices against both the HSV-1 and human enzymes comparable to 28. Data for compound 52 show that the thiocarbonyl distal to the pyridine ring is not required for activity against either enzyme in vitro. (However, in later studies, compound 52 did not potentiate the antiherpetic activity of ACV in vivo.<sup>61</sup>) Carbonohydrazones 53 and 54 produced weak or no inhibition, indicating that the thiocarbonyl proximal to the pyridine ring is required for efficient inactivation of the HSV-1 enzyme. Pyridine N-oxide 55 also was not active against the herpes virus ribonucleotide reductase, suggesting that the lone pair of electrons on the pyridine nitrogen is required for inactivation of enzyme activity. Nonetheless, some steric hindrance can be tolerated around the pyridine nitrogen, as indicated by data for 6-methylpyridine derivative 50. The

(60) Blumenkopf, T. A. U.S. Patent 5 021 437, 1991. This compound is identified in refs 49 and 59 as BW 348U87.

(61) Lobe, D. C.; Ellis, M. N.; Blumenkopf, T. A.; Spector, T. Unpublished results.

Table II. Chemical Data for New Compounds<sup>a</sup>

no.	formula <sup>b,c</sup>	analyses <sup>b</sup>	synthetic method <sup>d</sup>	mp (°C) <sup>e</sup>	yield (%) <sup>f</sup>
4a	C <sub>8</sub> H <sub>11</sub> N <sub>5</sub> S	C,H,N,S		185–186	97
4b	C <sub>7</sub> H <sub>9</sub> N <sub>5</sub> S·0.4MeOH·0.1H <sub>2</sub> O	C,H,N,S		162–164	92
4c	C <sub>8</sub> H <sub>11</sub> N <sub>5</sub> S·0.5MeOH·0.2H <sub>2</sub> O	C,H,N,S		166–168	89
4e	C <sub>8</sub> H <sub>11</sub> N <sub>5</sub> OS·0.5H <sub>2</sub> O	C,H,N,S		167.5–168.5	96
4f	C <sub>8</sub> H <sub>11</sub> N <sub>5</sub> O	C,H,N		200.5–201.5	91
8 <sup>g</sup>	C <sub>10</sub> H <sub>14</sub> N <sub>6</sub> S <sub>2</sub>	C,H,N,S	A	178.0–178.5	93
9	C <sub>14</sub> H <sub>20</sub> N <sub>6</sub> S <sub>2</sub> ·0.5H <sub>2</sub> O	C,H,N,S	B	180–182	83
10 <sup>h</sup>	C <sub>18</sub> H <sub>26</sub> N <sub>6</sub> S <sub>2</sub> ·0.75C <sub>3</sub> H <sub>6</sub> O	C,H,N,S	B	172–177	11
11	C <sub>15</sub> H <sub>23</sub> N <sub>7</sub> OS <sub>2</sub>	C,H,N,S	A	161–162	69
12	C <sub>16</sub> H <sub>25</sub> N <sub>7</sub> OS <sub>2</sub>	C,H,N,S	A	195–197	85
13	C <sub>16</sub> H <sub>16</sub> N <sub>6</sub> OS <sub>2</sub>	C,H,N,S	A	195–196	95
14	C <sub>12</sub> H <sub>16</sub> N <sub>6</sub> O <sub>2</sub> S <sub>2</sub>	C,H,N,S	B	195–197	71
15	C <sub>15</sub> H <sub>16</sub> N <sub>6</sub> S <sub>2</sub>	C,H,N,S	A	178–180	87
16	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> S <sub>2</sub>	C,H,N,S	B	168.5–175.5	76
17	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> S <sub>2</sub> ·0.10C <sub>3</sub> H <sub>7</sub> NO·0.15H <sub>2</sub> O	C,H,N,S	B	173.5–177.0	98
18	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> S <sub>2</sub> ·0.05C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S	B	154.0–155.5	95
19	C <sub>16</sub> H <sub>24</sub> N <sub>6</sub> S <sub>2</sub>	C,H,N,S	B	187–188	56
20	C <sub>15</sub> H <sub>15</sub> N <sub>7</sub> O <sub>2</sub> S <sub>2</sub> ·0.95H <sub>2</sub> O	C,H,N,S	A	230	17
21	C <sub>16</sub> H <sub>14</sub> ClN <sub>7</sub> O <sub>2</sub> S <sub>2</sub> ·1.1C <sub>3</sub> H <sub>7</sub> NO·0.2H <sub>2</sub> O	C,H,N,S,Cl	B	151.5–152.5	97
22	C <sub>16</sub> H <sub>17</sub> N <sub>7</sub> O <sub>3</sub> S <sub>2</sub>	C,H,N,S	A	212–213	80
23	C <sub>16</sub> H <sub>16</sub> F <sub>3</sub> N <sub>6</sub> S <sub>2</sub> ·0.2C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S	B	118.8–120.5	95
24	C <sub>16</sub> H <sub>15</sub> F <sub>3</sub> N <sub>6</sub> S <sub>2</sub>	C,H,N,S	A	181–184	61
25	C <sub>16</sub> H <sub>15</sub> F <sub>3</sub> N <sub>6</sub> S <sub>2</sub> ·0.05C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S	B	165.5–183.5	99
26	C <sub>16</sub> H <sub>16</sub> N <sub>7</sub> S <sub>2</sub> ·0.9H <sub>2</sub> O	C,H,N,S	B	178–180	94
27	C <sub>16</sub> H <sub>15</sub> N <sub>7</sub> S <sub>2</sub> ·H <sub>2</sub> O	C,H,N,S	B	241–242	100
28	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> S <sub>2</sub>	C,H,N,S,Cl	B	159–160	79
29	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> S <sub>2</sub>	C,H,N,S,Cl	B	168–171	100
30	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> S <sub>2</sub>	C,H,N,S,Cl	B	181–182	91
31	C <sub>15</sub> H <sub>15</sub> BrN <sub>6</sub> S <sub>2</sub>	C,H,N,S,Br	B	155–158	100
32	C <sub>15</sub> H <sub>15</sub> BrN <sub>6</sub> S <sub>2</sub> ·0.07C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,Br	B	161.5–165.0	87
33	C <sub>15</sub> H <sub>15</sub> BrN <sub>6</sub> S <sub>2</sub> ·0.04C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,Br	B	167.0–168.5	98
34	C <sub>15</sub> H <sub>15</sub> IN <sub>6</sub> S <sub>2</sub> ·0.02C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,I	B	161.5–162.7	92
35	C <sub>15</sub> H <sub>15</sub> IN <sub>6</sub> S <sub>2</sub>	C,H,N,S,I	B	159.5–161.0	96
36	C <sub>15</sub> H <sub>15</sub> IN <sub>6</sub> S <sub>2</sub> ·0.03C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,I	B	173.5–174.5	97
37	C <sub>16</sub> H <sub>17</sub> ClN <sub>6</sub> S <sub>2</sub> ·0.02C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,Cl	B	165.5–166.5	88
38	C <sub>15</sub> H <sub>14</sub> Cl <sub>2</sub> N <sub>6</sub> S <sub>2</sub> ·0.85C <sub>3</sub> H <sub>7</sub> NO·0.1H <sub>2</sub> O	C,H,N,S,Cl	B	156.3–157.5	98
39	C <sub>15</sub> H <sub>14</sub> Cl <sub>2</sub> N <sub>6</sub> S <sub>2</sub> ·0.7C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,Cl	B	119–121	96
40	C <sub>15</sub> H <sub>13</sub> Cl <sub>3</sub> N <sub>6</sub> S <sub>2</sub> ·0.75C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,Cl	B	133.5–135.5	100
41	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> OS <sub>2</sub> ·0.15C <sub>3</sub> H <sub>7</sub> NO·0.15H <sub>2</sub> O	C,H,N,S,Cl	A	180–182	29
42	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> OS <sub>2</sub>	C,H,N,S	A	172–176	86
43	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> OS <sub>2</sub> ·0.05C <sub>3</sub> H <sub>7</sub> NO·0.45H <sub>2</sub> O	C,H,N,S	A	162–164	62
44	C <sub>16</sub> H <sub>17</sub> ClN <sub>6</sub> OS <sub>2</sub>	C,H,N,S,Cl	B	170–171	99
45	C <sub>16</sub> H <sub>17</sub> ClN <sub>6</sub> OS <sub>2</sub> ·0.02C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S,Cl	B	156.5–158.0	87
46	C <sub>14</sub> H <sub>15</sub> N <sub>7</sub> S <sub>2</sub> ·C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S	B	204–205	53
47	C <sub>14</sub> H <sub>15</sub> N <sub>7</sub> S <sub>2</sub> ·0.05C <sub>3</sub> H <sub>7</sub> NO	C,H,N,S	B	176–179	79
48	C <sub>14</sub> H <sub>15</sub> N <sub>7</sub> S <sub>2</sub> ·0.05C <sub>3</sub> H <sub>7</sub> NO·H <sub>2</sub> O	C,H,N,S	B	179–180	82
49 <sup>i</sup>	C <sub>14</sub> H <sub>13</sub> ClN <sub>6</sub> S <sub>2</sub> ·0.05CH <sub>4</sub> O·0.1H <sub>2</sub> O	C,H,N,S,Cl	B	167–168	84
50 <sup>i</sup>	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> S <sub>2</sub> ·0.7CH <sub>4</sub> O·0.2H <sub>2</sub> O	C,H,N,S,Cl	B	151–153	84
51	C <sub>18</sub> H <sub>21</sub> ClN <sub>6</sub> S <sub>2</sub>	C,H,N,S,Cl	B	75	18 <sup>j</sup>
52	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> OS	C,H,N,S,Cl	B	177–179	67
53	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> OS·0.4C <sub>3</sub> H <sub>7</sub> NO·0.85H <sub>2</sub> O	C,H,N,S,Cl	B	132.5–135.0	100
54	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> O <sub>2</sub> ·0.1C <sub>3</sub> H <sub>7</sub> NO·0.05H <sub>2</sub> O	C,H,N,S,Cl	B	191.5–193.5	68
55	C <sub>15</sub> H <sub>15</sub> ClN <sub>6</sub> OS <sub>2</sub> ·0.2C <sub>3</sub> H <sub>7</sub> NO·0.3H <sub>2</sub> O	C,H,N,S,Cl	B	127–143	83

<sup>a</sup> Data for 6 and 7 are presented in the Experimental Section. <sup>b</sup> Satisfactory elemental analyses ( $\pm 0.4\%$ ) were obtained for all elements (C, H, N; S, Cl, Br, and I, where present). <sup>c</sup> C<sub>3</sub>H<sub>6</sub>O indicates acetone; C<sub>3</sub>H<sub>7</sub>NO, *N,N*-dimethylformamide; CH<sub>4</sub>O, methanol. <sup>d</sup> Methods used for the preparation of target thiocarbonohydrazones from 4a–f are indicated as A or B (see Experimental Section). <sup>e</sup> All compounds decomposed on melting (melting points are not corrected). <sup>f</sup> Yields shown for 4a–c and 4e,f are from corresponding ketones 3a–c and 3e,f. Yields for 8–55 are from the corresponding intermediates 4a–f, unless otherwise indicated. <sup>g</sup> The assigned structure is supported by correlation of the UV and <sup>13</sup>C NMR spectra of 8 with those of 2, for which an X-ray crystal structure was obtained (R. Morrison, unpublished results). <sup>h</sup> Recrystallized from acetone. <sup>i</sup> Washed with methanol. <sup>j</sup> Overall yield from isobutyl 2-pyridyl ketone.

inhibition index of 7, the thiadiazole analogue of 28, against the HSV-1 enzyme was comparable to that of 28.

## Conclusions

We have reported a series of 2-acetylpyridine thiocarbonohydrazones that have potent and apparently selective activity against herpes simplex virus ribonucleotide reductases. Compounds with side chains terminated by phenyl rings substituted with weak electron-attracting groups offer the best combination of potency and apparent selectivity against the HSV-1 enzyme. The combination of 28 and ACV is currently being developed as topical therapy for cutaneous herpes virus infections.

Structures and enzyme data for 11 additional thiocarbonohydrazones are reported in the supplementary material. The data for these additional analogues are consistent with the structure/activity relationships presented herein.

## Experimental Section

All solvents were reagent grade, dried over molecular sieves, and used without further purification. Chemicals were reagent grade and were used without purification unless noted. 2-Pyridyl,<sup>62</sup>



**Table III.** Physical Parameters of Selected Thiocarbonohydrazones

no.	pK <sub>a</sub> <sup>a</sup>	log P <sup>b</sup>	log k' <sup>c</sup>
2	4.1, 8.4	1.25	1.28
24	3.89, 6.79	ND <sup>d</sup>	2.94
25	3.87, 6.77	ND	3.26
28	3.94, 7.24	2.67	2.09
35	3.80, 7.03	ND	2.97
38	3.86, 7.09	ND	3.29
46	2.09, 5.87, 7.27	ND	2.8

<sup>a</sup> Determined spectrophotometrically; see ref 55. <sup>b</sup> 1-Octanol vs pH 5.2 acetate buffer; measured at Midwest Research Institute, Kansas City, MO. <sup>c</sup> Determined using the procedure in ref 56 and 57. <sup>d</sup> ND = not determined.

3-pyridyl,<sup>63</sup> and 4-pyridyl isothiocyanate<sup>64</sup> were prepared by the literature methods noted. All other isocyanates and isothiocyanates were purchased from commercial suppliers (Aldrich Chemical Co., Milwaukee, WI; Trans World Chemical Co., Chevy Chase, MD; or Fairfield Chemical Co., Blythewood, SC). Liquid isocyanates and isothiocyanates were distilled prior to use. When necessary, thiocarbonohydrazones were purified by dissolving in a minimal amount of *N,N*-dimethylformamide (DMF) at 0 °C and triturating the resulting solutions with cold H<sub>2</sub>O; the resulting precipitates were isolated by filtration. All final products were washed with H<sub>2</sub>O or methanol and dried in a vacuum desiccator at room temperature overnight (12–18 h). <sup>1</sup>H-NMR spectra were measured at 200 MHz with a Varian XL-200 spectrometer. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant data are tabulated in order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of hydrogens, coupling constant(s) in hertz, descriptor. Mass spectra were obtained at Oneida Research Services, Whitesboro, NY, via chemical ionization using methane as initiator; data for significant peaks are tabulated as *m/e* (intensity expressed as peak amplitude relative to base peak). Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

**Ribonucleotide Reductase Purifications and Assays.** HSV-1<sup>4</sup> and human<sup>65,66</sup> ribonucleotide reductases were partially purified as described in the indicated references. The enzymes were assayed by monitoring the reduction of purified [<sup>14</sup>C]CDP. Herpes virus ribonucleotide reductase was assayed in glass vials containing 200 mM Hepes Na<sup>+</sup> at pH 7.7, 10 mM dithiothreitol, and 2 μM (500 Ci/mol) CDP (3–4 × *K<sub>m</sub>*)<sup>4,5,67</sup> as previously described.<sup>4</sup> Human ribonucleotide reductase was assayed in glass vials containing 100 mM Hepes at pH 7.4, 5 mM dithiothreitol, 12 μM CDP (5 × *K<sub>m</sub>*) with a specific activity of 75 Ci/mol, 5 mM ATP, and 6 mM MgCl<sub>2</sub>.<sup>68</sup> The ATP, which was contaminated with iron, imparted about 3 μM iron in the reaction mixtures,<sup>46</sup> and ensured that the inhibitors would be in the iron-complexed form.<sup>46,59</sup> Although both iron-complexed and uncomplexed compounds 2 and 28 inhibit herpes virus ribonucleotide reductase, only the iron-complexed forms of these (and presumably the other) compounds inhibit human ribonucleotide reductase.<sup>46,59</sup> Multiple reactions were assayed simultaneously as described elsewhere.<sup>69</sup>

The reactions were terminated with hydroxyurea and EDTA, which quenched the reaction and decreased the blank rates to an insignificant level.<sup>4,65</sup> The product was dephosphorylated and isolated by chromatography on a Dowex-borate column according to the modified method<sup>4</sup> of Steeper and Stuart.<sup>70</sup> The reaction temperature was 37 °C.

**General Method for Preparation of Intermediates of Structure 4.** 2-Acetylpyridine Thiocarbonohydrazone (4a). To a solution of 33.3 g (275 mmol) of distilled 2-acetylpyridine (3a) in 300 mL of methanol was added 29.2 g (275 mmol) of thiocarbonohydrazide (Sigma Chemical Co., St. Louis, MO). The resulting mixture was heated at reflux, under nitrogen, overnight. A precipitate was collected by filtration, washed with cold methanol, and dried to give 56.0 g (97%) of 2-acetylpyridine thiocarbonohydrazone (4a) as a white crystalline solid: mp 185–186 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.34 (s, 3 H, CH<sub>3</sub>), 5.00 (br s, 2 H, NH<sub>2</sub>), 7.36 (ddd, 1 H, *J* = 1.0, 4.9, 7.5, aromatic CH), 7.77 (dt, 1 H, *J* = 1.7, 7.8, aromatic CH), 8.53 (m, 2 H, aromatic CH), 9.96 (br s, 1 H, NH), 10.31 (s, 1 H, NH). Anal. (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>S) C, H, N, S.

2-Acetylpyridine Thiocarbonohydrazone 1-Oxide (4e). To a solution of 34.0 g (248 mmol) of distilled 2-acetylpyridine 1-oxide (3e)<sup>71</sup> in 300 mL of methanol was added 26.3 g (248 mmol) of thiocarbonohydrazide. The resulting mixture was heated at reflux, under nitrogen, for 3 h. A precipitate was collected by filtration, washed with cold methanol, and dried to give 53.6 g (96%) of 2-acetylpyridine thiocarbonohydrazone 1-oxide (4e) as a yellow powder: mp 167.5–168.5 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.22 (s, 3 H, CH<sub>3</sub>), 4.92 (br s, 2 H, NH<sub>2</sub>), 7.39–7.42 (m, 2 H, aromatic CH), 7.72 (dd, 1 H, *J* = 2.5, 7, aromatic CH), 8.22 (d, 1 H, *J* = 7, aromatic CH), 9.72 (br s, 1 H, NH), 10.44 (s, 1 H, NH); mass spectrum, *m/e* 226 (100). Anal. (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>·0.5H<sub>2</sub>O) C, H, N, S.

**Preparation of Thiocarbonohydrazones. Method A.** 2-Acetylpyridine 5-[(Methylamino)thiocarbonyl]thiocarbonohydrazone (8). A 1-L, three-neck flask fitted with an overhead mechanical stirrer, condenser, glass stopper, and nitrogen line was charged with 5.37 g (25.7 mmol) of 2-acetylpyridine thiocarbonohydrazone (4a) and 500 mL of absolute ethanol. The resulting mixture was heated at reflux for 5 min to dissolve most of the solid material. The mixture was allowed to cool below reflux temperature and 2.20 mL (2.35 g, 32.1 mmol) of methyl isothiocyanate was added. The reaction mixture was heated at reflux for 30 min, allowed to cool to room temperature, and subsequently cooled in an ice-water bath. A precipitate was collected by filtration, washed with cold ethanol, and dried in vacuo to give 6.74 g (93.3%) of 2-acetylpyridine 5-[(methylamino)thiocarbonyl]thiocarbonohydrazone (8) as a white crystalline solid: mp 178.0–178.5 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.39 (s, 3 H, COCH<sub>3</sub>), 2.86 (d, 3 H, *J* = 4, NCH<sub>3</sub>), 7.38 (m, 1 H, aromatic CH), 7.85 (m, 1 H, NH), 8.56 (m, 2 H, aromatic CH), 9.43 (br s, 1 H, NH), 10.32 (br s, 1 H, NH), 10.82 (br s, 1 H, NH). Anal. (C<sub>10</sub>H<sub>14</sub>N<sub>6</sub>S<sub>2</sub>) C, H, N, S.

2-Acetylpyridine 5-[(4-Nitroanilino)thiocarbonyl]thiocarbonohydrazone (20) and 2-Acetylpyridine [5-(4-Nitroanilino)-1,3,4-thiadiazol-2-yl]hydrazones (6). Reaction by method A of 5.05 g (24.1 mmol) of 2-acetylpyridine thiocarbonohydrazone (4a) and 5.20 g (28.8 mmol) of 4-nitrophenyl isothiocyanate gave 6.72 g of a red solid. The material was suspended in 3 L of methanol and stirred at 40 °C for 2 h. The undissolved red solid was collected by filtration (the filtrate was treated as indicated below), taken up in 175 mL of DMF, and filtered hot. Addition of 50 mL of H<sub>2</sub>O caused precipitation of a red solid, which was collected by filtration, washed with H<sub>2</sub>O, ethanol, and ether, and dried to give 0.95 g (11%) of 2-acetylpyridine [5-(4-nitroanilino)-1,3,4-thiadiazol-2-yl]hydrazones (6) as an orange-red powder: mp > 260 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.37

- (63) Jochims, J. C. Über eine neue Synthese Aromatischer Isothiocyanate. *Chem. Ber.* 1968, 101, 1746–1752.  
 (64) Hansen, E. T.; Petersen, H. J. Preparation of *N*-Alkyl-*N'*-4-Pyridylthioureas Via Isolable 4-Pyridylisothiocyanate or Via Aerial Oxidation of 4-Pyridyldithiocarbamic Acid. *Synth. Commun.* 1984, 14, 537–536.  
 (65) Spector, T.; Averett, D. R. A Simple Method to Purify Ribonucleotide Reductase. *Anal. Biochem.* 1983, 134, 467–470.  
 (66) Spector, T. Improvement of a Simple Method to Purify Ribonucleotide Reductase. *Prep. Biochem.* 1985, 15, 183–188.  
 (67) Spector, T.; Stonehurner, J. G.; Biron, K. K.; Averett, D. R. Ribonucleotide Reductase Induced by Varicella Zoster Virus. Characterization, and Potentiation of Acyclovir by its Inhibition. *Biochem. Pharmacol.* 1987, 36, 4341–4346.  
 (68) Harrington, J. A.; Miller, W. H.; Spector, T. Effector Studies With 3'-Azidothymidine Nucleotides With Human Ribonucleotide Reductase. *Biochem. Pharmacol.* 1987, 36, 3757–3761.

- (69) Spector, T.; Harrington, J. A. Rapid Sampling of Multiple Enzyme Reactions. *J. Virol. Methods* 1989, 26, 237–244.  
 (70) Steeper, J. R.; Stuart, C. D. A Rapid Assay for CDP Reductase Activity in Mammalian Cell Extracts. *Anal. Biochem.* 1970, 34, 123–130.  
 (71) Winterfeld, K.; Zickel, W. Synthese von 1-Hydroxy-2-chlor-chinolizidin und 1-Hydroxy-2-amino-4-oxochinolizidin. *Arch. Pharm. Ber. Dtsch. Pharm.* 1969, 302, 900–908.



(s, 3 H, CH<sub>3</sub>), 7.37 (m, 1 H, aromatic CH), 7.72 (d, 2 H, *J* = 9, aromatic CH), 7.83 (m, 1 H, aromatic CH), 7.92 (m, 1 H, aromatic CH), 8.23 (d, 2 H, *J* = 9, aromatic CH), 8.58 (m, 1 H, aromatic CH), 10.71 (br s, 1 H, NH), 11.65 (br s, 1 H, NH). Anal. (C<sub>15</sub>H<sub>13</sub>N<sub>7</sub>O<sub>2</sub>S·0.35H<sub>2</sub>O) C, H, N, S.

The filtrate (indicated above) was concentrated in vacuo to a volume of ca. 800 mL and cooled, and the resulting precipitate was collected by filtration, washed with cold methanol, and dried to give 1.59 g (17%) of 2-acetylpyridine 5-[(nitroanilino)thiocarbonyl]thiocarbonohydrazone (20) as a tan powder: mp 230 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.49 (s, 3 H, CH<sub>3</sub>), 7.36–8.62 (m, 8 H, aromatic CH), 10.05 (br s, 1 H, NH), 10.25 (br s, 1 H, NH), 10.55 (br s, 1 H, NH), 11.06 (br s, 1 H, NH). Anal. (for C<sub>15</sub>H<sub>15</sub>N<sub>7</sub>O<sub>2</sub>S<sub>2</sub>·0.95H<sub>2</sub>O) C, H, N, S.

**Preparation of Thiocarbonohydrazones. Method B.** 4-[1-[[[1-(2-Pyridyl)ethylidene]hydrazino]thiocarbonyl]-4-thiosemicarbazido]benzonitrile (27). To a solution of 4.80 g (22.9 mmol) of 2-acetylpyridine thiocarbonohydrazone (4a) in 170 mL of dry DMF was added 3.67 g (22.9 mmol) of 4-cyanophenyl isothiocyanate and the resulting mixture was stirred at room temperature for 30 min. The solution was cooled in an ice bath and H<sub>2</sub>O was added. A precipitate was collected, washed with H<sub>2</sub>O, and dried in vacuo to give 8.5 g (100%) of 4-[1-[[[1-(2-pyridyl)ethylidene]hydrazino]thiocarbonyl]-4-thiosemicarbazido]benzonitrile (27) as a yellow powder: mp 241–242 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.42 (s, 3 H, CH<sub>3</sub>), 7.38 (dt, H, *J* = 1.6, 6.5, aromatic CH), 7.28–8.59 (m, 7 H, aromatic CH), 9.90 (br s, 1 H, NH), 10.12 (br s, 1 H, NH), 10.50 (s, 1 H, NH), 11.01 (s, 1 H, NH). Anal. (C<sub>15</sub>H<sub>15</sub>N<sub>7</sub>S<sub>2</sub>·H<sub>2</sub>O) C, H, N, S.

**2-Acetylpyridine 5-[(2-Chloroanilino)thiocarbonyl]thiocarbonohydrazone (28).** Reaction by method B of 14.00 g (66.9 mmol) of 2-acetylpyridine thiocarbonohydrazone (4a) and 12.0 g (70.7 mmol) of 2-chlorophenyl isothiocyanate in 500 mL of dry DMF gave a yellow solid. Recrystallization from DMF/H<sub>2</sub>O afforded 20.1 g (79%) of 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl]thiocarbonohydrazone (28) as an off-white powder: mp 159–160 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.48 (s, 3 H, CH<sub>3</sub>), 7.17–8.58 (m, 8 H, aromatic CH), 9.39 (br s, 1 H, NH), 9.95 (br s, 1 H, NH), 10.52 (br s, 1 H, NH), 10.96 (s, 1 H, NH). Anal. (C<sub>15</sub>H<sub>15</sub>ClN<sub>6</sub>S<sub>2</sub>) C, H, N, S, Cl.

**2-(3-Methylbutyryl)pyridine 5-[(2-Chloroanilino)thiocarbonyl]thiocarbonohydrazone (51).** To a solution of 4.06 g (24.8 mmol) of isobutyl 2-pyridyl ketone [2-(3-methylbutyryl)pyridine (Alfred Bader Chemicals, Aldrich Chemical Co., Milwaukee, WI)] in 150 mL of methanol was added 2.64 g (24.8 mmol) of thiocarbonohydrazide. The resulting mixture was heated at reflux, under nitrogen, overnight. A precipitate was collected by filtration, washed with cold methanol, and dried to give 4.17 g of crude 2-(3-methylbutyryl)pyridine thiocarbonohydrazone (4d). The crude material was dissolved in 100 mL of dry DMF and 4.48 g (26.4 mmol) of 2-chlorophenyl isothiocyanate was added. The resulting mixture was stirred at room temperature for 45 min. The solution was cooled in an ice bath and H<sub>2</sub>O was added. A precipitate was collected, washed with H<sub>2</sub>O, and dried. Recrystallization from ether/hexane afforded 1.30 g (18%) of 2-(3-methylbutyryl)pyridine 5-[(2-chloroanilino)thiocarbonyl]thiocarbonohydrazone (51) as yellow-orange crystals: mp 75 °C (dec, turned bright red and evolved H<sub>2</sub>S to resolidify and at 98–101 °C decomposed again to give a dark red melt); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 0.86 (d, 6 H, *J* = 6.6, CH<sub>3</sub>), 1.98 (m, 1 H, aliphatic CH), 3.06 (d, 2 H, *J* = 7.3, CH<sub>2</sub>), 7.34 (m, 5 H, aromatic CH), 7.78 (m, 2 H, aromatic CH), 8.56 (d, 1 H, *J* = 4.1, aromatic CH), 9.25–10.80 (br, 3 H, NH), 10.91 (br s, 1 H, NH). Anal. (C<sub>18</sub>H<sub>21</sub>ClN<sub>6</sub>S<sub>2</sub>) C, H, N, S, Cl.

**2-Acetylpyridine 5-[(2-Chloroanilino)thiocarbonyl]thiocarbonohydrazone 1-Oxide (55).** After reaction by method B of 1.79 g (7.64 mmol) of 2-acetylpyridine thiocarbonohydrazone

1-oxide hemihydrate (4e) and 1.30 g (7.66 mmol) of 2-chlorophenyl isothiocyanate in 65 mL of dry DMF for 4 days, removal of solvent in vacuo gave an orange oil. To this oil was added 10 mL of DMF, the resulting solution was chilled to 0 °C, and 200 mL of H<sub>2</sub>O was added dropwise. The resulting precipitate was collected by filtration. Recrystallization from DMF/H<sub>2</sub>O afforded 1.54 g (83%) of 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl]thiocarbonohydrazone 1-oxide (55) as a yellow powder: mp 127–143 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.28 (s, 3 H, CH<sub>3</sub>), 7.17–7.76 (m, 7 H, aromatic CH), 8.25 (d, 1 H, *J* = 6, aromatic CH), 9.37 (br s, 1 H, NH), 9.90 (br s, 1 H, NH), 10.42 (br s, 1 H, NH), 11.11 (s, 1 H, NH). Anal. (C<sub>15</sub>H<sub>15</sub>ClN<sub>6</sub>OS<sub>2</sub>·0.2C<sub>3</sub>H<sub>7</sub>NO·0.3H<sub>2</sub>O) C, H, N, S, Cl.

**2-Acetylpyridine [5-(2-Chloroanilino)-1,3,4-thiadiazol-2-yl]hydrazone (7).** To a solution of 0.51 g (1.35 mmol) of 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl]thiocarbonohydrazone (28) in 50 mL of MeOH were added 1 mL of triethylamine and 160 μL of 90% *tert*-butyl hydroperoxide (Aldrich). The resulting mixture was stirred at room temperature for 75 min and the resulting precipitate was collected by filtration to afford 0.42 g (85%) of 2-acetylpyridine [5-(2-chloroanilino)-1,3,4-thiadiazol-2-yl]hydrazone (7) as an off-white powder: mp 238–240 °C dec; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 2.35 (s, 3 H, CH<sub>3</sub>), 7.00 (dt, 1 H, *J* = 1.5, 6.5, aromatic CH), 7.32 (m, 2 H, aromatic CH), 7.44 (dd, 1 H, *J* = 1.4, 8, aromatic CH), 7.80 (dt, 1 H, *J* = 1.7, 8, aromatic CH), 7.93 (d, 1 H, *J* = 8, aromatic CH), 8.21 (br d, 1 H, *J* = 8, aromatic CH), 8.55 (m, 1 H, aromatic CH), 9.24 (br s, 1 H, NH), 11.46 (s, 1 H, NH); mass spectrum, *m/e* 345 (59.64), 227 (37.60), 121 (100). Anal. (C<sub>15</sub>H<sub>13</sub>ClN<sub>6</sub>S) C, H, N, S, Cl.

**Acknowledgment.** We thank Mr. Douglas Minick for p*K*<sub>a</sub> and log *k'*<sub>w</sub> measurements, Ms. Donna Eagleson and Mr. Derrick Justice for assistance in preparation of the tables, and Dr. David Porter for helpful discussions. We gratefully acknowledge the excellent technical assistance of cooperative education students Mr. Paul Friedrich, Ms. Amy Stout, Ms. Lisa Hefner, Mr. Tom Breeden, Mr. Ken Davis, and Mr. Boyd Safrit, as well as summer student Mr. Mark Harris. We thank Drs. Thomas Krenitsky and David Barry for their continued interest and support.

**Registry No.** 2, 96860-23-0; 3a, 1122-62-9; 3d, 6952-53-0; 3e, 2457-50-3; 4a, 96699-87-5; 4b, 106834-99-5; 4c, 128409-79-0; 4d, 127142-68-1; 4e, 140835-37-6; 4f, 140835-38-7; 6, 127142-11-4; 7, 127142-30-7; 8, 127142-67-0; 9, 127142-21-6; 10, 127142-19-2; 11, 127142-00-1; 12, 127142-03-4; 13, 127142-08-9; 14, 127142-22-7; 15, 127142-02-3; 16, 127142-49-8; 17, 128409-78-9; 18, 127142-50-1; 19, 140835-39-8; 20, 127142-10-3; 21, 127142-58-9; 22, 127142-09-0; 23, 127142-35-2; 24, 127142-05-6; 25, 127142-36-3; 26, 127157-38-4; 27, 127142-16-9; 28, 127142-14-7; 29, 127142-13-6; 30, 127142-12-5; 31, 127142-15-8; 32, 127142-48-7; 33, 140835-40-1; 34, 127142-34-1; 35, 127142-44-3; 36, 127142-45-4; 37, 127142-51-2; 38, 127142-37-4; 39, 127142-39-6; 40, 127142-43-2; 41, 127142-04-5; 42, 127142-07-8; 43, 127142-25-0; 44, 127142-56-7; 45, 127142-55-6; 46, 127142-23-8; 47, 127142-59-0; 48, 127142-24-9; 49, 127157-39-5; 50, 127157-40-8; 51, 127142-27-2; 52, 127142-26-1; 53, 140835-41-2; 54, 140835-42-3; 55, 140835-43-4; H<sub>2</sub>NNHC(S)NHNH<sub>2</sub>, 2231-57-4; MeN=C=S, 556-61-6; 4-MeC<sub>6</sub>H<sub>4</sub>N=C=S, 2131-61-5; 4-CNC<sub>6</sub>H<sub>4</sub>N=C=S, 2719-32-6; 2-ClC<sub>6</sub>H<sub>4</sub>N=C=S, 2740-81-0; ribonucleotide reductase, 9047-64-7.

**Supplementary Material Available:** Enzyme inhibition (Table IV) and chemical data (Table V) for 11 additional thiocarbonohydrazones, enzyme inhibition data at inhibitor concentrations not reported herein (Table VI), and <sup>1</sup>H-NMR and analytical data for all new compounds are provided (13 pages). Ordering information is given on any current masthead page.