Quantitative Structure–Activity Relationships of N^2-Phenylguanines as Inhibitors of Herpes Simplex Virus Thymidine Kinases

Joseph Gambino,† Federico Focher,‡ Catherine Hildebrand,† Giovanni Maga,‡ Timothy Noonan,† Silvio Spadari,‡ and George Wright*,†

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01655, and Istituto di Genetica Biochimica ed Evoluzionistica, Consiglio Nazionale della Ricerche, Pavia, Italy. *Received December 26, 1991*

Quantitative structure–activity relationships of the Hansch-type were developed to account for inhibition of thymidine kinases from Herpes simplex viruses types 1 and 2 (HSV1,2) by N^2-phenylguanines. Derivatives with meta and/or para substituents on the phenyl ring display a wide range of overlapping, but not identical, potencies as inhibitors of the enzymes. IC_{50} values for 36 (HSV1) and 35 inhibitors (HSV2) were used to develop equations using hydrophobic (π), electronic (σ, $\mathcal{R}$), and group size (MR) parameters. Equations 1 and 2 with correlation coefficients of 0.797 and 0.805, respectively, were obtained for inhibitors of the types 1 and 2 enzymes. Potencies were correlated positively with π values of meta substituents but negatively with π values of para substituents in the phenyl ring. Positive correlations were also obtained with the resonance parameter $\mathcal{R}$ of para substituents and with σ constants of meta substituents. The most potent inhibitor of both enzymes was N^2-[*m*-(trifluoromethyl)phenyl]guanine, although HSV2 thymidine kinase was more sensitive to certain compounds than the HSV1 enzyme.

A series of N^2-phenylguanines (PG) has been found to inhibit herpes simplex virus type 1 (HSV1) and herpes simplex virus type 2 (HSV2) thymidine kinases (TK) without inhibiting the corresponding mammalian cell TK.[1,2] The compounds inhibit the viral TKs competitively with respect to the enzyme substrates 2′-deoxythymidine (TdR) and 2′-deoxycytidine. Among a series of compounds substituted on the phenyl ring, the most potent TK inhibitor, N^2-[*m*-(trifluoromethyl)phenyl]guanine (1), inhibited the reactivation of latent HSV1 in explanted mouse trigeminal ganglia.[3] Observations that HSV TK expression may be implicated in recurrent infections and in pathogenicity of herpes simplex virus have prompted our and others' interest in development of selective viral TK inhibitors.[4]

Quantitative structure–activity relationships to describe the N^2-phenylguanine TK inhibitors were sought for two reasons. The first was to predict the type of substituents and their position on the phenyl ring which would provide more potent inhibitors as potential antiviral agents. The second was to facilitate an understanding of the structural differences in the inhibitor binding sites of HSV1 and HSV2 TKs.

Chemistry and Enzymology

The syntheses and inhibitory activities of many of the subject compounds have been reported.[1,2] Yields and physical properties of new N^2-phenylguanines are presented in Table I. Most were prepared by a standard method involving reaction of the aniline with 2-bromohypoxanthine in refluxing 2-methoxyethanol (see Experimental Section). Halomethyl derivatives 4 and 6 were prepared from the hydroxymethyl compound 20 (*m*-CH_2OHPG) and the appropriate inorganic acid (see Experimental Section).

The thymidine kinases from HSV1- and HSV2-infected HeLa cells were isolated and assayed as described previously.[1,2,5] IC_{50} values for inhibitors are the concentrations of inhibitor necessary to cause 50% inhibition of phosphorylation of [^{3}H]TdR at a fixed concentration of the substrate: 1 and 2 μM for HSV1 and HSV2 TKs, respectively. The IC_{50} values are close to and proportional to K_i, the actual inhibitor binding constants.[1,5]

QSAR of Inhibitors of HSV1 Thymidine Kinase

Equations were derived for the inhibitors with the use of the substituent parameters listed in Table II, taken from Hansch,[6] and multiple regression analysis. Table III lists the experimental potencies, as log $(1/IC_{50})$, and those calculated using eq 1. Equation 1 was developed by the

$$\log (1/IC_{50}) = 0.56\ (\pm 0.16)\pi_m - 0.45\ (\pm 0.14)\pi_p + 2.45\ (\pm 0.65)\mathcal{R} + 0.73\ (\pm 0.49)\sigma_m + 5.10\ (\pm 0.16) \quad (1)$$

$$n = 36,\ r = 0.797,\ F_{(4,31)} = 13.46_{(0.005)}$$

addition and deletion of physicochemical parameters thought to be important for inhibitor potency, and by use of the F test to determine the statistical significance of each change. The final sequence used is shown in Table IV. In all equations the parameter coefficients are included with standard deviations in parentheses.

π_m, the hydrophobic parameter for meta substituents, was the most important single parameter. This accounted for 34% (r^2) of the variance in the data (eq 1a, Table IV) and agrees with our earlier qualitative study on a limited set of inhibitors.[2] Adding π_p to the equation resulted in a negative correlation for this term (eq 1b, Table IV). This showed that the para position will not accommodate hydrophobic substituents without a loss in enzyme binding affinity.

The resonance parameter for para substituents, $\mathcal{R}$, was the next term added, and the resulting eq 1c (Table IV) accounted for 40% of the variance. While this term is positive in this equation, only one compound (22) in the

* Author to whom correspondence should be addressed.
† University of Massachusetts Medical School.
‡ Consiglio Nazionale della Ricerche.

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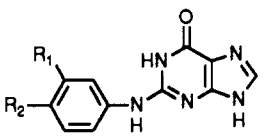
Table I. Physical Data for N^2-Phenylguanine Derivatives

no.[a]	% yield	mp, °C	crystn solvent	formula	^{1}H NMR,[b] δ (ppm) 2-NH	8-H
5	35	260–262	MeOH	$C_{11}H_8N_5OI \cdot {}^1/_2H_2O$	8.81	7.94
8	54	326–328	MeOH	$C_{11}H_8N_5OBr \cdot {}^1/_2H_2O$	8.84	7.87
12	47	>350	MeOH	$C_{12}H_8N_6O \cdot {}^1/_2H_2O$	9.01	7.83
19	50	287–289	EtOH	$C_{12}H_7N_5OClF_3 \cdot H_2O$	9.11	7.90
23	61	298–300	DMF/H_2O, 3:1	$C_{14}H_{15}N_5O \cdot {}^1/_4H_2O$	8.55	7.72
25	64	338–340	80% HAc	$C_{16}H_{19}N_5O \cdot {}^1/_6H_2O$	8.40	7.76
26	53	318–320	DMF	$C_{11}H_8N_5OI$	8.77	7.87
31	50	>350	DMF	$C_{11}H_8N_5OCl \cdot {}^2/_3H_2O$	8.79	7.86
33	61	318–321	DMF/H_2O, 1:1	$C_{14}H_{15}N_5O \cdot {}^1/_4H_2O$	8.52	7.80
34	48	>350	DMF/H_2O, 1:4	$C_{13}H_{13}N_5O \cdot {}^1/_3H_2O$	8.59	7.72
36	29	>350	MeOH	$C_{16}H_{19}N_5O \cdot {}^1/_6H_2O$	8.52	7.81
2	72	>350	EtOH	$C_{12}H_{10}N_8O \cdot DMSO$	8.77	7.86
4	82	>350	EtOH	$C_{12}H_{10}N_5OBr \cdot {}^1/_4H_2O$	8.61	7.98
6	80	>350	EtOH	$C_{12}H_{10}N_5OCl \cdot {}^1/_4H_2O$	8.83	7.65
21	60	>350	EtOH	$C_{12}H_{12}N_6O \cdot {}^1/_3H_2O$	9.74	7.74

[a] Substituents indicated in Table II. For methods see Experimental Section. [b] All other resonances as expected.

Table II. Parameters Used in the Derivation of Equations 1 and 2



no.	R_1	R_2	substituent parameters[a] π_m	π_p	$\mathcal{R}$	σ_m
1	CF_3	H	0.88	0	0	0.43
2	CH_2N_3	H	0.72	0	0	0
3	H	Br	0	0.86	−0.17	0
4	CH_2Br	H	0.79	0	0	0.12
5	I	H	1.12	0	0	0.35
6	CH_2Cl	H	0.64	0	0	0.10
7	Cl	H	0.71	0	0	0.37
8	Br	H	0.86	0	0	0.39
9	Et	H	1.02	0	0	−0.07
10	Br	Br	0.86	0.86	−0.17	0.39
11	*n*-Pr	H	1.55	0	0	−0.07
12	CN	H	−0.57	0	0	0.56
13	Et	Me	1.02	0.56	−0.13	−0.07
14	Cl	F	0.71	0.14	−0.34	0.37
15	H	H	0	0	0	0
16	Cl	Me	0.71	0.56	−0.13	0.37
17	$-CH_2CH_2CH_2-$[b]		0.60	0.60	−0.01	−0.26
18	H	Et	0	1.02	−0.10	0
19	CF_3	Cl	0.88	0.71	−0.15	0.43
20	CH_2OH	H	−1.03	0	0	0
21	CH_2NH_2	H	−1.04	0	0	0
22	H	CF_3	0	0.88	0.19	0
23	H	*n*-Pr	0	1.55	−0.10	0
24	$-CH{=}CHCH{=}CH-$[b]		0.66	0.66	0.01	0.04
25	Me	*n*-Bu	0.56	2.13	−0.13	−0.07
26	H	I	0	1.12	−0.19	0
27	H	Me	0	0.56	−0.13	0
28	F	F	0.14	0.14	−0.34	0.34
29	H	*n*-Bu	0	2.13	−0.13	0
30	Cl	Cl	0.71	0.71	−0.15	0.37
31	H	Cl	0	0.71	−0.15	0
32	H	OH	0	−0.67	−0.64	0
33	H	*i*-Pr	0	1.53	−0.10	0
34	CH_2OH	Me	−1.03	0.56	−0.13	0
35	H	F	0	0.14	−0.34	0
36	H	n-C_5H_{11}	0	2.39	−0.15	0

[a] Substituent parameters were obtained from ref 6. [b] π values for these groups were divided by 2 to give values for meta and para positions.

Table III. Experimental and Calculated IC_{50} of N^2-Phenylguanines against HSV1 Thymidine Kinase from Equation 1

no.	log (1/IC_{50}) exptl[a]	calcd	Δlog (1/IC_{50})
1	6.82 (±0.10)	5.91	0.91
2	6.10 (±0.10)	5.50	0.60
3	6.00 (±0.12)	4.30	1.70
4	6.00 (±0.05)	5.63	0.37
5	5.96 (±0.04)	5.98	−0.02
6	5.85 (±0.21)	5.53	0.32
7	5.82 (±0.10)	5.77	0.05
8	5.82 (±0.13)	5.87	−0.04
9	5.60 (±0.25)	5.62	−0.02
10	5.52 (±0.02)	5.06	0.46
11	5.48 (±0.07)	5.92	−0.44
12	5.30 (±0.10)	5.19	0.11
13	5.16 (±0.05)	5.11	0.05
14	5.10 (±0.01)	4.87	0.23
15	5.10 (±0.01)	5.10	0.00
16	4.92 (±0.08)	5.20	−0.28
17	4.82 (±0.05)	4.95	−0.13
18	4.70 (±0.01)	4.40	0.30
19	4.70 (±0.05)	5.22	0.52
20	4.60 (±0.01)	4.52	0.08
21	4.52 (±0.03)	4.52	0.00
22	4.52 (±0.01)	5.17	−0.65
23	4.52 (±0.04)	4.16	0.36
24	4.40 (±0.01)	5.23	−0.83
25	4.40 (±0.04)	4.09	0.31
26	4.40 (±0.01)	4.13	0.27
27	4.30 (±0.03)	4.53	−0.23
28	4.30 (±0.01)	4.53	−0.23
29	4.30 (±0.05)	3.82	0.48
30	4.00 (±0.01)	5.08	−1.08
31	4.00 (±0.05)	4.41	−0.41
32	3.85 (±0.05)	3.83	0.02
33	3.70 (±0.01)	4.17	−0.47
34	3.57 (±0.04)	3.95	−0.38
35	3.52 (±0.03)	4.20	−0.68
36	3.30 (±0.02)	3.66	−0.36
		standard deviation	0.51

[a] Numbers in parentheses are standard deviations from two experiments for each compound, except 1, 3, 6, 15, and 20, which are the results of four experiments.

data set has a $\mathcal{R}$ that is not less than or equal to 0. As a consequence, most para substituted compounds display a decrease in binding affinity for the enzyme. This equation has a correlation coefficient 15% higher than the equivalent equation with σ_p in place of $\mathcal{R}$. The addition of the inductance parameter F to eq 1c gave no improvement.

The last term added was σ_m, the Hammett electronic parameter, and the final equation (1) accounted for 64% of the variance. The inclusion of σ_m was, however, significant only to the 10% level. This result indicates a need for a substituent that is electron withdrawing as well as hydrophobic in the meta position for optimal enzyme binding. This is demonstrated in the data by the greater

Table IV. Development of Equation 1

eq no.	coefficients of variables π_m	π_p	$\mathcal{R}$	σ_m	constant	r	std dev	F	F of difference between eq
1a	0.75 (±0.20)				4.62 (±0.14)	0.540	0.709	$[F_{(1,34)}]$ $14.12_{(0.005)}$	
1b	0.68 (±0.18)	−0.49 (±0.16)			4.91 (±0.16)	0.670	0.626	$[F_{(2,33)}]$ $13.70_{(0.005)}$	$[F_{(1,33)}]$ $7.71_{(0.01)}$
1c	0.61 (±0.16)	−0.50 (±0.14)	2.39 (±0.66)		5.19 (±0.15)	0.781	0.526	$[F_{(3,32)}]$ $16.44_{(0.005)}$	$[F_{(1,32)}]$ $9.22_{(0.005)}$
1	0.56 (±0.16)	−0.45 (±0.14)	2.45 (±0.65)	0.73 (±0.49)	5.10 (±0.16)	0.797	0.509	$[F_{(4,31)}]$ $13.46_{(0.005)}$	$[F_{(1,31)}]$ $2.31_{(0.10)}$

Table V. Experimental and Calculated IC_{50} of N^2-Phenylguanines against HSV2 Thymidine Kinase from Equation 2

no.	log $(1/IC_{50})$ exptl[a]	log $(1/IC_{50})$ calcd	Δlog $(1/IC_{50})$
1	7.00 (±0.03)	6.36	0.64
2	5.82 (±0.03)	5.56	0.26
3	6.22 (±0.04)	4.56	1.66
4	5.92 (±0.05)	5.79	0.13
5	5.96 (±0.05)	6.32	−0.36
6	5.87 (±0.01)	5.70	0.17
7	6.46 (±0.04)	6.19	0.27
8	6.22 (±0.02)	6.29	−0.07
9	5.40 (±0.02)	5.56	−0.16
10	5.89 (±0.03)	5.58	0.31
11	5.52 (±0.02)	5.77	−0.25
12	6.40 (±0.02)	6.01	0.39
13	5.10 (±0.02)	5.07	0.03
14	5.60 (±0.02)	5.61	−0.01
15	5.80 (±0.03)	5.27	0.53
16	5.60 (±0.02)	5.70	−0.10
17	5.00 (±0.00)	4.73	0.27
18	4.60 (±0.01)	4.58	0.02
19	5.30 (±0.03)	5.76	−0.46
20	4.70 (±0.02)	4.86	−0.16
21	4.70 (±0.01)	4.85	−0.15
22	4.40 (±0.02)	5.09	−0.69
23	4.33 (±0.01)	4.30	0.03
24	5.00 (±0.01)	5.27	−0.27
26	5.05 (±0.00)	4.39	0.66
27	5.22 (±0.03)	4.78	0.44
28	5.66 (±0.01)	5.33	0.33
29	4.30 (±0.02)	3.95	0.35
30	4.52 (±0.02)	5.59	−1.07
31	4.30 (±0.00)	4.67	−0.37
32	4.70 (±0.02)	4.67	0.03
33	4.00 (±0.01)	4.31	−0.31
34	3.70 (±0.01)	4.37	−0.67
35	3.59 (±0.01)	4.69	−1.10
36	3.60 (±0.01)	3.78	−0.18
		standard deviation	0.51

[a] Numbers in parentheses are standard deviations from two experiments for each compound, except 1, 3, 6, 15, and 20, which are the results of four experiments.

activity of compounds with meta halo substituents compared with meta alkyl substituents with similar π values.

The final equation (1) gave a poor fit for compounds **3** and **30**. The IC_{50} of compound **3**, *p*-BrPG, was underestimated by 10-fold, and that of compound **30**, 3,4-Cl_2PG, was overestimated by 10-fold (Table III). Removal of these two compounds from the data set and reiteration of the experimental data gave eq 1d. This equation has a much higher correlation coefficient than eq 1, yet the only significant change between the equations is a 44% increase in the contribution to binding of the σ_m term in eq 1d. The facts that both compounds removed have halogen substituents and that the one term changed is σ_m suggest that there is some electronic interaction with the binding site that is not adequately explained by eq 1.

$$\log (1/IC_{50}) = 0.61\ (\pm 0.12)\pi_m - 0.44\ (\pm 0.11)\pi_p + 2.53\ (\pm 0.49)\mathcal{R} + 1.05\ (\pm 0.38)\sigma_m + 5.03\ (\pm 0.12) \quad (1d)$$

$$n = 34,\ r = 0.887,\ F_{(4,29)} = 26.21_{(0.001)}$$

QSAR of Inhibitors of HSV2 Thymidine Kinase

HSV2 TK showed a slightly different pattern of response to this set of inhibitors (compound **25** was not tested against HSV2 TK). Two compounds in particular, **12** and **28**, showed large decreases in IC_{50} values compared to HSV1 TK. Equation 2 was derived for the compounds

$$\log (1/IC_{50}) = 1.72\ (\pm 0.49)\sigma_m - 0.53\ (\pm 0.15)\pi_p + 0.40\ (\pm 0.16)\pi_m + 1.49\ (\pm 0.64)\mathcal{R} + 5.27\ (\pm 0.16) \quad (2)$$

$$n = 35,\ r = 0.805,\ F_{(4,30)} = 13.89_{(0.005)}$$

listed in Table II (less **25**). The experimental and calculated potencies are listed in Table V, and the development of eq 2 is shown in Table VI. This equation contains the same parameters as eq 1, but the two electronic parameters, σ_m and $\mathcal{R}$, show large changes in their contribution to inhibitor binding.

σ_m was the most important single term, accounting for 31% of the variance (eq 2a, Table VI). This indicates that for HSV2 TK inhibitors electrostatic interactions at the meta position predominate over hydrophobic interactions. As found for the HSV1 TK data, π_p is the second term added. It again showed a negative correlation, and the resulting eq 2b (Table VI) accounted for 49% of the variance.

π_m was the next term added, and its inclusion accounted for 59% of the variance (eq 2c, Table VI). The decreased value for this term from eq 1 again showed the lower contribution to binding by hydrophobic meta substituents with HSV2 TK. The last term added was $\mathcal{R}$, and the final equation (eq 2) accounted for 65% of the variance. The contribution of $\mathcal{R}$ to inhibitor binding affinity was lower for the HSV2 than for the HSV1 enzyme. The addition of σ_p to eq 2c resulted in a correlation coefficient 6% less than that of eq 2d. The addition of F to eq 2d gave no significant improvement.

As found for HSV1 TK (eq 1) compounds **3** and **30** were poorly correlated by eq 2 for the HSV2 enzyme. Removal of these compounds from the data set and reiteration gave eq 2d, which accounted for 79% of the variance. The σ_m

$$\log(1/IC_{50}) = 2.05\ (\pm 0.38)\sigma_m - 0.52\ (\pm 0.11)\pi_p + 0.45\ (\pm 0.12)\pi_m + 1.57\ (\pm 0.49)\mathcal{R} + 5.21\ (\pm 0.12) \quad (2d)$$

$$n = 33,\ r = 0.893,\ F_{(4,28)} = 27.61_{(0.001)}$$

Table VI. Development of Equation 2

eq no.	coefficients of variables π_m	π_p	$\mathcal{R}$	σ_m	constant	r	std dev	F	F of difference between eq
2a				2.40 (±0.62)	4.90 (±0.14)	0.561	0.704	$[F_{(1,33)}]$ $15.21_{(0.005)}$	
2b		−0.57 (±0.16)		1.96 (±0.56)	5.24 (±0.16)	0.700	0.607	$[F_{(2,32)}]$ $15.51_{(0.005)}$	$[F_{(1,32)}]$ $8.52_{(0.01)}$
2c	0.44 (±0.16)	−0.52 (±0.16)		1.65 (±0.52)	5.11 (±0.16)	0.765	0.547	$[F_{(3,31)}]$ $14.49_{(0.005)}$	$[F_{(1,31)}]$ $5.77_{(0.025)}$
2	0.40 (±0.16)	−0.53 (±0.15)	1.49 (±0.64)	1.72 (±0.49)	5.27 (±0.16)	0.805	0.504	$[F_{(4,30)}]$ $13.89_{(0.005)}$	$[F_{(1,30)}]$ $4.89_{(0.05)}$

Table VII. Squared Correlation between Parameters Used To Derive Equations 1 and 2

	π_m	π_p	$\mathcal{R}$	σ_m
π_m	1	0.016	0.011	0.057
π_p		1	0	0.076
$\mathcal{R}$			1	0.001
σ_m				1

term increased by only 20% but is still the only parameter to change significantly. As in the case of the HSV1 TK data, it appears that an electronic effect of these halo-substituted compounds is not fully modeled. The *p*-fluoro compound **35** is also poorly correlated in eq 2.

No strong correlations were found among the parameters used to derive both eqs 1 and 2 (Table VII). Other trial parameters used in the development of these equations included group size (molar refraction, MR) for both the meta and para substituents, as well as the sum of MR terms, σ_p, and the inductance parameter *F* for other electronic affects. None of the terms gave equations as significant as those described above, nor did any improve the correlations when added to eq 1 or 2.

Discussion

The equations that we have developed to describe inhibition of HSV thymidine kinases by N^2-phenylguanines give some insight to the nature of inhibitor binding sites for both of the enzymes. For compounds with meta substituents in the phenyl ring, binding to both enzymes is due to a combination of hydrophobic and electrostatic interactions. The results show that the electrostatic contribution is relatively more important in HSV2 TK, as reflected in a 140% increase in the σ_m term and a 29% decrease in π_m from eq 1 to eq 2.

Reduced potency for all compounds containing para substituents was observed with both enzymes except for compound **3**, *p*-BrPG. [This compound was resynthesized and retested, and the new sample had the same IC_{50} values as previously determined.] If this was due to a lack of physical space for binding of para substituents, then the group size parameter MR*p* should have shown a significant (negative) contribution. Therefore, the space into which para substituents fit is electrostatic in nature and possibly hydrophilic. Additional compounds bearing electron-attracting para substituents need to be synthesized and tested before the nature of this region of the binding site is better understood.

HSV1 and HSV2 thymidine kinases are 41-kDA proteins consisting of 376 amino acids, with considerable conserved homology between them.[7] A binding pocket for ATP, the phosphate donor, has been identified by its homology with nucleotide binding pockets of ATP and GTP binding proteins,[8] but, although HSV1 TK has been crystallized,[9] details of the 3D structure of the protein have not been reported. It appears likely that N^2-phenylguanines bind the active sites of the HSV TKs, based on the competitive inhibition kinetics measured for several analogs,[1,5] and we propose that the "inhibitor binding site" overlaps with the enzyme active site.[10] The similarity in the parameters found to contribute to binding of phenyl substituents to both HSV1 and HSV2 enzymes (eqs 1 and 2) suggests strong conservation of the inhibitor binding sites. However, quantitative differences in substituent contributions, as reflected in differences in potency of individual compounds (Tables III and V), indicate that these sites are not identical.

Experimental Section

The following compounds listed in Table II were synthesized as described previously: **1**, **3**, **7**, **10**, **11**, **14**, **16**, **18**, **20**, **22**, **24**, **28**, **30**, and **32**;[2] **9**, **15**, and **27**;[1] **13**;[11] **17**;[12] **29**,[13] and **35**.[14] All new compounds were fully characterized by ^{1}H NMR and elemental analysis (C, H, N); analyses were done by the Microanalysis Laboratory, University of Massachusetts, Amherst, MA, and agree to within ±0.4% of the calculated values. NMR spectra were determined on either a Varian Unity 300 or a Perkin-Elmer R12B instrument. Melting points were determined with a Mel-temp apparatus and are uncorrected.

All new N^2-phenylguanines except **2**, **4**, **6**, and **21** were prepared from 2-bromohypoxanthine and the appropriate aniline in refluxing 2-methoxyethanol as described.[13] The yields and properties of these compounds are presented in Table I.

***N*²-[3-(Bromomethyl)phenyl]guanine (4).** A solution of N^2-[3-(hydroxymethyl)phenyl]guanine (**20**) (200 mg, 0.778 mmol) in 49% aqueous HBr (5 mL) was stirred for 24 h at room temperature. Dilution with an equal volume of water gave a tan precipitate which was collected by filtration and washed with water. Crystallization from ethanol gave 204 mg (82%) of **4**.

***N*²-[3-(Chloromethyl)phenyl]guanine (6).** Compound **20** (257 mg, 1.0 mmol) was heated with concentrated hydrochloric acid (10 mL) in a pressure bomb at 50 °C for 24 h. An equal volume of water was added to the reaction mixture, and the resultant precipitate was collected by filtration. Crystallization from ethanol yielded 220 mg (80%) of **6**.

***N*²-[3-(Aminomethyl)phenyl]guanine (21).** N^2-[3-(Chloromethyl)phenyl]guanine (**6**) (100 mg, 0.364 mmol) was treated with ammonium hydroxide (5 mL) in a pressure bomb at 50 °C for 24 h. The reaction mixture was evaporated to dryness and the resulting residue was dissolved in ethanol. The desired product (56 mg) crystallized in 60% yield.

***N*²-[3-(Azidomethyl)phenyl]guanine (2).** N^2-[3-(Bromomethyl)phenyl]guanine (**4**) (100 mg, 0.313 mmol) was dissolved in DMSO (5 mL). Sodium azide (97 mg, 1.77 mmol) was added, and the mixture was stirred for 3 h at room temperature. Addition

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(10) Evidence that guanine, i.e. *purine*, derivatives can bind to the active site of HSV TKs, enzymes that phosphorylate *pyrimidine* nucleosides, includes the fact that "acyclonucleoside" derivatives of guanine both inhibit and are substrates for HSV1 TK (Keller, P. M.; Fyfe, J. A.; Beauchamp, L.; Lubbers, C. M.; Furman, P. A.; Schaeffer, H. J.; Elion, G. B. Enzymatic Phosphorylation of Acyclic Nucleoside Analogs and Correlations with Antiherpetic Activities. *Biochem. Pharmacol.* **1981**, *30*, 3071–3077).

(11) Wright, G. E.; Dudycz, L. W.; Kazimierczuk, Z.; Brown, N. C.; Khan, N. N. Synthesis, Cell Growth Inhibition and Antitumor Screening of 2-(*p*-n-Butylanilino)purines and their Nucleoside Analogs. *J. Med. Chem.* **1987**, *30*, 109–116.

(12) Wright, G. E.; Brown, V. M.; Baril, E. F.; Brown, N. C. Design and Characterization of N²-Arylaminopurines which Selectively Inhibit Replicative DNA Synthesis and Replication-Specific DNA Polymerases: Guanine Derivatives Specific for Mammalian DNA Polymerase Alpha and Bacterial DNA Polymerase III. *Nucl. Acids Res.* **1982**, *10*, 4431–4440.

(13) Wright, G. E.; Dudycz, L. W. Synthesis and Characterization of N²-(*p*-n-Butylphenyl)-2′-deoxyguanosine 5′-triphosphate and its Inhibition of HeLa DNA Polymerase Alpha. *J. Med. Chem.* **1984**, *27*, 175–184.

(14) Noonan, T.; Brown, N.; Dudycz, L.; Wright, G. Interaction of GTP Derivatives with Cellular and Oncogenic *Ras* p21 Proteins. *J. Med. Chem.* **1991**, *34*, 1302–1307.

of an equal volume of water gave an off-white precipitate which was collected by filtration. Crystallization from ethanol yielded 64 mg (72%) of 2.

Enzyme Isolation and Assay. The thymidine kinases from HSV1- and HSV2-infected HeLa cells were isolated by the use of a thymidine 3′-(*p*-aminophenylphosphate) affinity column as described previously.[5] The enzymes were assayed with limiting concentrations of [^{3}H]thymidine as described.[2] Stock solutions of inhibitors in dimethyl sulfoxide were diluted into assay mixtures; control assays contained an identical concentration of the compound solvent.

Calculations. Derivation of equations and statistical analyses were done on a IBM PC using a BASIC program written by one of the authors (J.G.).

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DNA-Directed Alkylating Agents. 5. Acridinecarboxamide Derivatives of (1,2-Diaminoethane)dichloroplatinum(II)

Ho H. Lee,† Brian D. Palmer,† Bruce C. Baguley,† Michael Chin,† W. David McFadyen,‡ Geoffrey Wickham,§ Deborah Thorsbourne-Palmer,§ Laurence P. G. Wakelin,∥ and William A. Denny*,†

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand, School of Science and Mathematics Education, Institute of Education, University of Melbourne, Parkville, Australia 3052, and Department of Medicinal Chemistry, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Australia 3052.
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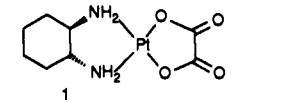
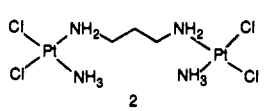
A series of acridine-2- and -4-carboxamide-linked analogues of $PtenCl_2$ has been prepared and evaluated for biological activity against several tumor cell lines in vitro and in vivo. The platinum complexes were generally more cytotoxic than the corresponding ligands against wild-type P388 leukemia cells in vitro, with acridine-4-carboxamide complexes being the more effective. In contrast to cisplatin and $PtenCl_2$, the complexes were equally active in vitro against both wild-type and cisplatin-resistant P388 lines. The 4-carboxamide complexes showed high levels of in vivo activity (ILS >100%) against wild-type P388 using a single-dose protocol, and one compound was also significantly active in vivo in a cisplatin-resistant line, against which cisplatin and $PtenCl_2$ are inactive.

One focus of work on developing analogues of cisplatin (*cis*-diamminedichloroplatinum(II)) is to improve activity against cell lines which are (for a variety of reasons) resistant to cisplatin itself. Novel examples are the DACH compounds (e.g. 1-OHP; 1)[1] and the bis-cisplatin derivatives (e.g. 2).[2] Members of these classes show almost equal cytotoxicity in cisplatin-sensitive and -resistant lines, probably (in the case of the DACH compounds) by providing DNA adducts of enhanced lethality.[3]



1



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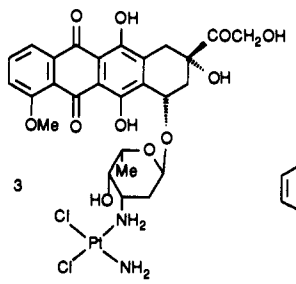

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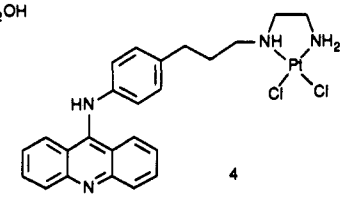
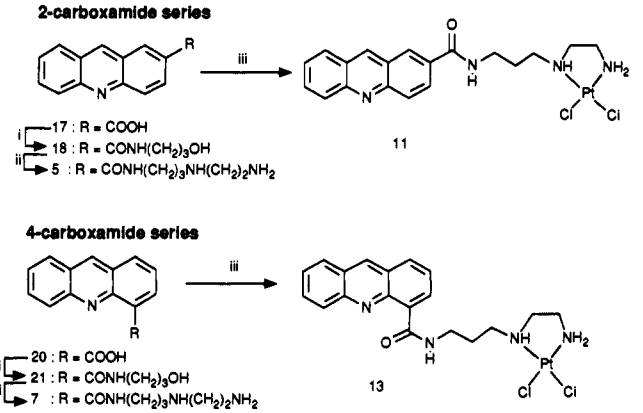


4

Another way of providing platinum–DNA adducts of high cytotoxicity is to "target" the platinum moiety to DNA by attachment to a suitable carrier ligand. This general concept has been discussed in detail,[4–6] and demonstrable effects with targeted aniline mustards include altered regiospecificity and sequence-specificity of DNA alkylation,[6,7] together with increased cytotoxicity and improved in vivo antitumor activity compared with the

Scheme I[a]



[a] (i) 1,1′-Carbonyldiimidazole/DMF/50 °C; $NH_2(CH_2)_nOH$; (ii) MsCl/py; excess $NH_2(CH_2)_2NH_2$; (iii) aqueous Na_2CO_3; K_2PtCl_4/20 °C/20 h; aqueous KCl.

† Cancer Research Laboratory.
‡ University of Melbourne.
§ Victorian College of Pharmacy.
∥ Present address: St. Luke's Cancer Research Fund, Highfield Rd., Rathgar, Dublin 6, Ireland.

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