Tubulin Polymerization Inhibition Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[42] Determination of IC_{50} values for the polymerization of purified tubulin was performed as described in detail elsewhere.[6] 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 μM) 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.

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

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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.[1] HSV-1 encodes a unique ribonucleotide reductase (EC 1.17.4.1) in infected cells[2,3] that catalyzes the reduction of all four ribonucleoside diphosphates to 2′-deoxynucleoside diphosphates.[4,5] In marked contrast to the mammalian enzyme, which is highly regulated by nucleoside triphosphates,[6,7] the viral enzyme is insensitive to allosteric control.[8-10] Indeed, HSV-1 is able to replicate in the presence of thymidine at concentrations that are inhibitory to host cell DNA synthesis.[4] 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.[10]

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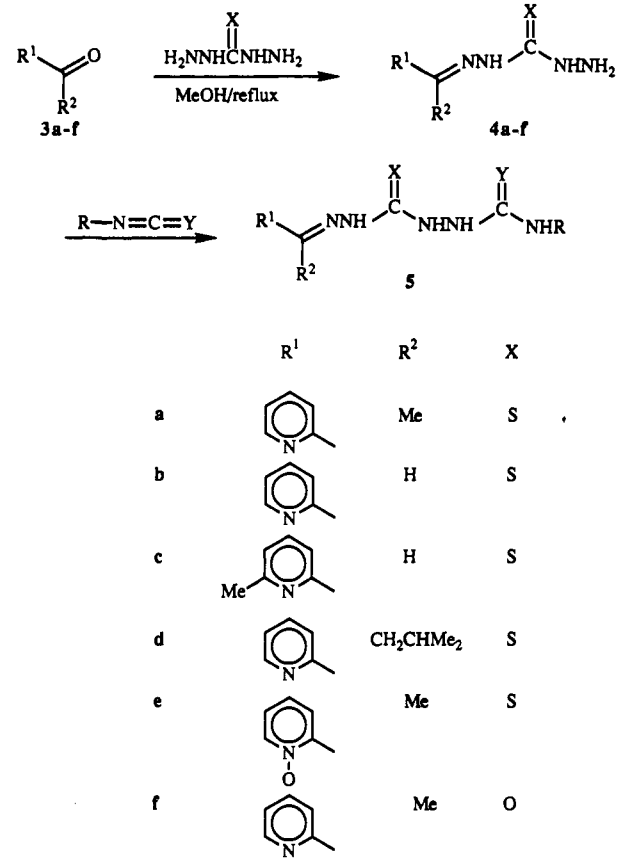
The value of herpes virus ribonucleotide reductase as a target for antiviral drugs used as single-agent therapies, however, is controversial.[11-19] Nevertheless, herpes virus infected cells treated with the antiviral drug acyclovir (ACV)[20-23] develop increased pools of deoxynucleotide dGTP, which can compete with the binding of acyclovir triphosphate (ACVTP) to herpes DNA polymerases.[24-27] Therefore, herpes virus ribonucleotide reductase inactivators in combination with ACV have been studied in these laboratories to prevent the build-up of dGTP.[10,28] 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.[29] While ACV is a potent and selective antiherpetic agent that is clinically effective against systemic and local infections caused by herpes simplex viruses,[30] treatment of herpes labialis in-

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



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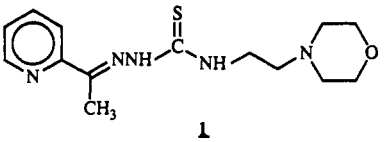
fections is one area where its effectiveness could be improved.[31-35] Futhermore, antiviral drug resistance has been observed with increasing frequency in patients with profound immune deficiency.[36-39] Clearly, improved therapies are needed for immunocompromised patients.

Recently, derivatives of 2-acetylpyridine thiosemicarbazone have been reported that inhibit HSV-1 ribonucleotide reductase[40,41] and demonstrate antiviral activity in vitro.[42-44] 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.[25,45] Subsequently, thiocarbonohydrazone 2, a more potent inactivator,[46] was found to potentiate ACV both in vitro[26] and as topical treatment in vivo.[47,48] Both compounds were found to decrease the dGTP pools in herpes virus infected cells and to markedly increase the pools of ACVTP.[25,26] Further studies, however, indicated hematological toxicity in rats dosed orally with 60 mg/kg of 2 (details of these studies will be published elsewhere[49]); 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.



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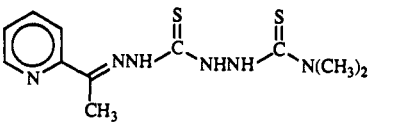

2

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).[50-53] Intermediates 4a–f can be readily prepared in large quantity, without contamination from possible bis-adducts,[54] by treating the corresponding aldehydes and ketones with carbohydrazide (X = O) or thiocarbohydrazide (X = S) in methanol at reflux.

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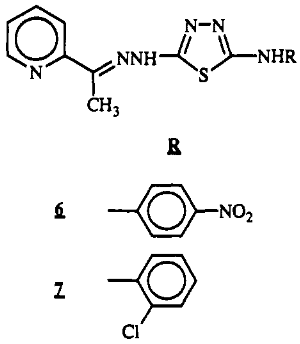
Reaction of intermediates **4a–f** with isothiocyanates initially were conducted in ethanol at reflux (method A).[53] We later found that changing the solvent to DMF facilitated reaction at room temperature (method B).[50–52] This discovery was useful for the preparation of analogues of **5** where R 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_2S, 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[53] 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[58] as described previously.[46,59] 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.

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

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

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 ±5 μM 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.[46,59] 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

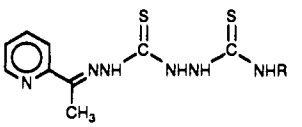
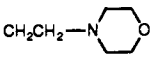
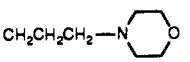
New thiocarbonohydrazones listed in Table I (data for lead compound **2** is provided for reference) are divided into four sets. Three of these sets are based on the R group at the terminus of the thiocarbonohydrazone side chain, where R is (1) an alkyl group, compounds **8–12**, (2) an acyl group, compounds **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**,[25,45] 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 no.	R	HSV-1 enzyme k_{inact}[a]	HSV-1 enzyme % inhib[a]	HSV-1 enzyme inhib index[b]	human enzyme k_{inact}[a]	human enzyme % inhib[a]	human enzyme inhib index[b]	apparent enzyme selectivity[c]
								
Set 1. R = alkyl group								
2[d]		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		11	24	15	0.4	31	1.0	15
12		6.1	12	7.0	0	23	0.29	24
Set 2. R = acyl group								
13	COPh	15[e]	38[e]	24[e]	2	54	5.4	4.5[f]
14	CO_2Et	6	14	7	2	40	3.9	1.8
Set 3. R = aryl group								
15	Ph	20	7	21	3	59	8.5	2.5
16	2-MeC_6H_4	10	27	14	1	51	3	4.6
17	3-MeC_6H_4	19	28	26	0	54	1.1	23
18	4-MeC_6H_4	16	25	21	2	64	7	3.0
19	4-t-Bu-C_6H_4	16[g]	10[g]	18[g]	ND[h]	ND	ND	ND
20	4-$NO_2C_6H_4$	33[i]	14[i]	38[i]	1.3	56	4.2	9.1[f]
21	3-NO_2-4-ClC_6H_3	45	7	48	0	20	0.24	200
22	2-MeO-5-$NO_2C_6H_3$	40	44	71	3	11	3.5	21
23	2-$CF_3C_6H_4$	10	6	11	3	43	5.9	1.8
24	3-$CF_3C_6H_4$	34	15	40	1	31	1.9	21
25	4-$CF_3C_6H_4$	33	59	80	0	19	0.23	350
26	3-CNC_6H_4	32	0	32	0.6	80	6.6	4.8
27	4-CNC_6H_4	29	0	29	0	64	1.7	17
28	2-ClC_6H_4	27	0	27	0	57	1.3	21
29	3-ClC_6H_4	36	50	72	0	48	0.9	80
30	4-ClC_6H_4	39	61	99	0	73	2.6	38
31	2-BrC_6H_4	21	38	34	0	14	0.16	210
32	3-BrC_6H_4	40	15	47	2	21	2.8	17
33	4-BrC_6H_4	44	23	57	2	29	3.2	18
34	2-IC_6H_4	29	18	35	4	23	5.4	6.5
35	3-IC_6H_4	25	27	34	3	7	3.3	10
36	4-IC_6H_4	22	24	29	0	5	0.05	560
37	2-Me-4-ClC_6H_3	29	31	42	1	55	3.4	12
38	2,3-$Cl_2C_6H_3$	49	29	68	0	30	0.42	160
39	2,5-$Cl_2C_6H_3$	55	8	59	0	20	0.24	240
40	2,4,5-$Cl_3C_6H_2$	16	22	21	0	3	0.03	680
41	2-$MeOC_6H_4$	23	0	23	2.1	44	4.5	5.1
42	3-$MeOC_6H_4$	21	6.7	22	7[j]	58[j]	1.7[j]	1.3[f]
43	4-$MeOC_6H_4$	16	45	29	0	52	1.1	28
44	2-MeO-3-ClC_6H_3	25	0	25	0	50	1.0	26
45	2-MeO-5-ClC_6H_3	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

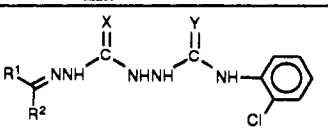
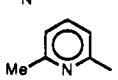
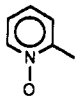
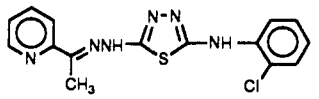
compound no.	R^1	R^2	X	Y	HSV-1 enzyme k_{inact}[a]	HSV-1 enzyme % inhib[a]	HSV-1 enzyme inhib index[b]	human enzyme k_{inact}[a]	human enzyme % inhib[a]	human enzyme inhib index[b]	apparent enzyme selectivity[c]
											
Set 4. Analogues of 28											
49		H	S	S	12	51	25	0	45	0.80	31
50		H	S	S	16	41	27	0	65	1.8	15
51		CH_2CHMe_2	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)

compound					HSV-1 enzyme			human enzyme			apparent enzyme selectivity[c]
no.	R^1	R^2	X	Y	k_{inact}[a]	% inhib[a]	inhib index[b]	k_{inact}[a]	% inhib[a]	inhib index[b]	
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

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

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_{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_{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_{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_{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_{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 relatvie 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 in vivo models as potentiators of ACV; details of these studies will be reported elsewhere.[49] From these studies, compound 28, which also inactivates VZV and HSV-2 ribonucleotide reductase,[59] was selected for development as combination topical therapy with ACV for cutaneous herpes virus infections.[60]

A study of the mode of inactivation of HSV-1 and human ribonucleotide reductases by 28 was recently published.[59] 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.[61]) 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 5021437, 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[a]

no.	formula[b,c]	analyses[b]	synthetic method[d]	mp (°C)[e]	yield (%)[f]
4a	$C_8H_{11}N_5S$	C,H,N,S		185–186	97
4b	$C_7H_9N_5S \cdot 0.4MeOH \cdot 0.1H_2O$	C,H,N,S		162–164	92
4c	$C_8H_{11}N_5S \cdot 0.5MeOH \cdot 0.2H_2O$	C,H,N,S		166–168	89
4e	$C_8H_{11}N_5OS \cdot 0.5H_2O$	C,H,N,S		167.5–168.5	96
4f	$C_8H_{11}N_5O$	C,H,N		200.5–201.5	91
8[g]	$C_{10}H_{14}N_6S_2$	C,H,N,S	A	178.0–178.5	93
9	$C_{14}H_{20}N_6S_2 \cdot 0.5H_2O$	C,H,N,S	B	180–182	83
10[h]	$C_{19}H_{26}N_6S_2 \cdot 0.75C_3H_6O$	C,H,N,S	B	172–177	11
11	$C_{15}H_{23}N_7OS_2$	C,H,N,S	A	161–162	69
12	$C_{16}H_{25}N_7OS_2$	C,H,N,S	A	195–197	85
13	$C_{16}H_{16}N_6OS_2$	C,H,N,S	A	195–196	95
14	$C_{12}H_{16}N_6O_2S_2$	C,H,N,S	B	195–197	71
15	$C_{15}H_{16}N_6S_2$	C,H,N,S	A	178–180	87
16	$C_{16}H_{18}N_6S_2$	C,H,N,S	B	168.5–175.5	76
17	$C_{16}H_{18}N_6S_2 \cdot 0.10C_3H_7NO \cdot 0.15H_2O$	C,H,N,S	B	173.5–177.0	98
18	$C_{16}H_{19}N_6S_2 \cdot 0.05C_3H_7NO$	C,H,N,S	B	154.0–155.5	95
19	$C_{19}H_{24}N_6S_2$	C,H,N,S	B	187–188	56
20	$C_{15}H_{15}N_7O_2S_2 \cdot 0.95H_2O$	C,H,N,S	A	230	17
21	$C_{15}H_{14}ClN_7O_2S_2 \cdot 1.1C_3H_7NO \cdot 0.2H_2O$	C,H,N,S,Cl	B	151.5–152.5	97
22	$C_{16}H_{17}N_7O_3S_2$	C,H,N,S	A	212–213	80
23	$C_{16}H_{15}F_3N_6S_2 \cdot 0.2C_3H_7NO$	C,H,N,S	B	118.8–120.5	95
24	$C_{16}H_{15}F_3N_6S_2$	C,H,N,S	A	181–184	61
25	$C_{16}H_{15}F_3N_6S_2 \cdot 0.05C_3H_7NO$	C,H,N,S	B	165.5–183.5	99
26	$C_{16}H_{15}N_7S_2 \cdot 0.9H_2O$	C,H,N,S	B	178–180	94
27	$C_{16}H_{15}N_7S_2 \cdot H_2O$	C,H,N,S	B	241–242	100
28	$C_{15}H_{15}ClN_6S_2$	C,H,N,S,Cl	B	159–160	79
29	$C_{15}H_{15}ClN_6S_2$	C,H,N,S,Cl	B	168–171	100
30	$C_{15}H_{15}ClN_6S_2$	C,H,N,S,Cl	B	181–182	91
31	$C_{15}H_{15}BrN_6S_2$	C,H,N,S,Br	B	155–158	100
32	$C_{15}H_{15}BrN_6S_2 \cdot 0.07C_3H_7NO$	C,H,N,S,Br	B	161.5–165.0	87
33	$C_{15}H_{15}BrN_6S_2 \cdot 0.04C_3H_7NO$	C,H,N,S,Br	B	167.0–168.5	98
34	$C_{15}H_{15}IN_6S_2 \cdot 0.02C_3H_7NO$	C,H,N,S,I	B	161.5–162.7	92
35	$C_{15}H_{15}IN_6S_2$	C,H,N,S,I	B	159.5–161.0	96
36	$C_{15}H_{15}IN_6S_2 \cdot 0.03C_3H_7NO$	C,H,N,S,I	B	173.5–174.5	97
37	$C_{16}H_{17}ClN_6S_2 \cdot 0.02C_3H_7NO$	C,H,N,S,Cl	B	165.5–166.5	88
38	$C_{15}H_{14}Cl_2N_6S_2 \cdot 0.85C_3H_7NO \cdot 0.1H_2O$	C,H,N,S,Cl	B	156.3–157.5	98
39	$C_{15}H_{14}Cl_2N_6S_2 \cdot 0.7C_3H_7NO$	C,H,N,S,Cl	B	119–121	96
40	$C_{15}H_{13}Cl_3N_6S_2 \cdot 0.75C_3H_7NO$	C,H,N,S,Cl	B	133.5–135.5	100
41	$C_{16}H_{18}N_6OS_2 \cdot 0.15C_3H_7NO \cdot 0.15H_2O$	C,H,N,S,Cl	A	180–182	29
42	$C_{16}H_{18}N_6OS_2$	C,H,N,S	A	172–176	86
43	$C_{16}H_{18}N_6OS_2 \cdot 0.05C_3H_7NO \cdot 0.45H_2O$	C,H,N,S	A	162–164	62
44	$C_{16}H_{17}ClN_6OS_2$	C,H,N,S,Cl	B	170–171	99
45	$C_{16}H_{17}ClN_6OS_2 \cdot 0.02C_3H_7NO$	C,H,N,S,Cl	B	156.5–158.0	87
46	$C_{14}H_{15}N_7S_2 \cdot C_3H_7NO$	C,H,N,S	B	204–205	53
47	$C_{14}H_{15}N_7S_2 \cdot 0.05C_3H_7NO$	C,H,N,S	B	176–179	79
48	$C_{14}H_{15}N_7S_2 \cdot 0.05C_3H_7NO \cdot H_2O$	C,H,N,S	B	179–180	82
49[i]	$C_{14}H_{13}ClN_6S_2 \cdot 0.05CH_4O \cdot 0.1H_2O$	C,H,N,S,Cl	B	167–168	84
50[i]	$C_{15}H_{15}ClN_6S_2 \cdot 0.7CH_4O \cdot 0.2H_2O$	C,H,N,S,Cl	B	151–153	84
51	$C_{18}H_{21}ClN_6S_2$	C,H,N,S,Cl	B	75	18[j]
52	$C_{15}H_{15}ClN_6OS$	C,H,N,S,Cl	B	177–179	67
53	$C_{15}H_{15}ClN_6OS \cdot 0.4C_3H_7NO \cdot 0.85H_2O$	C,H,N,S,Cl	B	132.5–135.0	100
54	$C_{15}H_{15}ClN_6O_2 \cdot 0.1C_3H_7NO \cdot 0.05H_2O$	C,H,N,S,Cl	B	191.5–193.5	68
55	$C_{15}H_{15}ClN_6OS_2 \cdot 0.2C_3H_7NO \cdot 0.3H_2O$	C,H,N,S,Cl	B	127–143	83

[a] Data for 6 and 7 are presented in the Experimental Section. [b] Satisfactory elemental analyses (±0.4%) were obtained for all elements (C, H, N; S, Cl, Br, and I, where present). [c] C_3H_6O indicates acetone; C_3H_7NO, *N,N*-dimethylformamide; CH_4O, methanol. [d] Methods used for the preparation of target thiocarbonohydrazones from 4a–f are indicated as A or B (see Experimental Section). [e] All compounds decomposed on melting (melting points are not corrected). [f] 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. [g] The assigned structure is supported by correlation of the UV and ^{13}C NMR spectra of 8 with those of 2, for which an X-ray crystal structure was obtained (R. Morrison, unpublished results). [h] Recrystallized from acetone. [i] Washed with methanol. [j] 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 supplmentary 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,[62]

(62) Fairfull, A. E. S.; Peak, D. A. Dithiobiurets. Part I. Some 1- and 1,5-Substituted Derivatives. *J. Chem. Soc.* 1955, 796–802.

Table III. Physical Parameters of Selected Thiocarbonohydrazones

no.	pK_a[a]	log P[b]	log $k'w$[c]
2	4.1, 8.4	1.25	1.28
24	3.89, 6.79	ND[d]	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

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

3-pyridyl,[63] and 4-pyridyl isothiocyanate[64] 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, thiocarbonohydrazone products were purified by dissolving in a minimal amount of *N,N*-dimethylformamide (DMF) at 0 °C and triturating the resulting solutions with cold H_2O; the resulting precipitates were isolated by filtration. All final products were washed with H_2O or methanol and dried in a vacuum desiccator at room temperature overnight (12–18 h). ^{1}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 tabluated 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[4] and human[65,66] ribonucleotide reductases were partially purified as described in the indicated references. The enzymes were assayed by monitoring the reduction of purified[4] [^{14}C]CDP. Herpes virus ribonucleotide reductase was assayed in glass vials containing 200 mM Hepes Na^+ at pH 7.7, 10 mM dithiothreitol, and 2 μM (500 Ci/mol) CDP ($3–4 \times K_m$)[4,5,67] as previously described.[4] Human ribonucleotide reductase was assayed in glass vials containing 100 mM Hepes at pH 7.4, 5 mM dithiothreitol, 12 μM CDP ($5 \times K_m$) with a specific activity of 75 Ci/mol, 5 mM ATP, and 6 mM $MgCl_2$.[68] The ATP, which was contaminated with iron, imparted about 3 μM iron in the reaction mixtures,[46] and ensured that the inhibitors would be in the iron-complexed form.[46,59] 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.[46,59] Multiple reactions were assayed simultaneously as described elsewhere.[69] The reactions were terminated with hydroxyurea and EDTA, which quenched the reaction and decreased the blank rates to an insignificant level.[4,65] The product was dephosphorylated and isolated by chromatography on a Dowex-borate column according to the modified method[4] of Steeper and Steuart.[70] 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 thiocarbohydrazide (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; ^{1}H-NMR (DMSO-d_6) δ 2.34 (s, 3 H, CH_3), 5.00 (br s, 2 H, NH_2), 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. ($C_8H_{11}N_5S$) 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**)[71] in 300 mL of methanol was added 26.3 g (248 mmol) of thiocarbohydrazide. 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; ^{1}H-NMR (DMSO-d_6) δ 2.22 (s, 3 H, CH_3), 4.92 (br s, 2 H, NH_2), 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. ($C_8H_{11}N_5OS \cdot 0.5H_2O$) 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; ^{1}H-NMR (DMSO-d_6) δ 2.39 (s, 3 H, $COCH_3$), 2.86 (d, 3 H, J = 4, NCH_3), 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. ($C_{10}H_{14}N_6S_2$) C, H, N, S.

2-Acetylpyridine 5-[(4-Nitroanilino)thiocarbonyl]thiocarbonohydrazone (20) and 2-Acetylpyridine [5-(4-Nitroanilino)-1,3,4-thiadiazol-2-yl]hydrazone (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_2O caused precipitation of a red solid, which was collected by filtration, washed with H_2O, ethanol, and ether, and dried to give 0.95 g (11%) of 2-acetylpyridine [5-(4-nitroanilino)-1,3,4-thiadiazol-2-yl]hydrazone (**6**) as an orange-red powder: mp > 260 °C; ^{1}H-NMR (DMSO-d_6) δ 2.37

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(s, 3 H, CH_3), 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. ($C_{15}H_{13}N_7O_2S \cdot 0.35H_2O$) 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; ^{1}H-NMR (DMSO-d_6) δ 2.49 (s, 3 H, CH_3), 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 $C_{15}H_{15}N_7O_2S_2 \cdot 0.95H_2O$) 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_2O was added. A precipitate was collected, washed with H_2O, 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; ^{1}H-NMR (DMSO-d_6) δ 2.42 (s, 3 H, CH_3), 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. ($C_{16}H_{15}N_7S_2 \cdot H_2O$) 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_2O afforded 20.1 g (79%) of 2-acetylpyridine 5-[(2-chloroanilino)-thiocarbonyl]thiocarbonohydrazone (**28**) as an off-white powder: mp 159–160 °C dec; ^{1}H-NMR (DMSO-d_6) δ 2.48 (s, 3 H, CH_3), 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. ($C_{15}H_{15}ClN_6S_2$) C, H, N, S, Cl.

2-(3-Methylbutyryl)pyridine 5-[(2-Chloroanilino)-carbonyl]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 thiocarbohydrazide. 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_2O was added. A precipitate was collected, washed with H_2O, 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_2S to resolidify and at 98–101 °C decomposed again to give a dark red melt); ^{1}H-NMR (DMSO-d_6) δ 0.86 (d, 6 H, J = 6.6, CH_3), 1.98 (m, 1 H, aliphatic CH), 3.06 (d, 2 H, J = 7.3, CH_2), 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. ($C_{18}H_{21}ClN_6S_2$) 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_2O was added dropwise. The resulting precipitate was collected by filtration. Recrystallization from DMF/H_2O 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; ^{1}H-NMR (DMSO-d_6) δ 2.28 (s, 3 H, CH_3), 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. ($C_{15}H_{15}ClN_6OS_2 \cdot 0.2C_3H_7NO \cdot 0.3H_2O$) 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; ^{1}H-NMR (DMSO-d_6) δ 2.35 (s, 3 H, CH_3), 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. ($C_{15}H_{13}ClN_6S$) C, H, N, S, Cl.

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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 ^{1}H-NMR and analytical data for all new compounds are provided (13 pages). Ordering information is given on any current masthead page.