Synthesis, Biological Evaluation, and Quantitative Structure–Activity Relationship Analysis of New Schiff Bases of Hydroxysemicarbazide as Potential Antitumor Agents[†]

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Thirty Schiff bases of hydroxysemicarbazide (Ar–CH=NNHCONHOH) have been synthesized and tested against L1210 murine leukemia cells. The IC_{50} values were found to be in a range from 2.7×10^{-6} to 9.4×10^{-4} M. A total of 17 out of the 30 compounds had higher inhibitory activities than hydroxyurea (an anticancer drug currently used for the treatment of melanoma, leukemia, and ovarian cancer) against L1210 cells. Six compounds with IC_{50} values in micromolar range were 11- to 30-fold more potent than hydroxyurea ($IC_{50} = 8.2 \times 10^{-5}$ M). The partition coefficient (log P) and ionization constants (pK_a) of a model compound [1-(3-trifluoromethylbenzylidene)-4-hydroxysemicarbazide, **1**] were measured by the shake-flask method, and the measured log P was used to derive Hansch–Fujita π constant of –CH=NNHCONHOH. On the basis of the newly derived π and those of other moieties, the partition coefficients (SlogP) of the other 29 compounds were calculated by the summation of π values. Quantitative structure–activity relationship (QSAR) analysis showed that, besides the essential pharmacophore (–NHCONHOH), hydrophobicity (SlogP), molecular size/polarizability (calculated molar refractivity), and the presence of an oxygen-containing group at the ortho position (I) were important determinants for the antitumor activities. In conclusion, the results obtained in this study show that several Schiff bases of hydroxysemicarbazide are potent inhibitors of tumor cells and warrant further investigation as cancer chemotherapeutic agents.

Introduction

Ribonucleotide reductase (RR) is a key enzyme that catalyzes the reduction of ribonucleotides to deoxyribonucleotides and provides the building blocks for the de novo DNA synthesis in all dividing cells. Previous studies have clearly indicated that RR plays a fundamental role in the critical early events involved in tumor promotion, and the RR activity is tightly linked to the neoplastic expression state.[1] Therefore, RR is a promising target for the design and development of anticancer drugs.

Several different classes of compounds such as hydroxyguanidine, thiosemicarbazone, and substituted benzohydroxamic acid and their derivatives have been shown to be RR inhibitors by Lien's group,[2–10] Sartorelli's group,[11,12] and others.[1,13–15] Submicromolar median inhibitory concentrations (IC_{50}) have been observed against various tumor cell lines and viruses for several compounds. Some of the active compounds have shown in vivo anticancer activities and clinical potential.[5,12] On the basis of previous studies, an essential pharmacophore [–C(=X)NHOH; X = O, NH] has been identified for antitumor/antiviral activities.[2,10]

Among all RR inhibitors investigated, only hydroxyurea (HU) has been marketed and used for the treatment of melanoma, chronic myelocytic leukemia, and recurrent, metastatic, or inoperable ovarian cancer. HU is also used in the local control of primary squamous cell carcinomas of the head and neck.[16] Investigation of HU has gained new momentum in the wake of success stories about combination therapy in cancer and AIDS treatment.[17–19] At the present time, HU is the first-line chemotherapeutic agent in the chronic phase of myelogenous leukemia. Clinical data have clearly shown the superiority of HU over busulfan.[18] HU has also been used in the treatment of AIDS in combination with didanosine, showing no viral rebound after 1 year's treatment.[19] HU causes an immediate inhibition of DNA synthesis by acting on the R_2 subunit of the RR enzyme by one-electron transfer from HU to the tyrosyl free radical and by destabilizing the iron center.[20,21] Therapeutic application of HU has several disadvantages such as short half-life (1.9–3.9 h) in patients due to its small molecular size (MW = 76.06) and extremely polar nature (log $P_{o/w} = -1.80$), the necessity of using high

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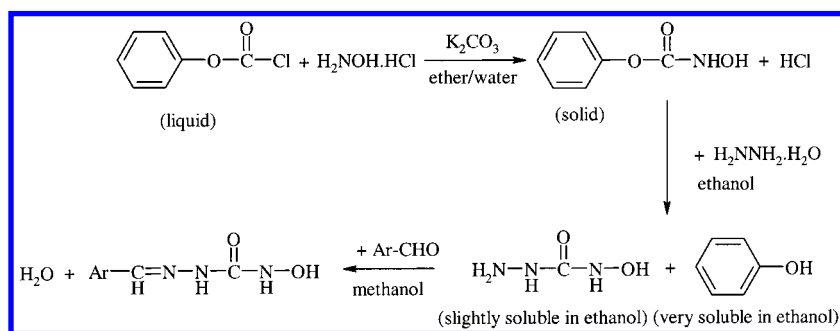[‡] School of Pharmacy, University of Southern California.
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Scheme 1

dosage (80 mg/kg every third day or 20−30 mg/kg daily), and the rapid development of resistance.[16,22,23]

To obtain more potent and selective RR inhibitors, a series of Schiff bases of hydroxysemicarbazide (SB-HSC) with the essential pharmacophore (−NHCONHOH) and diverse hydrophobic, electronic, and steric properties are designed. We now report the synthesis, in vitro antitumor activity against murine leukemia L1210 cells, and quantitative structure−activity relationship (QSAR) analysis of the SB-HSCs.

Results and Discussion

Chemistry. The SB-HSCs were prepared by a dehydration reaction of hydroxysemicarbazide (HSC) with different aromatic and heterocyclic aldehydes. HSC, which was not commercially available, was synthesized in two steps using modified procedures of Grobner and Steinberg (see Scheme 1).[24,25] The percent yields and corrected melting points are summarized in Table 1.

The logarithm of partition coefficient (log P) in 1-octanol/phosphate buffer (pH 7.3) and ionization constant (pK_a) values of compound **1** were measured. The log P value of compound **1** was found to be 1.64. There are two pK_a values for compound **1**, with a basic group (= N−, pK_a = 1.87) and an acidic group (−OH, pK_a = 10.16).

Hansch−Fujita π substituent constants of =NNH-CONHOH and −CH=NNHCONHOH were derived from the measured log P of compound **1** and other π constants as the following:

$$\pi_{=NNHCONHOH} = \log P - \pi_{-C6H4} - \pi_{-CF3} - \pi_{-CH=} = $$
$$1.64 - 1.96 - 0.88 - 0.33 = -1.53$$

$$\pi_{-CH=NNHCONHOH} = \log P - \pi_{-C6H4} - \pi_{-CF3} = 1.64 - $$
$$1.96 - 0.88 = -1.20$$

From these, the SlogP values of other 29 compounds were calculated by taking the sum of π values of constituent groups and were used in subsequent QSAR analysis as well as ClogP from CQSAR program (vide infra).

The stability study in phosphate buffers demonstrated that there was no change in UV absorbance in 72 h at pH 3 for compound **1**. At higher pH, 2.7−21.3% changes in UV absorbance were observed. At 25 °C 97.3% of compound **1** remained at pH 7 and 78.7% remained at pH 11 at the end of 72 h. The data indicated that less than 3% of compound **1** was degraded in 72 h at neutral pH at room temperature. Since the formation of Schiff base is reversible, the change in the UV absorbance may
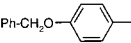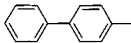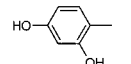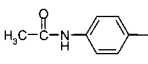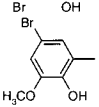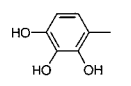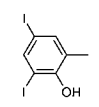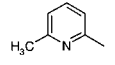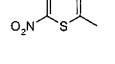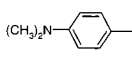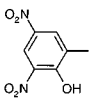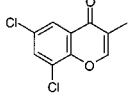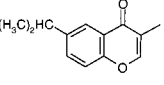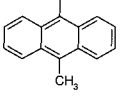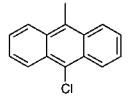
be due to breakage of the Schiff base bond (−CH=N−) in aqueous solution at higher pH.

Biological Study. The IC$_{50}$ values of the newly synthesized compounds were determined against murine leukemia L1210 cells using MTS/PES [MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, PES = phenazine ethosulfate] microculture tetrazolium assay in 96-well plates.[26−29] HU (H$_2$NCONHOH), HSC (H$_2$NHNCON-HOH), and semicarbazide (SC, H$_2$NHNCONH) were used as reference compounds for comparison. The IC$_{50}$ values of the compounds (see Table 2) range from 2.7 × 10^{-6} to 9.4 × 10^{-4} M. Compounds **2**, **4**, **7**, **21**, **29**, and **30** are the most active compounds with IC$_{50}$ values in the micromolar range. According to the chemical structures, the six most active compounds can be divided into three groups. Compounds **2**, **4**, and **7** containing 2-OH-3,5-dihalogen-substituted phenyl rings consist of the first group. Compound **21** with a 2,3,4-OH-substituted phenyl ring represents the second group. Compounds **29** and **30** belong to the third group because both of them have substituted anthryl moieties. The IC$_{50}$ ratios of HU over compounds **2**, **4**, **7**, **21**, **29**, and **30** range from 11 to 30, indicating that the six compounds are 11- to 30-fold more potent than HU against L1210 cells. It is interesting to note that compound **24** with a nitrothienyl group has an IC$_{50}$ value of 1.1 × 10^{-5} M. Compounds **10**, **20**, and **23** with IC$_{50}$ values greater than 8.0 × 10^{-4} M are the least active of the 30 Schiff bases. The ranking order (SC ≫ HSC > HU) of the IC$_{50}$ values of the three reference compounds reveals that the (N)−OH function is essential for antitumor activity (see Table 2).

QSAR. The CQSAR program of BioByte Corp. was utilized to calculate ClogP (calculated log P) and CMR (calculated molar refractivity) and to derive all regression equations.[30] SlogP was calculated by taking the sum of the π of −CH=NNHCONHOH derived from the experimental log P value of compound **1** and those of other moieties.[31−33]

The correlation between the inhibitory activities (log-($^1/_{IC50}$)) against L1210 cells and the physicochemical properties of the 30 SB-HSCs and two reference compounds (HU, HSC) was analyzed by a nonweighted least-squares method. Another compound, SC, was not included in the QSAR analysis because it lacked the common (N)−OH group and the IC$_{50}$ value was greater than 2.2 × 10^{-3} M. The physicochemical parameters examined included hydrophobic (ClogP, SlogP), steric (CMR), and electronic (e.g., dipole moment) parameters, as well as hydrogen bonding capacity (Hb). An indicator

Table 1. Percent Yields and Melting Points of Schiff Bases of Hydroxysemicarbazide Synthesized (Ar−CH=NNHCONHOH)

No.	Ar	formula	recry. solvent	yield, %	mp, °C (corrected)
1	(CF$_3$-, Cl-substituted aryl)	C$_9$H$_8$F$_3$N$_3$O$_2$	MeOH	72	205-207
2	(Cl, Cl, OH-substituted aryl)	C$_8$H$_7$Cl$_2$N$_3$O$_3$	MeOH	80	204-206
3	(Br, OH-substituted aryl)	C$_8$H$_8$BrN$_3$O$_3$	MeOH	69	225-227 (dec.)
4	(Br, Br, OH-substituted aryl)	C$_8$H$_7$Br$_2$N$_3$O$_3$	MeOH	67	210-212
5	(Br, H$_3$CO, OH-substituted aryl)	C$_9$H$_{10}$BrN$_3$O$_4$	MeOH	66	200-202
6	(I-substituted aryl)	C$_8$H$_8$IN$_3$O$_2$	MeOH	79	210-212
7	(I, I, OH-substituted aryl)	C$_8$H$_7$I$_2$N$_3$O$_3$	MeOH	74	205-208
8	(NC-substituted aryl)	C$_9$H$_8$N$_4$O$_2$·H$_2$O	MeOH	72	186-188
9	((CH$_3$)$_2$N-substituted aryl)	C$_{10}$H$_{14}$N$_4$O$_2$	MeOH	79	159-162
10	(O$_2$N, O$_2$N, OH-substituted aryl)	C$_8$H$_7$N$_5$O$_7$	MeOH	79	171-173
11	(O$_2$N-substituted aryl)	C$_8$H$_8$N$_4$O$_4$	MeOH	70	213-215
12	(H$_3$CO-substituted aryl)	C$_9$H$_{11}$N$_3$O$_3$	MeOH	64	175-178
13	(H$_3$CO-substituted aryl)	C$_9$H$_{11}$N$_3$O$_3$	MeOH	61	164-166
14	(H$_3$CO, OCH$_3$-substituted aryl)	C$_{10}$H$_{13}$N$_3$O$_4$	MeOH	62	150-153
15	(CH$_3$O, OH-substituted aryl)	C$_9$H$_{11}$N$_3$O$_4$	MeOH	77	155-157
16	(OCH$_3$, H$_3$CO, OH-substituted aryl)	C$_{10}$H$_{13}$N$_3$O$_5$	MeOH	79	169-171 (dec.)
17	Ph-CH$_2$O-substituted aryl	C$_{15}$H$_{15}$N$_3$O$_3$	MeOH	86	155-157
18	(biphenyl)	C$_{14}$H$_{13}$N$_3$O$_2$	MeOH	73	158-160
19	(HO, OH-substituted aryl)	C$_8$H$_9$N$_3$O$_4$	EtOH	62	179-181
20[a]	H$_3$C-C(=O)-N(H)-substituted aryl	C$_{10}$H$_{12}$N$_4$O$_3$	EtOH	64	205-207
21	(HO, HO, OH-substituted aryl)	C$_8$H$_9$N$_3$O$_5$	EtOH	88	182-185
22	(pyridyl)	C$_7$H$_8$N$_4$O$_2$	MeOH	70	166-168
23	(H$_3$C, CH$_3$ pyridyl)	C$_8$H$_{10}$N$_4$O$_2$	MeOH	69	164-166
24	(O$_2$N, S thienyl)	C$_6$H$_6$N$_4$O$_4$S·H$_2$O	MeOH	76	185-187
25	(indolyl)	C$_{10}$H$_{10}$N$_4$O$_2$	MeOH	64	150-152
26	(Cl, Cl chromone)	C$_{11}$H$_7$Cl$_2$N$_3$O$_4$	MeOH	70	184-186
27	((H$_3$C)$_2$HC chromone)	C$_{14}$H$_{15}$N$_3$O$_4$	MeOH	83	177-179
28	(benzodioxane)	C$_{10}$H$_{11}$N$_3$O$_4$	MeOH	79	167-169
29	(dimethylanthracene)	C$_{17}$H$_{15}$N$_3$O$_2$	MeOH	90	>250
30	(Cl, methylanthracene)	C$_{16}$H$_{12}$ClN$_3$O$_2$	MeOH	84	210 (dec.)

[a] Compound **20** is a known compound.[24]

variable I was used for the presence (I =1) or absence (I = 0) of an oxygen-containing group (−OH, −OCH$_3$, keto) at the ortho position of −CH=NNHCONHOH.

The antitumor activities and physicochemical parameters used in the regression analysis are summarized in Table 2. The quantitative relationship between the antitumor activities (represented by log($^1/_{IC_{50}}$)) and the structures (represented by the physicochemical properties) can be described by the stepwise regression equations in Table 3.

The statistical parameters describing the regression are *n*, the number of data points upon which the equation is based; *R*, the correlation coefficient; Q^2, a measure of the predictive power of the equation (also called cross-validated R^2);[34] and *s*, the standard deviation from the regression. The level of significance of single- or multiple-variable equations and that of addition of a single variable were examined by overall and stepwise *F* tests, respectively. The probability of being a chance correlation is designated as *p*. The number in

Table 2. Inhibitory Activities and Physicochemical Properties of HU, SC, HSC, and SB-HSCs Analyzed in the Regression Analysis

compd	IC$_{50}$ ± sd[a] (×10^{-6} M)	log($^1/_{IC_{50}}$) (M) obsd	calcd[b]	deviation (obsd − calcd)	ClogP [c]	SlogP [d]	CMR[c]	I [e]
HU	82.0 ± 6.0 (60.6)[f]	4.086	3.856	0.230	−1.80[g]	−1.80[g]	1.57	0
SC[h]	>2192				−2.75[g]	−2.75[g]	1.78	
HSC	281.6 ± 21.4	3.550	3.680	−0.130	−2.91	−3.26	1.94	0
1	39.5 ± 2.7	4.403	4.332	0.071	1.94	1.64[i]	5.55	0
2	6.5 ± 0.6	5.187	5.092	0.095	1.88 (1.75)[j]	1.61 (1.48)[j]	6.17	1
3	10.1 ± 1.0	4.996	4.905	0.091	1.55	1.16	5.97	1
4	7.2 ± 1.7	5.143	5.191	−0.048	2.23 (2.03)[j]	1.97 (1.77)[j]	6.75	1
5	37.7 ± 5.7	4.424	4.536	−0.112	1.32	1.01	6.59	1
6	80.4 ± 3.5	4.095	4.271	−0.176	2.18	1.91	6.34	0
7	4.7 ± 1.5	5.328	5.250	0.078	2.68 (2.48)[j]	2.39 (2.19)[j]	7.80	1
8	208.5 ± 1.0	3.681	3.357	0.324	0.49	0.19	5.52	0
9	446.3 ± 44.0	3.350	3.540	−0.190	1.22	0.94	6.33	0
10[k]	820.6 ± 73.1	3.086	4.214 (3.117)[l]	−1.128[k] (−0.031)[l]	0.73 (−2.68)[j]	−0.47 (−3.88)[j]	6.41	1
11	399.3 ± 88.1	3.399	3.494	−0.095	0.80	0.48	5.65	0
12	368.8 ± 1.5	3.433	3.596	−0.163	0.97	0.74	5.66	0
13	316.7 ± 13.1	3.499	3.596	−0.097	0.97	0.74	5.66	0
14	30.1 ± 5.0	4.521	4.465	0.056	1.06	0.72	6.27	1
15	60.2 ± 2.4	4.220	4.274	−0.054	0.49	0.07	5.81	1
16	91.4 ± 0.9	4.039	4.087	−0.048	0.52	0.05	6.43	1
17	48.1 ± 9.9	4.318	4.173	0.145	2.74	2.42	8.17	0
18	42.7 ± 7.9	4.370	4.570	−0.200	2.94	2.72	7.55	0
19	130.8 ± 12.3	3.883	4.056	−0.173	−0.28	−0.58	5.34	1
20	944.1 ± 78.7	3.025	2.862	0.163	0.07	−0.21	6.37	0
21[k]	2.7 ± 0.6	5.565	3.786	1.779[k]	−0.88	−1.25	5.50	1
22	607.0 ± 69.5	3.217	3.148	0.069	−0.44	−0.70	4.83	0
23	891.0 ± 128.3	3.050	3.212	−0.162	0.06	−0.20	5.29	0
24[k]	10.6 ± 0.3	4.975	3.399	1.576[k]	0.53	0.13	5.46	0
25	476.6 ± 37.3	3.322	3.477	−0.155	1.04	0.71	6.16	0
26	28.2 ± 2.6	4.550	4.530	0.020	1.72	1.38	7.50	1
27	32.2 ± 0.4	4.492	4.398	0.094	1.72	1.38	7.90	1
28	178.3 ± 4.5	3.749	3.461	0.288	0.98	0.58	6.09	0
29	4.4 ± 0.0	5.357	5.158	0.199	3.90	3.64	8.88	0
30	5.4 ± 0.4	5.268	5.394	−0.126	4.11	3.84	8.91	0

[a] The average median inhibitory concentration obtained from two or three measurements. [b] Calculated from eq 5. [c] Calculated by using CQSAR program of BioByte.[30] [d] Calculated by taking the sum of the π of −CH=NNHCONHOH derived from the experimental log P value of compound **1** and other constants.[31,32] [e] An indicator variable indicating the presence ($I = 1$) or absence ($I = 0$) of an oxygen-containing substituent at the ortho position of the Ar group. [f] From ref 4. [g] Measured log P values obtained from the CQSAR program.[30] [h] Not included in the regression analysis. [i] Experimental log P value from this study. [j] Calculated distribution coefficients (log D).[32] [k] Statistical outliers, excluded from eqs 5 and 7. [l] Calculated from eq 9.

parentheses is the 95% confidence interval of each coefficient in the equations.

Nonweighted least-squares regression analysis of the data reveals three parameters to be the best predictors of the inhibitory activities of the compounds against L1210 cells. These parameters are ClogP (or SlogP), CMR, and the indicator variable *I*. First, ClogP (or SlogP), a measure of the hydrophobicity of the compounds, makes a positive contribution to the inhibitory activities of the compounds tested. The inhibitory activities (log($^1/_{IC_{50}}$)) nonlinearly depend on ClogP (or SlogP). Second, CMR, a measure of molecular size and polarizability, makes a negative contribution to the inhibitory activities against L1210 cells, suggesting that as CMR increases, the inhibitory activity decreases after correcting for the contribution of hydrophobicity (ClogP or SlogP). Third, the indicator variable (*I*) makes a positive contribution to the inhibitory activities. This indicates that the presence of an oxygen-containing substituent at the ortho position is beneficial to the inhibitory activity. The OH groups on the phenyl ring act as free radical scavengers and thus improve the antitumor activity.

Compounds **10**, **21**, and **24** were statistical outliers with deviations greater than 2*s*. Upon deletion of the three outliers, better equations with higher R^2 values of 0.955 and 0.948 and lower *s* values of 0.166 and 0.177 were obtained for eqs 5 and 7, respectively. By these two equations, 95.5% and 94.8% of the variances in the data can be accounted for. Here, the QSAR analysis reveals that compounds **21** and **24** might have different limiting steps from other compounds as inhibitors of the tumor cells. Besides the common pharmacophore of −NHCONHOH, three phenolic OH groups of compound **21** may act as additional free radical scavengers and/or chelator of the Fe^{3+} ions, which are required for the RR enzyme activity. This explanation is consistent with the findings of van't Riet,[35,36] Tihan,[37] and Szekeres.[14] Among the different benzohydroxamic acid derivatives investigated, hydroxy-substituted benzohydroxamic acid derivatives turned out to be more effective RR inhibitors than HU. Further structure−activity analysis revealed that addition of at least two hydroxyls on the benzene ring could dramatically increase the enzyme inhibitory capacity of the compound. Addition of a third hydroxyl group could even further enhance the enzyme inhibitory

Table 3. Stepwise Regression Equations

no.	equation	n	R^2	Q^2	s	overall F test	stepwise F test
1	$\log(^1/_{IC_{50}}) = 0.252(0.169)\,ClogP + 3.903(0.305)$	32	0.231	0.122	0.685	$F_{1,30} = 9.02$, $p < 0.01$	
2	$\log(^1/_{IC_{50}}) = 0.245(0.153)\,ClogP + 0.647(0.450)I + 3.647(0.328)$	32	0.407	0.245	0.612	$F_{2,29} = 9.96$ $p < 0.0005$	$F_{1,29} = 8.62$ $p < 0.01$
3	$\log(^1/_{IC_{50}}) = 0.082(0.162)\,ClogP + 0.097(0.058)(ClogP)^2 + 0.832(0.400)I + 3.431(0.308)$	32	0.585	0.399	0.521	$F_{3,28} = 13.14$ $p < 0.0005$	$F_{1,28} = 11.96$ $p < 0.005$
4	$\log(^1/_{IC_{50}}) = 0.296(0.337)\,ClogP + 0.092(0.057)(ClogP)^2 + 0.933(0.417)I - 0.217(0.302)\,CMR + 4.512(1.532)$	32	0.616	0.399	0.510	$F_{4,27} = 10.81$ $p < 0.0005$	$F_{1,27} = 2.18$ $p < 0.25$
5	$\log(^1/_{IC_{50}}) = 0.465(0.120)\,ClogP + 0.088(0.019)(ClogP)^2 + 1.012(0.142)I - 0.329(0.104)\,CMR + 4.924(0.524)$	29^a	0.955	0.921	0.166	$F_{4,24} = 126.08$ $p < 0.0005$	
6	$\log(^1/_{IC_{50}}) = 0.489(0.282)\,SlogP + 0.084(0.054)(SlogP)^2 + 0.987(0.408)I - 0.248(0.264)\,CMR + 4.779(1.417)$	32	0.644	0.440	0.492	$F_{4,27} = 12.20$ $p < 0.0005$	
7	$\log(^1/_{IC_{50}}) = 0.499(0.117)\,SlogP + 0.084(0.020)(SlogP)^2 + 1.035(0.153)I - 0.311(0.104)\,CMR + 4.978(0.555)$	29^a	0.948	0.915	0.177	$F_{4,24} = 109.59$ $p < 0.0005$	
8^b	$\log(^1/_{IC_{50}}) = 0.431(0.196)\,ClogP + 0.075(0.047)(ClogP)^2 + 1.055(0.389)I - 0.312(0.212)\,CMR + 4.996(1.079)$	32	0.684	0.548	0.463	$F_{4,27} = 14.62$ $p < 0.0005$	
9^b	$\log(^1/_{IC_{50}}) = 0.500(0.075)\,ClogP + 0.088(0.018)(ClogP)^2 + 1.059(0.149)I - 0.355(0.080)\,CMR + 5.042(0.404)$	30^c	0.952	0.929	0.172	$F_{4,25} = 124.75$ $p < 0.0005$	
10	$\log(^1/_{IC_{50}}) = 0.698(0.232)\,ClogP + 0.856(0.294)I - 0.413(0.218)\,CMR + 5.541(1.082)$	29^a	0.784	0.653	0.354	$F_{3,25} = 30.20$ $p < 0.0005$	
11	$ClogP = 0.954(0.051)\,SlogP + 0.386(0.079)$	30	0.981	0.978	0.166	$F_{1,28} = 1454.8$ $p < 0.0005$	

^{a} Compounds **10**, **21**, and **24** were deleted as statistical outliers with deviations greater than 2s. ^{b} The calculated log D values of compounds **2**, **4**, **7**, and **10** were used in the equations. ^{c} Compounds **21** and **24** were deleted as statistical outliers with deviations greater than 2s.

potency of the compound. A good correlation has been demonstrated between the ability to scavenge the free radical and inhibition of the RR enzyme activity.[38] It has been reported that resveratrol (3,5,4′-trihydroxystilbene) and p-propoxyphenol are active RR inhibitors with IC_{50} values of 1.0×10^{-4} and 3.0×10^{-4} M in soluble extracts of murine leukemia L1210-R2 cells.[39,40] Further study is needed to clarify the outlier behavior of compound **24**, which contains a 5-nitrothienyl as Ar group.

The outlier behavior (observed $\log(^1/_{IC_{50}})$ < calculated $\log(^1/_{IC_{50}})$, deviation = −1.128) of compound **10** probably resulted from the ionization of the phenolic OH group. The phenolic OH group with a pK_a of 3.99 (obtained from ref 28) is 99.96% ionized at the physiological condition (pH = 7.4) because of the strong electron-withdrawing effect of 3,5-dinitro groups. The degrees of ionization of compounds **2**, **4**, and **7** range from 26% to 36%. The distribution coefficients (log D) of these ionized compounds were calculated according to the relationship of log D and log P,[32] and then they were used in the regression analysis. Compound **10** with a deviation of −0.031 was no longer an outlier in eq 9 when its log D was used in the regression analysis because of the considerable difference between log P (0.73) and log D (−2.68). However, the use of the log D values of compounds **2**, **4**, and **7** did not significantly improve the correlation (see eq 9) simply because only 0.13−0.20 log unit differences were observed between the log D and log P values for compounds **2**, **4**, and **7**.

Because both log P and (log $P)^2$ terms have positive coefficients, this is different from the traditional parabolic or reversed parabolic dependence of biological activity on log P. Upon deletion of the (log $P)^2$ term, eq 10 was obtained with a correlation coefficient of 0.885, which was still statistically highly significant (see Table 3).

It is interesting to note that HU and HSC fit eqs 5, 7, and 9 very well, suggesting that HU, HSC, and SB-HSCs most likely have the same rate-limiting step and

Table 4. Squared Correlation Matrix (R^2) of the Physicochemical Parameters Used in the Regression Analysis for HU, HSC, and SB-HSCs ($n = 32$)

	ClogP	SlogP	CMR	I
ClogP	1.000	0.986	0.806	0.001
SlogP		1.000	0.758	0.000
CMR			1.000	0.038
I				1.000

mechanism of action as inhibitors of the tumor cells. Here, the QSAR analysis provides an insight for the action of the different compounds against tumor cells.

The squared correlation matrix of the physicochemical properties used in the regression analysis is shown in Table 4. From Table 4, one can see that ClogP (or SlogP) and CMR are highly interdependent, and there is no covariance between ClogP (or SlogP) and I and between CMR and I ($R^2 < 0.04$).

The ClogP values of the 30 SB-HSCs are highly correlated with the SlogP values, with a mean difference of 0.34(ClogP − SlogP), as shown by eq 11 (see Table 3). It should be noted that in a ClogP calculation many correction factors are used (e.g., 3,5-dinitro), while in our SlogP calculation no correction factors are used.

In conclusion, on the basis of the pharmacophore of −NHCONHOH and the nature of RR enzyme, we have designed and synthesized a series of HU-based RR inhibitors with in vitro antitumor activity. A total of 17 compounds have been shown to have higher in vitro inhibitory activities than HU against L1210 murine leukemia cells. A total of 6 of the 17 compounds with IC_{50} values in micromolar range are 11- to 30-fold more potent than HU. The QSAR study suggests that, besides the essential pharmacophore (−NHCONHOH), both hydrophobicity and molecular size/polarizability are important determinants for the inhibitory activity against L1210 cells. The presence of an oxygen-containing group at the ortho position also makes a positive contribution to the antitumor activity. From the chemical structure point of view, the SB-HSCs most likely target the same enzyme as HU. This hypothesis is further supported by

the QSAR analysis in which HU fits the regression equations very well.

Testing results of compounds **4**, **21**, and **29** against human leukemia CCRF-CEM cells have shown that compounds **4**, **21**, and **29** have IC$_{50}$ values comparable to those observed in murine leukemia L1210 cells.[41] The IC$_{50}$ values ranging from 2.7×10^{-6} to 1.2×10^{-5} M have been observed for compound **29** against four different solid tumor cell lines.[41] Compounds **2** and **29** have no cross-resistance with HU in HU-resistant human oropharyngeal carcinoma KB cells.[41] These in vitro data as first-line evidence indicate that compounds **2** and **29** may have potential in the treatment of solid tumors and HU-resistant cancers. It has been shown that in a variety of tumor and nontumor cell lines compound **29** has more favorable selectivity than HU against tumor cells. The relative selectivity indices span from 8 to 1350 for the eight tumor cell lines tested vs a nontumor cell line (3T3 Swiss mouse embryo fibroblasts),[41] suggesting that the SB-HSCs have better selectivity against tumor cell growth and thus may have lower toxicity in vivo. The enzyme inhibition capacity at a single concentration (5×10^{-5} M) has been correlated with the cell growth inhibition potency by Zhou et al. in a preliminary study.[42]

The disadvantages associated with HU's physicochemical properties, e.g., very high hydrophilicity (log P = -1.80) and small molecular size, may be overcome by the structural modification employed in this study. The most active SB-HSCs possess more suitable physicochemical properties, namely, higher hydrophobicity (log P) and molecular size (M_R or MW) than HU. We thus expect that the most active compounds should have better pharmacokinetic and pharmacodynamic profiles, such as longer half-lives, higher permeability through various biological membranes, and higher binding affinity to the RR enzyme. The SB-HSC compounds synthesized are structurally related to HU, a well-known anticancer drug that is currently used in the treatment of different cancers and has been shown to have clinical efficacy and tolerance in patients. The efficacies of the SB-HSC compounds against both leukemia and solid tumor cells have been significantly improved by the structural modifications compared with HU.

The most active hydroxybenzohydroxamic acid derivatives, didox (3,4-dihydroxybenzohydroxamic acid) and trimidox (3,4,5-trihydroxybenzohydroxamidoxime), inhibited growth of wild-type L1210 cells with IC$_{50}$ values of 6.0×10^{-5} and 3.3×10^{-5} M, respectively.[37] However, it is commonly known that mono- or polyphenols are not very stable in an aqueous solution and can be oxidized by the air readily. A phase II clinical trial using didox in advanced breast cancer revealed no therapeutic responses.[43] Heterocyclic carboxaldehyde thiosemicarbazones have been reported to be potent RR and tumor cell inhibitors, but because of the low efficacy in patients, metabolic instability, and high toxicity, there are no further clinical applications at this point.[44-48] Thiosemicarbozones exert an RR inhibitory effect only through the chelating of the non-heme iron of the R2 subunit because they have no (N)−OH groups in the pharmacophore.[48] Our compounds possess the essential pharmacophore of −NHCONHOH, which has both iron

chelating and free radical scavenging properties as shown by the studies on HU.[1,20,21] We expect that the SB-HSCs may inhibit the RR enzyme more effectively. A series of Schiff bases of *N*-hydroxy-*N'*-aminoguanidine tosylate (SB-HAG.T) have been synthesized and tested for antitumor activities. Micromolar and submicromolar IC$_{50}$ values have been observed, but only limited in vivo activity has been obtained on one of the series of compounds.[2,49]

Selassie et al.[50] revealed that anticancer drugs at the extreme ends of the molecular weight and hydrophobicity scales showed minimal cross-resistance with each other in a QSAR analysis of cross-resistant anticancer drugs. For example, small drugs (such as HU) exhibited the least cross-resistance with large drug molecules (like mithramycin). This interesting finding provides insight into better understanding of the lack of cross-resistance between HU and compound **29** (large and hydrophobic molecule).

To compare the title compounds with other compounds currently being explored,[1-15] further in vivo activity and toxicity studies are needed to assess the potentials of these new compounds as cancer chemotherapeutic agents. The results obtained from this study can be used as guidelines for further improvement of RR inhibitors as anticancer agents.

Experimental Section

Chemistry. Melting points were determined in melting point capillary tubes with a MEL-TEMP melting point apparatus and were corrected. All elemental analyses, conducted by Galbraith Laboratories, Tennessee, were within 0.4% of the theoretical values for C, H, and N after taking solvent of crystallization into account for compounds **5**, **12**, **18**, **25**, **29**, and **30**. To further establish the correct structures of these six compounds, mass spectra were obtained from Mass Spectrometry Laboratory of University of California Riverside, using the DCI (desorption chemical ionization) method on a VG 7070 high-resolution mass spectrometer. IR spectra were recorded with a Beckman IR-4210 spectrophotometer using KBr pellets. The new compounds were characterized by ^{1}H, ^{13}C {^{1}H}, and ^{13}C NMR on a Bruker AMX 500 MHz FT NMR spectrometer. Some compounds were further characterized by ^{1}H, ^{1}H correlation spectroscopy (COSY) NMR (compounds **1**, **11**), and ^{13}C,^{1}H correlated NMR spectroscopy (compounds **1**, **6**, **7**, **11**, **12**).

***N*-Hydroxyphenyl Carbamate.** *N*-hydroxyphenyl carbamate was synthesized by using modified procedures of Grobner and Steinberg.[24,25] Yield 18.6 g (89%); mp 105−107 °C (reported mp 105-107 °C[24]).

Hydroxysemicarbazide. A modified procedure of Grobner and Steinberg[24,25] was used in the synthesis of hydroxysemicarbazide. Yield 7.8 g (86%); mp 129−131 °C (reported mp 126 °C[24]). ^{1}H NMR: δ 3.94, 7.51, 8.36, 8.40. ^{13}C NMR: δ 162.78 (*C*=O). IR, cm^{-1}: 3340, 3200, 1675, 1620, 1520.

General Procedure for Syntheses of Compounds 1−30. A mixture of an aldehyde (0.0098−0.0308 mol) and an equimolar quantity of hydroxysemicarbazide in 100−200 mL of anhydrous methanol was refluxed for 24 h. After checking for product formation via TLC in methanol/ethyl acetate/chloroform (2:3:4), the mixture was concentrated to 5−20 mL in vacuo. The precipitate was collected by filtration and recrystallized in methanol (or ethanol) to give compounds **1−30**.

1-(3-Trifluoromethylbenzylidene)-4-hydroxysemicarbazide (1). ^{1}H NMR: δ 7.59 (t, ^3J = 7.3 Hz, 1H, 5-Ar*H*), 7.64 (d, ^3J = 7.1 Hz, 1H, 4-Ar*H*), 7.92 (d, ^3J = 7.5 Hz, 1H, 6-Ar*H*), 8.05 (s, 1H, −C*H*=), 8.10 (s, 1H, 2-Ar*H*), 8.53, 9.48, 10.46 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 122.65 (2-Ar*C*), 124.07 (−*C*F$_3$), 125.17 (4-Ar*C*), 129.47 (5-Ar*C*), 129.69 (3-Ar*C*), 130.68 (6-Ar*C*), 135.90

(1-Ar*C*), 139.96 (−*C*H=, 156.96 (*C*=O). IR, cm⁻¹: 3430, 3200, 3120, 2980, 1705, 1660, 1530, 1330. Anal. (C₉H₈F₃N₃O₂) C, H, N.

1-(2-Hydroxy-3,5-dichlorobenzylidene)-4-hydroxysemicarbazide (2). ¹H NMR: δ 7.45, 7.52 (d, ⁴*J* = 2.5 Hz, 1H; d, ⁴*J* = 2.5 Hz, 1H; 4,6-Ar*H*), 8.33 (s, 1H, −C*H*=), 8.78, 9.33, 10.96 11.98 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR: δ 121.47, 121.85 (1,3-Ar*C*), 122.93 (5-Ar*C*), 127.21 (6-Ar*C*), 129.16 (4-Ar*C*), 142.06 (−*C*H=), 151.46 (2-Ar*C*), 156.70 (*C*=O). IR, cm⁻¹: 3360, 1695, 1665, 1540. Anal. (C₈H₇Cl₂N₃O₃) C, H, N.

1-(2-Hydroxy-5-bromobenzylidene)-4-hydroxysemicarbazide (3). ¹H NMR: δ 6.82 (d, ³*J* = 8.7 Hz, 1H, 3-Ar*H*), 7.31 (dd, ³*J* = 8.7 Hz, ⁴*J* = 2.5 Hz, 1H, 4-Ar*H*), 7.81 (d, ⁴*J* = 2.5 Hz, 1H, 6-Ar*H*), 8.25 (s, 1H, −C*H*=), 8.58, 9.32, 10.58, 10.75 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR: δ 110.50 (1-Ar*C*), 118.30 (3-Ar*C*), 122.19 (5-Ar*C*), 129.66 (6-Ar*C*), 132.47 (4-Ar*C*), 139.50 (−*C*H=), 155.55 (2-Ar*C*), 156.86 (*C*=O). IR, cm⁻¹: 3360, 1695, 1660, 1525. Anal. (C₈H₈BrN₃O₃) C, H, N.

1-(2-Hydroxy-3,5-dibromobenzylidene)-4-hydroxysemicarbazide (4). ¹H NMR: δ 7.63, 7.68 (d, ⁴*J* = 2.4 Hz, 1H; d, ⁴*J* = 2.4 Hz, 1H, 4,6-Ar*H*), 8.31 (s, 1H, −C*H*=), 8.81, 9.31, 11.01, 12.24 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR: δ 110.30 (5-Ar*C*), 111.18, 121.93 (1,3-Ar*C*), 130.98 (6-Ar*C*), 134.52 (4-Ar*C*), 142.45 (−*C*H=), 152.94 (2-Ar*C*), 156.65 (*C*=O). IR, cm⁻¹: 3350, 1700, 1670, 1525. Anal. (C₈H₇Br₂N₃O₃) C, H, N.

1-(2-Hydroxy-3-methoxy-5-bromobenzylidene)-4-hydroxysemicarbazide (5). ¹H NMR: δ 3.81 (s, 3H, −OC*H₃*), 7.05 (d, ⁴*J* = 2.2 Hz, 1H, 4-Ar*H*), 7.43 (d, ⁴*J* = 2.2 Hz, 1H, 6-Ar*H*), 8.27 (s, 1H, −C*H*=), 8.65, 9.43, 10.25, 10.66 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR δ 56.21 (−O*C*H₃), 109.93 (5-Ar*C*), 115.33 (4-Ar*C*), 121.22 (6-Ar*C*), 121.66 (1-Ar*C*), 139.86 (−*C*H=), 145.32 (2-Ar*C*), 148.87 (3-Ar*C*), 156.66 (*C*=O). IR, cm⁻¹: 3360, 1695, 1670, 1525, 1105. MS (DCI, *m/e*): 304 (M + H)⁺. Anal. (C₉H₁₀BrN₃O₄·0.2CH₃OH) C, H, N.

1-(3-Iodobenzylidene)-4-hydroxysemicarbazide (6). ¹H NMR: δ 7.17 (t, ³*J* = 7.8 Hz, 1H, 5-Ar*H*), 7.62 (dt, ³*J* = 7.8 Hz, ⁴*J* = 1.3 Hz, 1H, 6-Ar*H*), 7.68 (ddd, ³*J* = 7.9 Hz, ⁴*J* = 1.7 Hz, ⁴*J* = 1.0 Hz, 1H, 4-Ar*H*), 8.16 (t, ⁴*J* = 1.6 Hz, 1H, 2-Ar*H*), 7.88 (s, 1H, −C*H*=), 8.48, 9.40, 10.38 (N*H*, N*H*, O*H*). ¹³C NMR: δ 94.97 (3-Ar*C*), 126.44 (6-Ar*C*), 130.56 (5-Ar*C*), 134.33 (2-Ar*C*), 137.06 (1-Ar*C*), 137.45 (4-Ar*C*), 139.62 (−*C*H=), 156.76 (*C*=O). IR, cm⁻¹: 3380, 1700, 1670, 1525. Anal. (C₈H₈-IN₃O₂) C, H, N.

1-(2-Hydroxy-3,5-diiodobenzylidene)-4-hydroxysemicarbazide (7). ¹H NMR: δ 7.68 (d, ⁴*J* = 2.4 Hz, 1H, 6-Ar*H*), 7.95 (d, ⁴*J* = 2.1 Hz, 1H, 4-Ar*H*), 8.24 (s, 1H, −C*H*=), 8.82, 9.27, 11.01, 12.55 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR: δ 81.65, 87.43 (3,5-Ar*C*), 121.11 (1-Ar*C*), 137.84 (6-Ar*C*), 143.01 (−*C*H=), 145.55 (4-Ar*C*), 156.01 (2-Ar*C*), 156.68 (*C*=O). IR, cm⁻¹: 1675, 1540. Anal. (C₈H₇I₂N₃O₃) C, H, N.

1-(4-Cyanobenzylidene)-4-hydroxysemicarbazide (8). ¹H NMR: δ 7.79, 7.88 (d, ³*J* = 8.3 Hz, 2H, Ar-*H*; d, ³*J* = 8.4 Hz, 2H, Ar*H*), 8.00 (s, 1H, −C*H*=), 8.65, 9.60, 10.70 (N*H*, N*H*, O*H*). ¹³C NMR: δ 110.82 (4-Ar*C*), 118.47 (−*C*N), 127.00 (2,6-Ar*C*), 132.17 (3,5-Ar*C*), 139.11 (1-Ar*C*), 139.29 (−*C*H=), 156.36 (*C*=O). IR, cm⁻¹: 2230, 1675, 1550. Anal. (C₉H₈N₄O₂·1H₂O) C, H, N.

1-(4-Dimethylaminobenzylidene)-4-hydroxysemicarbazide (9). ¹H NMR: δ 2.93 [s, 6H, −N(C*H₃*)₂], 6.69 (d, ³*J* = 9.0 Hz, 2H, 3,5-Ar*H*), 7.48 (d, ³*J* = 8.9 Hz, 2H, 2,6-Ar*H*), 7.86 (s, 1H, −C*H*=), 8.41, 8.96, 9.97 (N*H*, N*H*, O*H*). ¹³C NMR: δ 39.76 [−N(CH₃)₂], 111.77 (3,5-Ar*C*), 122.34 (1-Ar*C*), 127.85 (2,6-Ar*C*), 142.74 (−*C*H=), 151.04 (4-Ar*C*), 157.33 (*C*=O). IR, cm⁻¹: 1680, 1610, 1520, 1360. Anal. (C₁₀H₁₄N₄O₂) C, H, N.

1-(2-Hydroxy-3,5-dinitrobenzylidene)-4-hydroxysemicarbazide (10). ¹H NMR: δ 8.50 (s, 1H, −C*H*=), 8.68, 8.71 (d, ⁴*J* = 2.8 Hz, 1H; d, ⁴*J* = 2.5 Hz, 1H, Ar*H*), 8.50, 8.71, 9.46, 11.19 (N*H*, N*H*, O*H*, ArO*H*). ¹³C NMR: δ 121.22 (4-Ar*C*), 123.65 (1-Ar*C*), 127.44 (6-Ar*C*), 137.12, 137.59 (3,5-Ar*C*), 140.50 (−*C*H=), 156.43 (*C*=O), 156.92 (2-Ar*C*). IR, cm⁻¹: 1640, 1615, 1530, 1345. Anal. (C₈H₇N₅O₇) C, H, N.

1-(3-Nitrobenzylidene)-4-hydroxysemicarbazide (11). ¹H NMR: δ 7.66 (t, ³*J* = 8.0 Hz, 1H, 5-Ar*H*), 8.08 (s, 1H, −C*H*=), 8.10 (dt, ³*J* = 7.8 Hz, ⁴*J* = 1.1 Hz, 1H, 6-Ar*H*), 8.15 (ddd, ³*J* = 8.5 Hz, ⁴*J* = 2.3 Hz,⁴*J* = 1.1 Hz, 1H, 4-Ar*H*), 8.54 (t, ⁴*J* = 2.0 Hz 1H, 2-Ar*H*), 8.53, 9.51, 10.55 (N*H*, N*H*, O*H*). ¹³C NMR: δ 120.56 (2-Ar*C*), 123.21 (4-Ar*C*), 130.01 (5-Ar*C*), 132.87 (6-Ar*C*), 136.72 (1-Ar*C*), 139.09 (−*C*H=), 148.38 (3-Ar*C*), 156.65 (*C*=O). IR, cm⁻¹: 1705, 1660, 1525, 1350. Anal. (C₈H₈N₄O₄) C, H, N.

1-(3-Methoxybenzylidene)-4-hydroxysemicarbazide (12). ¹H NMR: δ 3.79 (s, 3H, −OC*H₃*), 6.91 (ddd, ³*J* = 8.2 Hz, ⁴*J* = 2.6 Hz, ⁴*J* = 1.0 Hz, 1H, 4-Ar*H*), 7.19 (dt, ³*J* = 7.6 Hz, ⁴*J* = 1.1 Hz, 1H, 6-Ar*H*), 7.28 (t, ³*J* = 7.8 Hz, 1H, 5-Ar*H*), 7.31 (t, ⁴*J* = 1.5 Hz, 1H, 2-Ar*H*), 7.94 (s, 1H, −C*H*=), 8.44, 9.24, 10.25 (N*H*, N*H*, O*H*). ¹³C NMR δ 55.03 (−O*C*H₃), 110.71 (2-Ar*C*), 115.31, 119.54 (4,6-Ar*C*), 129.34 (5-Ar*C*), 136.00 (1-Ar*C*), 141.36 (−*C*H=), 156.74 (*C*=O), 159.41 (3-Ar*C*). IR, cm⁻¹: 1690, 1670, 1525, 1275, 1140. MS (DCI, *m/e*): 210 (M + H)⁺. Anal. (C₉H₁₁N₃O₃·0.135CH₃OH) C, H, N.

1-(4-Methoxybenzylidene)-4-hydroxysemicarbazide (13). ¹H NMR: δ 3.76 (s, 3H, −OC*H₃*), 6.92 (d, ³*J* = 8.8 Hz, 2H, 3,5-Ar*H*), 7.62 (d, ³*J* = 8.8 Hz, 2H, 2,6-Ar*H*), 7.95 (s, 1H, −C*H*=), 8.51, 9.16, 10.18 (N*H*, N*H*, O*H*). ¹³C NMR: δ 55.18 (−O*C*H₃), 114.07 (3,5-Ar*C*), 127.34 (1-Ar*C*), 128.17 (2,6-Ar*C*), 141.86 (−*C*H=), 157.27 (*C*=O), 160.22 (4-Ar*C*). IR, cm⁻¹: 1670, 1615, 1500, 1250, 1130, 1035. Anal. (C₉H₁₁N₃O₃) C, H, N.

1-(2,5-Dimethoxybenzylidene)-4-hydroxysemicarbazide (14). ¹H NMR: δ 3.74 (s, 3H, −OC*H₃*), 3.76 (s, 3H, −OC*H₃*), 6.89 (dd, ³*J* = 9.0 Hz, ⁴*J* = 3.0 Hz, 1H, 4-Ar*H*), 6.95 (d, ³*J* = 9.0 Hz, 1H, 3-Ar*H*), 7.52 (d, ⁴*J* = 2.9 Hz, 1H, 6-Ar*H*), 8.26 (s, 1H, −C*H*=), 8.41, 9.30, 10.29 (N*H*, N*H*, O*H*). ¹³C NMR: δ 55.50, 56.20 (−O*C*H₃), 109.93, 113.00, 116.67 (3,4,6-Ar*C*), 123.51 (1-Ar*C*), 151.72, 153.38 (2,5-Ar*C*), 136.88 (−*C*H=), 156.89 (*C*=O). IR, cm⁻¹: 1675, 1535, 1225, 1045. Anal. (C₁₀H₁₃N₃O₄) C, H, N.

1-(2-Hydroxy-4-methoxybenzylidene)-4-hydroxysemicarbazide (15). ¹H NMR: δ 3.74 (s, 3H, −OC*H₃*), 6.43 (d, ⁴*J* = 2.5 Hz, 1H, 3-Ar*H*), 6.45 (dd, ³*J* = 8.5 Hz, ⁴*J* = 2.5 Hz, 1H, 5-Ar*H*), 7.31 (d, ³*J* = 8.6 Hz, 1H, 6-Ar*H*), 8.29 (s, 1H, −C*H*=), 8.66, 9.03, 10.46 11.28 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR: δ 55.16 (−O*C*H₃), 101.21 (3-Ar*C*), 106.02 (5-Ar*C*), 112.38 (1-Ar*C*), 130.30 (6-Ar*C*), 144.63 (−*C*H=), 157.17 (*C*=O), 158.59 (2-Ar*C*), 161.37 (4-Ar*C*). IR, cm⁻¹: 1630, 1565, 1250, 1140. Anal. (C₉H₁₁N₃O₄) C, H, N.

1-(2-Hydroxy-4,6-dimethoxybenzylidene)-4-hydroxysemicarbazide (16). ¹H NMR: δ 3.75 (s, 3H, −OC*H₃*), 3.79 (s, 3H, −OC*H₃*), 6.07, 6.09 (d, ⁴*J* = 2.3 Hz, 1H; d, ⁴*J* = 2.2 Hz, 1H; 3,5-Ar*H*), 8.65 (s, 1H, −C*H*=), 8.67, 8.93, 10.61, 12.17 (N*H*, N*H*, O*H*, 2-ArO*H*). ¹³C NMR: δ 55.27, 55.84 (−O*C*H₃), 90.27, 93.90 (3,5-Ar*C*), 101.06 (1-Ar*C*), 159.09, 160.20, 162.30 (2,4,6-Ar*C*), 142.62 (−*C*H=), 157.17 (*C*=O). IR, cm⁻¹: 1635, 1610, 1575, 1215, 1115. Anal. (C₁₀H₁₃N₃O₅) C, H, N.

1-(4-Benzyloxybenzylidene)-4-hydroxysemicarbazide (17). ¹H NMR: δ 5.13 (s, 2H, −C*H₂*O−), 7.02 (d, ³*J* = 8.8 Hz, 2H, 3,5-Ar*H*), 7.32 (tt, ³*J* = 7.3 Hz, ⁴*J* = 1.4 Hz, 1H, 4′-Ar*H*), 7.38 (td, ³*J* = 7.4 Hz, ⁴*J* = 1.4 Hz, 2H, 3′,5′-Ar*H*), 7.44 (dd, ³*J* = 7.5 Hz, ⁴*J* = 1.4 Hz, 2H, 2′,6′-Ar*H*), 7.62 (d, ³*J* = 8.7 Hz, 2H, 2,6-Ar*H*), 7.93 (s, 1H, −C*H*=), 8.44, 9.12, 10.15 (N*H*, N*H*, O*H*). ¹³C NMR: δ 69.39 (−*C*H₂O−), 114.94 (3,5-Ar*C*), 127.55, 127.67, 127.82, 128.13, 128.38 (1, 2, 6, 2′-6′-Ar*C*), 136.89 (1′-Ar*C*), 141.58 (−*C*H=), 157.14 (*C*=O), 159.28 (4-Ar*C*). IR, cm⁻¹: 1685, 1505, 1225, 1000. Anal. (C₁₅H₁₅N₃O₃) C, H, N.

1-(4-Phenylbenzylidene)-4-hydroxysemicarbazide (18). ¹H NMR: δ 7.35 (tt, ³*J* = 7.3 Hz, ⁴*J* = 1.1 Hz, 1H, 4′-Ar*H*), 7.45 (td, ³*J* = 7.4 Hz, ⁴*J* = 1.8 Hz, 2H, 3′,5′-Ar*H*), 7.67, 7.78 (d, ³*J* = 8.4 Hz, 4H; d, ³*J* = 8.3 Hz, 2H, 2,3,5,6,2′,6′-Ar*H*), 8.06 (s, 1H, −C*H*=), 8.51, 9.24, 10.33 (N*H*, N*H*, O*H*). ¹³C NMR: δ 126.34 (3′,5′-ArH), 126.55, 127.05, 128.69 (2,6,3,5,2′,6′-Ar*C*), 127.40 (4′-Ar*C*), 133.71, 139.33, 140.58 (1,1′,4-Ar*C*), 141.27 (−*C*H=), 156.86 (*C*=O). IR, cm⁻¹: 1650, 1555. MS (DCI, *m/e*): 256 (M + H)⁺. Anal. (C₁₄H₁₃N₃O₂·0.2CH₃OH) C, H, N.

1-(2,4-Dihydroxybenzylidene)-4-hydroxysemicarbazide (19). ¹H NMR: δ 6.28 (d, ³*J* = 2.4 Hz, 1H, 3-Ar*H*), 6.31 (dd, ³*J* = 8.4 Hz, ⁴*J* = 2.4 Hz, 1H, 5-Ar*H*), 7.19 (d, ³*J* = 8.4 Hz, 1H, 6-Ar*H*), 8.25 (s, 1H, −C*H*=), 8.66, 8.99, 9.75, 10.37, 11.02 (N*H*, N*H*, O*H*, 2,4-ArO*H*). ¹³C NMR: δ 102.66, 107.40 (3,5-Ar*C*), 111.06 (1-Ar*C*), 130.49 (6-Ar*C*), 145.23 (−*C*H=),

157.28 (*C*=O), 158.66, 159.86 (2,4-Ar*C*). IR, cm^{-1}: 1655, 1630, 1575. Anal. (C$_8$H$_9$N$_3$O$_4$) C, H, N.

1-(4-Acetamidobenzylidene)-4-hydroxysemicarbazide (20). ^{1}H NMR: δ 2.04 (−NHCOC*H$_3$*), 7.59, 7.60 (d, ^3J = 8.7 Hz, 2H; d, ^3J = 8.7 Hz, 2H, Ar*H*), 7.92 (s, 1H, −C*H*=), 8.47, 9.14, 9.94, 10.21 (N*H*, N*H*, O*H*, −N*H*CO−). ^{13}C NMR: δ 23.94 (−NHCO*C*H$_3$), 118.86, 127.17 (2,3,5,6-Ar*C*), 129.38 (1-Ar*C*), 140.17 (4-Ar*C*), 141.55 (−*C*H=), 157.11 (*C*=O), 168.33 (−NH*C*OCH$_3$). IR, cm^{-1}: 1680, 1640, 1535, 1335. Anal. (C$_{10}$H$_{12}$N$_4$O$_3$) C, H, N.

1-(2,3,4-Trihydroxybenzylidene)-4-hydroxysemicarbazide (21). ^{1}H NMR: δ 6.32, 6.65 (d, ^3J = 8.4 Hz, 1H; d, ^3J = 8.6 Hz, 1H, 5,6-Ar*H*), 8.24 (s, 1H, −C*H*=), 8.35, 8.76, 9.06, 9.29, 10.58, 11.21 (N*H*, N*H*, O*H*, 2,3,4-Ar*OH*). ^{13}C NMR: δ 107.35 (5-Ar*C*), 111.45 (1-Ar*C*), 120.17 (6-Ar*C*), 132.68 (3-Ar*C*), 146.25 (−*C*H=), 146.83, 147.85 (2,4-Ar*C*), 157.22 (*C*=O). IR, cm^{-1}: 1665, 1630, 1565, 1365, 1195. Anal. (C$_8$H$_9$N$_3$O$_5$) C, H, N.

1-(3-Pyridylmethylene)-4-hydroxysemicarbazide (22). ^{1}H NMR: δ 7.38 (dd, ^3J = 8.0 Hz, ^4J = 4.9 Hz, 1H, 5-Ar*H*), 8.00 (s, 1H, −C*H*=), 8.12 (dt, ^3J = 8.0 Hz, ^4J = 1.9 Hz, 1H, 4-Ar*H*), 8.51 (dd, ^3J = 4.9 Hz, ^4J = 1.9 Hz, 1H, 6-Ar*H*), 8.82 (d, ^4J = 1.8 Hz, 1H, 2-Ar*H*), 8.54, 9.40, 10.48 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 123.60 (5-Ar*C*), 130.57 (3-Ar*C*), 133.18 (4-Ar*C*), 138.60 (−*C*H=), 148.17, 149.70 (2,6-Ar*C*), 156.78 (*C*=O). IR, cm^{-1}: 1685, 1650, 1545, 1145. Anal. (C$_7$H$_8$N$_4$O$_2$) C, H, N.

1-[2-(6-Methylpyridyl)methylene]-4-hydroxysemicarbazide (23). ^{1}H NMR: δ 2.48 (s, 3H, −C*H$_3$*), 7.16, 7.86 (d, ^3J = 7.6 Hz, 1H; d, ^3J = 7.7 Hz, 1H, 3,5-Ar*H*), 7.66 (t, ^3J = 7.6 Hz, 1H, 4-Ar*H*), 7.97 (s, 1H, −C*H*=), 8.55, 9.35, 10.55 (N*H*, N*H*, O*H*). ^{13}C NMR δ 23.76 (−*C*H$_3$), 116.78, 122.74 (3,5-Ar*C*), 136.57 (4-Ar*C*), 142.31 (−*C*H=), 153.10, 156.68, 157.36 (2,6-Ar*C*, *C*=O). IR, cm^{-1}: 1675, 1640, 1520, 1450. Anal. (C$_8$H$_{10}$N$_4$O$_2$) C, H, N.

1-[2-(5-Nitrothienyl)methylene]-4-hydroxysemicarbazide (24). ^{1}H NMR: δ 7.37 (d, ^3J = 4.3 Hz, 1H, 3-Ar*H*), 8.02 (d, ^3J = 4.3 Hz, 1H, 4-Ar*H*), 8.23 (s, 1H, −C*H*=), 8.64, 9.35, 10.80 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 127.57, 130.26 (3,4-Ar*C*), 135.31 (−*C*H=), 147.51, 150.07 (2,5-Ar*C*), 156.17 (*C*=O). IR, cm^{-1}: 1680, 1560, 1325. Anal. (C$_6$H$_6$N$_4$O$_4$S·1H$_2$O) C, H, N.

1-(3-Indolylmethylene)-4-hydroxysemicarbazide (25). ^{1}H NMR: δ 7.10, 7.16 (td, ^3J = 7.8 Hz, ^3J = 1.3 Hz, 1H; td, ^3J = 7.1 Hz, ^4J = 1.3 Hz, 1H, 5,6-Ar*H*), 7.40, 8.22 (d, ^3J = 8.1 Hz, 1H; d, ^3J = 7.9 Hz, 1H, 4,7-Ar*H*), 7.64 (s, 1H, 2-Ar*H*), 8.24 (s, 1H, −C*H*=), 8.45, 8.75, 9.92, 11.31 (N*H*, N*H*, O*H*, indole-N*H*). ^{13}C NMR: δ 111.39 (7-Ar*C*), 111.58 (3-Ar*C*), 119.89, 121.63, 122.13 (4,5,6-Ar*C*), 124.15 (9-Ar*C*), 128.53 (2-Ar*C*), 136.78 (8-Ar*C*), 139.84 (−*C*H=), 157.50 (*C*=O). IR, cm^{-1}: 1690, 1655, 1540, 1625, 1450. MS (DCI, *m/e*): 219 (M + H)$^+$. Anal. (C$_{10}$H$_{10}$N$_4$O$_2$·0.2CH$_3$OH) C, H, N.

1-[3-(6,8-Dichloro-4-oxo-4*H*-1-benzopyran)methylene]-4-hydroxysemicarbazide (26). ^{1}H NMR: δ 7.94, 8.11 (large peak, ^4J = 2.3 Hz, 1H; large peak, ^4J = 2.4 Hz, 1H, 5,7-Ar*H*), 7.99 (s, 1H, −CH=), 8.50 (s, 1H, 2-Ar*H*), 9.04, 9.39, 10.51 (N*H*, N*H*, O*H*). ^{13}C NMR δ 119.18 (3-Ar*C*), 123.28 (5-Ar*C*), 123.96 (8-Ar*C*), 125.41 (10-Ar*C*), 130.01 (6-Ar*C*), 131.81 (−*C*H=), 133.68 (7-Ar*C*), 150.24 (9-Ar*C*), 154.20 (2-Ar*C*), 156.53 (*C*=O), 173.24 (4-Ar*C*). IR, cm^{-1}: 1670, 1640, 1555, 1150. Anal. (C$_{11}$H$_7$Cl$_2$N$_3$O$_4$) C, H, N.

1-[3-(6-Isopropyl-4-oxo-4*H*-1-benzopyran)methylene]-4-hydroxysemicarbazide (27). ^{1}H NMR: δ 1.24, 1.26 [s, 6H, −CH(C*H$_3$*)$_2$], 3.04 [sep, ^3J = 7.0 Hz, 1H, −C*H*(CH$_3$)$_2$], 7.61 (d, ^3J = 8.7 Hz, 1H, 8-Ar*H*), 7.72 (dd, ^3J = 8.7 Hz, ^4J = 2.0 Hz, 1H, 7-Ar*H*), 7.92 (d, ^4J = 2.3 Hz, 1H, 5-Ar*H*), 8.07 (s, 1H, −C*H*=), 8.46 (d, ^4J = 1.0 Hz, 1H, 2-Ar*H*), 8.93, 9.35, 10.42 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 23.60 [−CH(*C*H$_3$)$_2$], 32.87 [−*C*H(CH$_3$)$_2$], 118.51 (3-Ar*C*), 118.55 (8-Ar*C*), 121.54, 121.58 (5,7-Ar*C*), 123.12 (10-Ar*C*), 133.05 (−*C*H=), 146.17 (6-Ar*C*), 154.05 (2-Ar*C*), 154.23 (9-Ar*C*), 156.70 (*C*=O), 174.83 (4-Ar*C*). IR, cm^{-1}: 1640, 1620, 1545, 1355, 1135. Anal. (C$_{14}$H$_{15}$N$_3$O$_4$) C, H, N.

1-(1,4-Benzodioxan-6-ylmethylene)-4-hydroxysemicarbazide (28). ^{1}H NMR: δ 4.24 [s, 4H, 2,3-C*H$_2$*−], 6.83 (d, ^3J = 8.5 Hz, 1H, 8-Ar*H*), 7.11 (d, ^3J = 8.5 Hz, 1H, 7-Ar*H*), 7.24 (s,

1H, 5-Ar*H*), 7.84 (s, 1H, −C*H*=), 8.42, 9.17, 10.14 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 63.99, 64.22 (2,3-*C*), 114.77, 117.04, 120.34 (5,7,8-Ar*C*), 128.21 (6-Ar*C*), 141.34 (−*C*H=), 143.54, 144.53 (9,-10-Ar*C*), 157.06 (*C*=O). IR, cm^{-1}: 1680, 1580, 1125, 1070. Anal. (C$_{10}$H$_{11}$N$_3$O$_4$) C, H, N.

1-[9-(10-Methylanthryl)methylene]-4-hydroxysemicarbazide (29). ^{1}H NMR: δ 3.10 (s, 3H, −C*H$_3$*), 7.58, 8.40, 8.59 (m, 8H, Ar*H*), 8.63 (s, 1H, −C*H*=), 8.97, 9.25, 10.62 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 13.99 (−*C*H$_3$), 124.95, 125.17, 125.45, 125.86 (Ar*C*H), 124.63, 129.07, 129.19, 132.15 (Ar-*C*), 142.17 (−*C*H=), 157.13 (*C*=O). IR, cm^{-1}: 1660, 1530, 1125. MS (DCI, *m/e*): 294 (M + H)$^+$. Anal. (C$_{17}$H$_{15}$N$_3$O$_2$·0.2CH$_3$OH) C, H, N.

1-[9-(10-Chloroanthryl)methylene]-4-hydroxysemicarbazide (30). ^{1}H NMR: δ 7.67, 7.73 (td, ^3J = 7.5 Hz, ^4J = 1.2 Hz, 2H; td, ^3J = 7.5 Hz, ^4J = 1.2 Hz, 2H, 2,3,6,7-Ar*H*), 8.48, 8.64 (d, ^3J = 8.7 Hz, 2H; d, ^3J = 8.7, 2H, 1,4,5,8-Ar*H*), 9.06 (s, 1H, −C*H*=), 8.67, 9.25, 10.72 (N*H*, N*H*, O*H*). ^{13}C NMR: δ 124.43, 125.76, 127.02, 127.47 (Ar-*C*H), 126.60, 127.79, 128.88, 129.70 (Ar-*C*), 141.29 (−*C*H=), 157.23 (*C*=O). IR, cm^{-1}: 1685, 1660, 1555. MS (DCI, *m/e*): 314 (M + H)$^+$. Anal. (C$_{16}$H$_{12}$-ClN$_3$O$_2$·0.3CH$_3$OH) C, H, N.

Measurements of Partition Coefficient (log *P*) and Ionization Constants (p*K*$_a$). Measurements of log *P* and p*K*$_a$ values of one representative compound were performed using the shake-flask method with two immiscible solvents (1-octanol/phosphate buffer).[33,51−53] Compound **1** was first dissolved in methanol (2 mg/mL). To 38.4 mL of phosphate buffers (pH 2.2, 7.3, 11.4) saturated with 1-octanol, 1.6 mL of compound **1** methanol solution and 40 mL of 1-octanol saturated with phosphate buffers were added. The mixtures were shaken 4 h and centrifuged 3 min at 1000 rpm (IEC Clinical Centrifuge, International Equipment Company). The concentrations of compound **1** in phosphate buffer and that in the 1-octanol phase were measured with a Hitachi U-2000 UV spectrophotometer at λ_{max}. The standard curves were obtained for 1-octanol and pH 2, pH 7, and pH 11 buffers and then used for measurement of concentrations of compound **1**.

The p*K*$_a$ values were calculated with the following equations derived from the buffer equation.

$$\text{at pH 11,} \quad \text{p}K_a(\text{acid}) = \text{pH} - \log\!\left(P\frac{[\text{D}]_{\text{pb}}}{[\text{D}]_{\text{o}}} - 1\right)$$
$$\text{[for (N)−OH group]}$$

$$\text{at pH 2,} \quad \text{p}K_a(\text{base}) = \text{pH} + \log\!\left(P\frac{[\text{D}]_{\text{pb}}}{[\text{D}]_{\text{o}}} - 1\right)$$
$$\text{(for the imino −N= nitrogen)}$$

where *P* is partition coefficient, [D]$_{pb}$ the drug concentration in phosphate buffer, and [D]$_o$ is the drug concentration in 1-octanal.

The stability of compound **1** was studied in pH 3, pH 7, and pH 11 phosphate buffers at room temperature up to 72 h. The concentrations at different time intervals were determined by using a Hitachi U-2000 UV spectrophotometer.

In Vitro Antitumor Activity. The lymphocytic murine leukemia cell line L1210 was obtained from Cell Culture Core Facility of the Norris Comprehensive Cancer Center at the Keck School of Medicine, University of Southern California. The cells were maintained in a 5% CO$_2$ humidified atmosphere at 37 °C in RPMI-1640 medium containing penicillin (170 IU/mL), streptomycin (170 μg/mL) (Mediatech-Cellgro, Herndon, VA), and 10% fetal bovine serum (FBS) (Omega Scientific, Tarzana, CA). The cell culture was passaged every 3 days using an initial density of 4 × 10^4 cells/mL.

The IC$_{50}$ values of the test compounds against L1210 cells were determined using MTS/PES microculture tetrazolium assay in 96-well microtiter plates.[26−29] For each experiment, the cells were suspended in RPMI-1640 medium with 10% FBS at 1 × 10^5/mL and seeded in 96-well plates at 50 μL/well. Each compound was initially dissolved in 100% DMSO immediately before use and further diluted into RPMI-1640 medium to obtain six gradual concentrations ranging from 2 × 10^{-3} to 1

$\times$ 10^{-8} M. Each dilution was added to the wells at 50 μL/well in triplicates. The final concentration of DMSO in the cell cultures was 1% for the highest drug concentration, and in the corresponding controls, all others had <0.25% to avoid the toxicity of DMSO. Hydroxyurea (Aldrich Chemical Co., St. Louis, MO) was used as a positive control. Negative control, containing only DMSO at identical dilutions, was run with each experiment. All experiments were repeated at least twice.

The plate was incubated in a 5% CO$_2$ humidified atmosphere at 37 °C for 65 h. A total of 20 μL/well of MTS/PES CellTiter 96 Aqueous One Solution reagent (Promega Corp., Madison, WI) was added to each well, and the plate was incubated for 3 more hours. The absorbance at 490 nm was recorded by using a microplate reader (Dynatech Laboratories, Revelation, version 3.04, Chantilly, VA).

The inhibition of L1210 cell growth at different concentrations was expressed as a percent of the negative control. After the percent inhibitions were converted into probits, IC$_{50}$ values were calculated from the regression equations by substituting a probit value of 5 to represent 50% inhibition.

Supporting Information Available: IR, ^{1}H, ^{13}C, 2-D NMR (compounds **1**, **6**, and **11**), MS (compounds **5**, **12**, **18**, **25**, **29** and **30**) spectra, and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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