Quantitative Structure–Activity Relationship (QSAR) Analysis of the Cytotoxicities Of Aminohydroxyguanidine Derivatives and Their Antiviral Activities *in Vitro*

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Substituted Schiff bases of 1-amino-3-hydroxyguanidine (SB-HAG) were tested for the first time against noninfected T4 lymphocytes (CEM-6 cells) and the same cell line infected by HIV-1 *in vitro*. Twenty-one of 23 compounds at micromolar levels did not inhibit the growth of the noninfected T4 cells, suggesting minimal cytotoxicity. The antiviral effects of these compounds in a micromolar concentration range have been shown to be nonsignificant (<30%) against HIV-1. Three-dimensional parameter focusing of the physicochemical properties (i.e., log P and V_w) and the marginal antiviral activities shows that the marginally active compounds lie in a region different from the inactive compounds. QSAR analysis of the two subsets shows that the cytotoxicity correlates well with the electronic and lipophilic parameters. The results of the QSAR analysis can serve as guidelines for further structural modification of this series of compounds to minimize the cytotoxicity against host cells.

KEY WORDS: HIV-1; virus; hydroxyaminoguanidines; quantitative structure–activity relationship (QSAR); antiviral activity; cytotoxicity.

INTRODUCTION

Hydroxyurea, hydroxyguanidine, and thiosemicarbazone derivatives have been reported to have antiviral and antitumor activities (1–4). Substituted Schiff bases of hydroxyaminoguanidine (SB-HAG) combine the structural features of hydroxyurea and thiosemicarbazone. A series of SB-HAG derivatives has been synthesized, tested, and shown to have antiviral as well as antitumor activities, in our laboratories (5–11).

Hydroxyguanidine and thiosemicarbazone derivatives are metal-chelating agents (12,13). Besides containing the essential pharmacophore of the above compounds, SB-HAG also possesses the same linkage [−CH=N−NH−C(=NH)NHOH] as guanoxabenz, which also chelates metal ions (14). These findings suggest that SB-HAG derivatives may inhibit virus-induced or virus-associated enzymes requiring a metal ion as a cofactor for enzyme activities.

Most virus-associated DNA polymerases or RNA polymerases are metalloenzymes and are often virus specific (15). These viral-specific enzymes requiring distinct metal ions are not utilized by uninfected normal cells (16–18) and, therefore, can serve as biotargets for the development of new antiviral agents. Heterocyclic and aromatic Schiff bases of 1-amino-3-hydroxyguanidine have been shown to be active against several viruses *in vitro*, e.g., Rous sarcoma virus (RSV), herpes simplex virus (HSV) type 1, and coronavirus (5,6,9,10). Therefore, SB-HAG can serve as a lead agent for the development of chemotherapeutic agents against various viral infections.

Viral integratase, which is an important enzyme in the life cycle of HIV-1 virus, contains a "zinc finger" structure (19) and can be a target site for viral chemotherapy. Oxford and Penin have suggested that thiosemicarbazone and related compounds may inhibit this enzyme (20,21). SB-HAG derivatives may have inhibitory activity similar to that of thiosemicarbazones, with fewer solubility problems. This series of compounds was tested by the National Cancer Institute, Bethesda, MD, against infection of CEM-6 cells by HIV-1. In this study, QSAR analysis based on the cytotoxicities and selected physicochemical properties was performed by the method of least squares.

MATERIALS AND METHODS

Partition Coefficient Determination of Two Selected Compounds

The compound was dissolved in a phosphate buffer containing 10% ethanol as a stock solution. The hydrophobic property of a compound can be described by its partition in octanol–phosphate buffer (pH 7.4). The partition coefficients were determined at room temperature by shaking 25 ml of the octanol phase with 25 ml of the aqueous phase (containing 2–3% ethanol as the final concentration) for 4 hr as described previously (22). The partition coefficient (log P) was calculated from

$$\log P = \log (C_{oct.}/C_{aq.})$$

where $C_{oct.}$ is the concentration of the drug in the octanol phase and $C_{aq.}$ is the concentration of the drug in the aqueous phase.

Preliminary Screening of SB-HAG Derivatives Against HIV *in Vitro*

The compounds tested are shown in Table I. The anti-HIV test of SB-HAG was conducted through the NCI's *In Vitro* Anti-AIDS Drug Discovery Program, using the methodology reported previously (23). The assay was run in duplicate and each value was the average of two counts.

Physicochemical Parameters

Several physicochemical parameters were obtained or calculated from Refs. 24–27. These parameters include the hydrophobic parameters (log P and π), the bulk of the substituent (e.g., V_w, the van der waals volume of substituent), and electronic parameters ($\Sigma\sigma$, μ, and $\Sigma\vec{\mu}$), where $\Sigma\sigma$ is the summation of Hammett substituent constants—its sign is positive for electron-withdrawing groups; μ is the group di-

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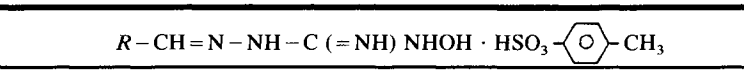
Table I. The Relative Ranking Order of Antiviral Activity and Cytotoxicity of SB-HAG Derivatives at Micromolar Levels Against HIV-1 *in Vitro*



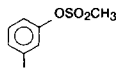
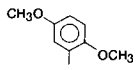
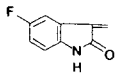
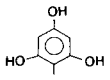
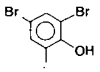
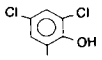
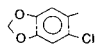
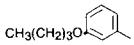
Ranking order of antiviral activity	*R*	Conc. (μM)	Antiviral activity[a] (% of control in infected cells)	Cytotoxicity[a] (% of control in noninfected cells)
1		2.12	27.36	102
2		6.11	23.96	106
3		5.95	22.46	105
4		7.04	20.77	100
5		6.86	20.56	108
6		4.82	19.65	97
7		4.33	18.53	105
8		6.17	5.83	98
9		6.29	5.68	111
10		2.49	5.12	95
11		3.00	4.29	107
12		1.91	4.15	103
13		2.26	3.93	99
14		2.16	3.83	96
15		2.49	3.51	97
16		7.04	3.45	107
17		6.34	3.27	94

Table I. Continued

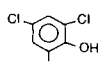
$R-CH=N-NH-C(=NH)\,NHOH \cdot HSO_3-C_6H_4-CH_3$

Ranking order of antiviral activity	R	Conc. (μM)	Antiviral activity[a] (% of control in infected cells)	Cytotoxicity[a] (% of control in noninfected cells)
18		3.05	3.02	16
19		2.69	2.71	97
20		2.36	2.61	102
21		2.24	2.52	99
22		6.59	2.46	99
23		6.25	−0.82	−1.3

[a] Calculated average from two counts.

Table II. Examples of the Inhibitory Activities of Several Schiff Bases of *N*-Hydroxy-*N'*-aminoguanidine Tosylate at Micromolar Concentrations Against Several Viruses *in Vitro*

$R-CH=N-NH-C(=NH)\,NHOH \cdot HSO_3-C_6H_4-CH_3$

	% of inhibition					
		HSV Type 1[a]				
R	HIV-1	Mckrae	RE6	KOS	RSV[b]	Coronavirus[b]
	20.6 (6.86)[c]	49 (1.0)	64 (1.0)	41 (1.0)	0.1 (5.15)	—
	2.46 (6.59)	44 (1.0)	45 (1.0)	49 (1.0)	11.6 (5.0)	—
	4.29 (3.0)	24 (1.0)	33 (1.0)	51 (1.0)	—	91.43 (9.7)
	5.83 (6.17)	—	—	—	—	91.00 (9.7)

[a] Obtained from Ref. 9.
[b] Obtained from Refs. 29 and 30.
[c] Concentration in 10^{-6} *M* in parentheses.

Table III. Physicochemical Constants and Cytotoxicity of Schiff Bases of 1-Amino-3-hydroxyguanidine Tosylate Against Noninfected CEM-6 Cells

$R-CH=N-NH-C(=NH)NHOH \cdot H_2SO_4-C_6H_4-CH_3$

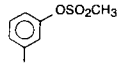
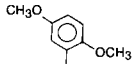
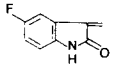
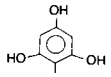
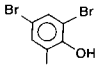
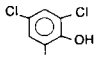
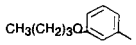
No.	R	log $1/IC_{50}$[e]		log P'	Physicochemical constant of R		
		Obs.	Cal.		π	μ	V_w
1		4.43	4.34[d]	−0.64[a]	1.25	4.11	85.38
2		3.94	4.04[d]	−0.29[a]	1.60	1.66	75.51
3		3.87	—	−0.74[a]	1.15	—	63.06
4		3.15	1.00[c]	−0.07[a]	1.82	0.59	44.08
5		3.85	3.67[c]	−0.78[a]	1.11	1.74	54.74
6		4.43	4.45[d]	−0.33[a]	1.56	4.01	57.95
7		<3.37	4.68[d]	1.12[a]	3.01	2.61	64.28
8		4.46	4.45[d]	0.72[b]	2.77	1.69	73.49
9		<3.20	3.66[d]	−1.73[a]	0.16	2.70	64.23
10		4.59	4.63[d]	1.33[a]	3.22	1.35	74.40
11		4.29	4.63[d]	1.35[b]	3.08	1.41	68.74
12		4.66	4.73[d]	0.35[a]	2.24	4.33	67.05
13		4.22	4.59[c]	−1.24[a]	0.65	2.14	44.26
14		<3.17	—	0.01[a]	1.90	0.21	56.34
15		4.88	4.75[d]	1.90[a]	3.79	1.22	103.89
16		3.44	—	1.55[a]	3.44	0.00	57.93
17		4.56	4.53[d]	−0.04[a]	1.85	3.95	58.34

Table III. Continued

R–CH=N–NH–C (=NH) NHOH · H_2SO_4–C_6H_4–CH_3							
		log 1/IC_{50}[e]			Physicochemical constant of *R*		
No.	*R*	Obs.	Cal.	log *P'*	π	μ	V_w
18		6.31	—	0.90[a]	2.79	—	67.11
19		4.85	4.69[d]	1.72[a]	3.61	1.22	91.57
20		5.79	5.66[c]	0.19[a]	2.08	2.60	70.98
21		4.02	4.06[c]	−0.68[a]	1.21	1.91	47.61
22		4.45	4.31[d]	0.22[a]	2.11	1.22	60.88
23		4.79	4.69[c]	0.41[a]	2.30	2.18	70.98

[a] Calculated log *P* based on the summation of π_R and average $\pi_{HAG \cdot tosylate}$ which was calculated from π_R and the log *P* of compounds 8 and 11.
[b] Experimental log *P*.
[c] Calculated from Eq. (1).
[d] Calculated from Eq. (2).
[e] The concentration required to inhibit the growth of normal cells by 50% in the tissue culture.

pole moment; and $\Sigma\vec{\mu}$ is the vector summation of the group dipole moments of the substituents—by definition its sign is negative for electron-withdrawing groups.

Multiple Regression Analysis

Stepwise multiple regression analysis was used in deriving the correlations.

RESULTS AND DISCUSSION

The inhibition of several compounds at the micromolar level against several viruses *in vitro* ranged from 0.1 to 91% (see Table II). The antiviral activities of SB-HAG derivatives differ for different test viruses. Previously, some SB-HAG derivatives were found to inhibit both ribonucleotide reductase in L1210 (9) and RNA polymerase in coronavirus in cell culture (28). Hence these compounds could inhibit different virus-encoded enzymes in different viruses, and QSAR analysis may assist future molecular modifications.

The physicochemical parameters and the cytotoxicity data used in the regression are summarized in Tables III and IV. The equations for quantitative structure–activity relationship between the cytotoxicity (log 1/IC_{50}) and the physicochemical parameters are given as follows.

N-Heterocyclic Compounds:

$$\log 1/IC_{50} = -0.366 + 2.318\,\mu_R$$
$$n = 5; \quad r = 0.96; \quad s = 0.26; \quad F_{1,3} = 33.63 > F_{1,3,0.95} = 10.13 \tag{1}$$

Aromatic Compounds:

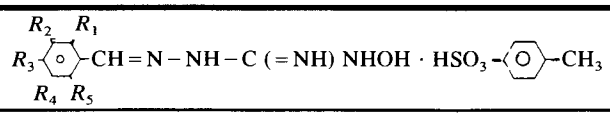
$$\log 1/IC_{50} = 4.116 + 0.263 \log P - 0.103\Sigma\vec{\mu}$$
$$n = 11; \quad r = 0.83; \quad s = 0.16; \quad F_{2,8} = 8.97 > F_{2,8,0.95} = 4.48 \tag{2}$$

Stepwise *F* test was used to evaluate the statistical significance of each equation.

The compounds were tested *in vitro* for the first time against noninfected and HIV-1-infected T4 lymphocytes (CME-6 cell line) by the NCI. The procedure of detecting anti-HIV activity in microculture has been reported by Weislow *et al.* (23). Twenty-one of the 23 compounds at the micromolar level did not inhibit growth of noninfected cells (Table I). Thus, cytotoxicity of these compounds at micromolar levels is minimal.

The relative ranking of anti-HIV activity of these compounds at the micromolar level is shown in Table I. Although the marginal antiviral effects of these compounds in a micro-

Table IV. Physicochemical Constants and Cytotoxicity of Schiff Bases of 1-Amino-3-hydroxyguanidine Tosylate Against Noninfected CEM-6 Cells



					log $1/IC_{50}$		Physicochemical constant of substituents				
R_1	R_2	R_3	R_4	R_5	Obs.	Cal.[a]	$\Sigma\pi$	$\Sigma\sigma$	ΣV_w[b]	$\Sigma\vec{\mu}$[c]	log P'
H	OC_4H_9	H	H	H	4.85	4.69	1.03	0.10	48.39	−1.19	1.72
H	OCH_3	H	H	H	4.45	4.31	−0.02	0.12	17.70	−1.30	0.22
OH	C_3H_5	H	H	H	4.46	4.45	0.43	−0.48	30.44	−1.39	0.72
OH	Cl	H	Cl	H	4.29	4.63	0.74	0.37	28.63	−1.59	1.35
H	H	$OCH_2\phi$	H	H	4.88	4.75	1.66	−0.23	60.61	−1.30	1.90
OCH_3	H	H	OCH_3	H	3.94	4.04	−0.04	−0.15	35.40	0.00	−0.29
H	H	N≡C	H	H	4.43	4.45	−0.57	0.66	15.06	−4.08	−0.33
H	OSO_2CH_3	H	H	H	4.43	4.34	−0.88	0.39	42.10	−3.77	−0.64
H	NO_2	H	H	H	4.56	4.53	−0.28	0.71	15.06	−4.13	−0.04
OH	Br	H	Br	H	4.59	4.63	1.05	0.39	34.29	−1.57	1.33
H	NO_2	Cl	H	H	4.66	4.73	0.43	0.94	26.94	−5.11	0.35

[a] Calculated from Eq. (2).
[b] $(V_{WR} - V_{W\phi})$.
[c] $\Sigma\vec{\mu} = [(AO)^2 + (BO)^2 + 2(AO)(BO)Cos\theta]^{1/2}$, where AO and BO are the group dipole moments of substituents excluding hydrogen, and θ is the angle between AO and BO.

molar concentration range (−0.82 to 27% inhibition) are considered "nonsignificant," there appears to be a clear division between the first seven compounds, with an inhibition range of 18 to 27%, and the rest of the compounds, with an inhibition range of −0.82 to 5.83%. The three-dimensional parameter focusing of the physicochemical properties (i.e., log P' and V_w) and antiviral activity (i.e., the percentage of control in infected cells) (Fig. 1) shows the relationship between the physicochemical parameters and the marginal antiviral activity. Six of the first seven compounds are localized in the critical area defined by V_w (a range of 44–85) and log P' (a range of 0.00 to −0.90) in Fig. 1. The nature of the substituent is critical for activity, but further study with more data points is needed to establish possible correlations between the antiviral activity and physicochemical properties.

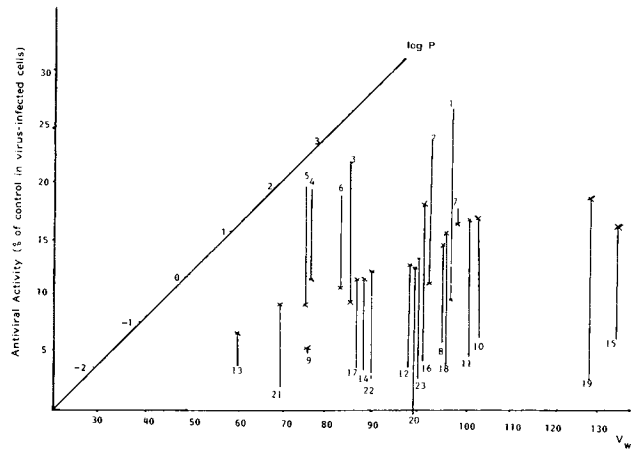


Fig. 1. Three-dimensional parameter focusing of the physicochemical parameters and antiviral activity of SB-HAG derivatives. Each compound number was obtained from Table I.

The correlation between the cytotoxicity against noninfected T4-cells and the physicochemical parameters of 19 compounds was analyzed. In Table III, compounds 7, 9, and 14 do not have accurate activity values, and the group dipole moments for compounds 3 and 18 were not available from the literature; therefore, these compounds were not included in the regression analysis. Equations obtained from the QSAR analysis between the cytotoxicity and the physicochemical constants of the 19 compounds (in Table III) are not significant, based on F test. The physicochemical properties of either the substituents or the full molecules can explain less than 50% of variance in the data. When the 19 compounds in Table III are divided into two subsets of N-heterocyclic and aromatic compounds, high correlations between the cytotoxicity and the physicochemical properties are obtained. For N-heterocyclic compounds, Eq. (1) can explain 92% of the variance in the data. Equation (1) shows that the cytotoxicity of the compound increases with increasing group dipole moment. In Eq. (2), the log P' and the vector summation of group dipole moments of the substituents can explain 69% of the variance in the data. Equation (2) shows that the cytotoxicity of aromatic SB-HAG derivatives is positively dependent on log P' and negatively dependent on $\Sigma\vec{\mu}$. These equations can be used as guidelines for further structural modification of this series of compounds to minimize the cytotoxicity against host cells.

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