Structure-Activity Relationships for Mitomycin C and Mitomycin A Analogues

Kenneth R. Kunz,† Bhashyam S. Iyengar,† Robert T. Dorr,‡ David S. Alberts,‡ and William A. Remers*,†

Department of Pharmaceutical Sciences and Cancer Center, University of Arizona, Tucson, Arizona 85721.
Received August 31, 1990

A set of 30 mitomycin C and mitomycin A analogues, including five new compounds, was screened against three different solid human tumor cell lines using the MTT tetrazolium dye assay. A statistically significant correlation among antitumor activity, quinone reduction potential ($E_{1/2}$), and the logarithm of the partition coefficient (log P) was obtained, with the most easily reduced and the most lipophilic compounds being the most potent. When these analogues were separated into mitomycin C and mitomycin A subsets, the former gave a correlation only with $E_{1/2}$, whereas the latter (which differ little in their $E_{1/2}$ values) gave a correlation only with log P. These correlations are in contrast to those made in the P388 leukemia assay in mice wherein the most active mitomycin C and mitomycin A analogues were the most hydrophilic ones. When the same compounds were tested against P388 leukemia cells in the MTT assay, the results were the same as those of the solid tumor assays. Thus, the substantial differences in relative potencies of mitomycins are related not to the kind of tumor cell, but to the type of assay performed, cell culture versus whole animal. No correlation was found between antitumor potency in the cell culture systems and calculated relative DNA binding strengths, probably because the limiting factors in antitumor potency of mitomycins appear to be tumor cell uptake (log P) and/or bioreductive activation ($E_{1/2}$).

The effectiveness of mitomycin C (1) as a clinical antitumor agent has stimulated the preparation and evaluation of hundreds of analogues.[1] This research has resulted in a substantial data base of antitumor activity, especially against P388 leukemia in mice. A number of attempts have been made to correlate this activity with structural features and physical properties of the mitomycins.[2-7] Among the structural features, the aziridine ring and the carbamate substituent are especially important for good activity. Mitosanes are more potent than the corresponding mitosenes, probably because the indoloquinone chromophores of the latter are more difficult to reduce than the benzoquinone chromophores of the former.[2] Reduction is essential to bioactivation of both mitosanes and mitosenes as bifunctional alkylating agents.[1,8,9] Attempts to correlate the antitumor activity of mitosanes with physical properties such as partition coefficient, quinone reduction potential, and substituent size have been generally disappointing. Early studies on mitosanes, wherein substituents varied at a number of positions on the molecule, suggested an inverse correlation between antitumor activity and quinone reduction potential[3] or the presumably related electron-withdrawing power of quinone-ring substituents.[4] Subsequent studies on mitomycin C analogues, with substituents varied only on the quinone-ring amino group (N^7) revealed no statistically significant correlations for sets of compounds consisting of substituted methylamines,[2] substituted ethylamines,[10] or secondary amines.[11] It was possible to obtain a correlation ($R^2 = 0.85$) between partition coefficient and antitumor potency [minimum effective dose (MED) against P388 leukemia in mice] within a set of N^7-phenyl analogues.[5] Correlations of antitumor potency with quinone reduction potential ($E_{1/2}$), which gave a linear plot against σ values for substituents on the phenyl group, or substituent size were statistically insignificant. More recently, two different sets of mitomycin A analogues did not give statistically significant correlations between partition coefficients and MED values against P388 murine leukemia.[6,7]

Despite the generally unsatisfactory previous results on mitomycin QSAR, we thought that it might be possible to obtain better correlations between antitumor activity and physical properties if a more reproducible test system and more accurate estimations of physical properties, including net binding enthalpies, were used. In particular, the P388 leukemia assay in mice caused problems because of variations in survival times from run to run. Consequently, we decided to use in vitro assays, which could give precise and reproducible IC_{50} values for QSAR. Three different human tumor cell lines, WiDr colon,[12] 2780 ovarian,[13] and MCF-7 breast cancer,[14] were chosen for this purpose. The physical properties investigated were log P, $E_{1/2}$, and the net binding enthalpies of drugs to the DNA segment, GC10, as determined by molecular mechanics calculations. These properties follow from the sequence of events that occur in mitomycin antitumor activity. Thus, log P may be related to the partitioning of mitomycins into tumor cell membranes, $E_{1/2}$ is related to their bioreductive activation, and net binding enthalpy is related to their ability to alkylate DNA in the nucleus once they reach it. Effects of substituent size should be accounted for in the net binding calculations, although they were found previously to be insignificant in the set of N^7-phenyl derivatives.[5] Although each of these three effects, cell penetration, bioactivation, and DNA binding, is essential for antitumor activity, they might not all turn out to be statistically significant in the QSAR. For example, if cell penetration

† Department of Pharmaceutical Sciences.
‡ Cancer Center.

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Table I. Preparation and Properties of New Mitomycin C Analogues[a]

no.	yield, %	solvents for silica gel chromatography	mp, °C	^{1}H NMR for the 7-substituent[b]
10[e]	80	$CHCl_3$/MeOH, 9:1, cryst from MeOH/EtOAc	128–131	7.04 (d, 2), 7.50 (d, 2), 8.06 (br s, 1)
11[f]	87	$CHCl_3$/MeOH, 9:1, extracted with EtOAc	121–125	7.10 (d, 2), 7.45 (s, 2), 8.05 (br s, 1)
15	70	$CHCl_3$/MeOH, 9:1, then acetone/$CHCl_3$, 7:3, extracted with acetone, EtOAc	>360 dec	6.80–7.15 (m, 3), 7.50 (s, 1), 8.00 (br s, 1)
16[d]	44	$CHCl_3$/MeOH, 19:1, extracted with MeOH/CH_2Cl_2	>300 dec	7.1 (d, 1, J = 9 Hz), 7.63 (dd, 1, J = 9, 2.5 Hz), 7.65 (d, 1, J = 2.5 Hz), 7.7 (br s, 1), 9.1 (s, 1)[c]
17[h]	89	$CHCl_3$/MeOH, 8:2 extracted with MeOH/$CHCl_3$	153–155 dec	6.5 (s, 1), 6.9 (s, 1), 7.2–7.5 (m, 3), 7.9 (s, 1, exchanges with D_2O), 8.4 (br s, 1, exchanges with D_2O)[d]

[a] Analytical results were within ±0.40% of theoretical value for C, H, N, and Br (for 10), unless stated otherwise. [b] The solvent was acetone-d_6 unless specified otherwise and TMS was the internal standard. [c] The solvent was $CDCl_3$. [d] The solvent was CD_3OD. [e] N: calcd 11.44, found 10.95. [f] C: calcd 48.27, found 47.72. [g] N: calcd 13.90, found 13.29. [h] N: calcd 14.30, found 13.75.

is difficult, but bioactivation and DNA binding are easy once the mitomycin enters the cell, it is possible that log P will be the only significant factor. In addition to a new test system, better log P estimations were possible than we had in previous QSAR studies because of the availability of Leo's CLOGP computer program.[15]

A set of 30 different mitomycin C and mitomycin A analogues was chosen for this study. Those already on hand were checked for purity by TLC and repurified by preparative TLC if necessary. Known compounds not available were prepared as described previously.[2,5-7] Compounds **10**, **11**, **15**, **16**, and **17** were new. Among them **16** and **17** were "rationally" designed to have enhanced DNA binding. As indicated in Table II, **16** and **17** have significant calculated net binding enthalpies. Compounds **11** and **15** were based on N^7-(4-iodophenyl) analogue **12**, after it showed unexpectedly high activity in the human tumor cell assays. Initially, we wished to prepare the diiodophenol derivative by reduction of 2,6-diiodo-4-nitrophenol. However, this compound lost one of the iodine atoms in the process.

Chemistry

The five new analogues **10**, **11**, **15**, **16**, and **17** were prepared by treating mitomycin A (**20**) in methanol with the appropriate arylamines. Triethylamine in water was used to promote the slow reaction of **16**. The yields and physical properties of the new analogues are given in Table I. 4-Amino-2-iodophenol was prepared by sodium dithionite reduction of 2,6-diiodo-4-nitrophenol. It has been reported,[16] but not characterized. Appropriate data are given in the Experimental Section.

Antitumor Activity

Antitumor potencies (IC_{50}) of the mitomycin analogues against the three human tumor cell lines were determined by the MTT assay, as described in the Experimental Section. The results are listed in Table II, which also gives the values for log P, $E_{1/2}$, and net binding energy to be used in QSAR. It can be seen that potencies of a particular drug against all three tumors are roughly comparable, suggesting that mitomycins are not cell-line selective in their toxicity. There are some exceptions to this generalization, but the variations in activity for a compound against the three tumors are usually much less than the variations among all compounds for a particular tumor. Among the more apparent relationships in Table II are the superior in vitro antitumor potencies for lipophilic mitomycin A analogues and certain N^7-aryl derivatives of mitomycin C. Only 14 of the compounds in Table II were characterized by calculated net binding energies because of the extensive time and computer costs involved. There is no obvious relationship between antitumor potency and net binding energy for these compounds. As described below, this effect is not statistically significant.

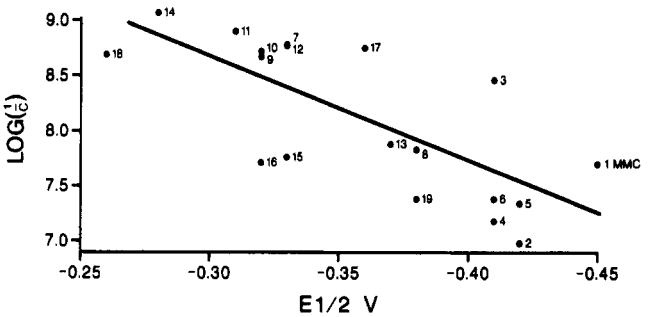

Figure 1. Mitomycin C analogues: log $(1/C)$ vs $E_{1/2}$. Data from Table I.

QSAR Studies

All 30 compounds in Table II were subjected to multiple linear regression analysis with log $(1/C)$ as the dependent variable and log P and $E_{1/2}$ as the independent variables. The IC_{50} values of the three human tumor cell lines were averaged to obtain C. A statistically significant correlation was obtained, affording the regression equation log $(1/C) = 10.1 + 6.59E_{1/2} + 0.35 \log P$: $N = 30$, $R = 0.83$, $R^2 = 0.69$, $S = 0.46$, $F = 30.3$, sig $F = 0.000$. The parameter $E_{1/2}$ accounted for 48% of the variance and log P accounted for the remaining 21%.

Regression analysis was next performed independently on data for the mitomycin C analogues (**1–19**) and the mitomycin A analogues (**20–30**). For the mitomycin C analogues, only $E_{1/2}$ was a significant independent variable. The equation was log $(1/C) = 11.50 + 9.45E_{1/2}$: $N = 19$, $R = 0.73$, $R^2 = 0.53$, $S = 0.48$, $F = 19.4$, sig $F = 0.0004$. A plot of these data is given in Figure 1, which shows some spread in the points about the least-squares line, but no unusual outliers. The two compounds with activity most higher than predicted by the equation were propargyl analogue **3** and 5-indolyl analogue **17**, whereas the four compounds with activity most below predicted values were the hydroxypropyl (**2**) and 3-pyrazolyl (**19**) analogues and the two disubstituted aniline analogues **15** and **16**. Possibly the last two compounds experience steric hindrance to DNA binding.

As mentioned above, a previous study on the correlation of antitumor potencies in the P388 leukemia assay in mice with physical properties of monosubstituted N^7-arylmitomycin C derivatives revealed that the π value for aryl ring substituents was the only property to give a statistically significant correlation, with the least lipophilic compounds being the most potent. This same correlation

(15) CLOGP in PCGEMS was provided by R. Kinnerson at the U.S. Environmental Protection Agency.

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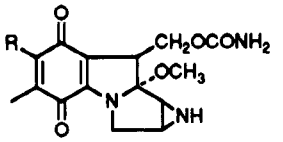
Table II. Activity of Mitomycin Analogues against Human Tumor Cells in Culture[a] (Parameters for QSAR)



no.	R	IC_{50} (nM) against colon WIDR	ovarian 2780	breast MCF-7	av	log (1/C)	log P	$E_{1/2}$, V	π	σ	net binding, kcal/mol
				Mit C Analogues							
1	H_2N	13.0	14.8	32.2	20.0	7.70	−0.38	−0.45			−115.3
2	$HO(CH_2)_3NH$	102	76.4	132.5	104	6.98	0.10	−0.42			−123.7
3	$HC{\equiv}CCH_2NH$	3.90	2.10	4.40	3.47	8.46	0.24	−0.41			−116.1
4	tetrahydrofurfuryl-NH	59.7	133.8	35.8	76.4	7.13	0.21	−0.41			−112.5
5	2-furyl-$(CH_2)_2NH$	40.3	59.5	36.6	45.5	7.34	1.90	−0.42			−109.2
6	2-pyridyl-$(CH_2)_2NH$	26.2	77.4	22.8	42.1	7.38	1.23	−0.41			−109.2
7	C_6H_5NH	1.09	2.60	1.32	1.67	8.78	1.30	−0.33	0.00	0.00	
8	$4\text{-}H_2NC_6H_4NH$	28.8	50.0	13.1	30.6	7.83	0.07	−0.38	−1.23	−0.66	
9	$4\text{-}FC_6H_4NH$	1.63	3.03	1.77	2.14	8.67	1.44	−0.32	0.14	0.06	
10	$4\text{-}BrC_6H_4NH$	1.19	1.84	2.66	1.90	8.72	2.16	−0.32	0.86	0.23	
11	$3\text{-}IC_6H_4NH$	0.21	1.68	1.87	1.25	8.90	2.42	0.31	1.12	0.35	
12	$4\text{-}IC_6H_4NH$	0.63	1.60	2.84	1.69	8.77	2.42	−0.33	1.12	0.18	−120.7
13	$4\text{-}HOC_6H_4NH$	6.30	16.4	16.9	13.2	7.88	0.63	−0.37	−0.67	−0.37	−127.6
14	$4\text{-}O_2NC_6H_4NH$	0.42	0.59	1.53	0.85	9.07	1.02	−0.28	−0.12	0.78	
15	$3\text{-}I\text{-}4\text{-}HOC_6H_3NH$	6.52	29.0	16.3	17.3	7.76	1.75	−0.33			
16	$4\text{-}HO\text{-}3\text{-}O_2NC_6H_3NH$	25.5	10.8	22.7	19.7	7.71	0.51	−0.32			−137.7
17	5-indolyl-NH	1.07	3.10	1.10	1.76	8.75	2.45	−0.36			−127.3
18	4-methylthiazolyl-NH	1.32	1.74	3.13	2.06	8.69	1.52	−0.26			
19	3-pyrazolyl-NH	13.0	38.5	72.4	41.3	7.38	0.56	−0.38			
				Mit A Analogues							
20	CH_3O	0.052	0.22	0.63	0.30	9.52	0.26	−0.21			−106.8
21	$c\text{-}C_3H_5O$	0.21	0.56	1.12	0.63	9.20	0.83	−0.21			
22	$c\text{-}C_3H_5CH_2O$	0.16	0.45	0.51	0.37	9.43	1.35	−0.21			
23	$c\text{-}C_4H_7CH_2O$	0.19	0.27	0.21	0.22	9.66	2.47	−0.21			
24	$C_6H_5CH_2O$	0.13	0.26	1.43	0.61	9.21	1.94	−0.20			
25	$HO(CH_2)_2O$	4.22	6.85	3.69	4.92	8.31	−1.10	−0.21			−110.0
26	$C_6H_5O(CH_2)_2O$	0.12	0.16	0.72	0.33	9.48	1.74	−0.21			
27	$HO(CH_2)_2O(CH_2)_2O$	25.8	36.6	80.6	47.7	7.32	−1.08	−0.21			−110.6
28	$CH_3O(CH_2)_2O(CH_2)_2O$	4.57	4.57	8.22	5.79	8.24	−0.46	−0.21			−110.1
29	$C_6H_5S(CH_2)_2O$	0.15	0.53	1.40	0.69	9.16	2.38	−0.21			
30	$HO(CH_2)_2SS(CH_2)_2O$	1.48	1.36	3.92	2.25	8.65	0.36	−0.21			

[a] Activities determined by the MTT assay recording to the procedure of Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55.

was attempted with the monosubstituted N^7-arylmitomycin C derivatives in Table II (compounds 7–14), using π and σ values of substituents as the independent variables. The result gave a statistically significant correlation between the σ values, with the most electron-withdrawing substituents conferring the greatest potency (lowest IC_{50}). The regression equation was log $(1/C) = 8.46 + 0.92\sigma$; $N = 8$, $R = 0.89$, $R^2 = 0.80$, $S = 0.22$, $F = 23.4$, sig $F = 0.0029$.[17] There was no statistically significant correlation with π. The difference between the correlations obtained from the P388 leukemia assay in mice and the assays with cultured human tumor cells is surprising. It suggests that the two test systems are substantially unlike in terms of sensitivity to different mitomycin analogues. Of course, the mouse system is much more complex than the cell cultures, but even so log P still accounted for 85% of the variance.

Statistical analysis was performed on the mitomycin A analogues **20–30** with log $(1/C)$ values from the human tumor cell assays (Table II) and log P as the independent variable. These compounds have little variation in $E_{1/2}$ because they all are 7-alkoxymitosanes. A statistically significant correlation was obtained: $N = 11$, $R = 0.80$, $R^2 = 0.64$; $S = 0.46$, $F = 15.9$, sig $F = 0.0032$. The equation was log $(1/C) = 8.58 + 0.44$ log P,[17] indicating that the most lipophilic compounds have the greatest potencies. These data are plotted in Figure 2. Most of the points lie close to the least-squares line, but mitomycin A (**20**) is substantially more potent than predicted and (hydroxyethoxy)ethoxy analogue **27** is substantially less potent than predicted. The reasons for these deviations are not obvious. In two earlier publications, we noted that mitomycin A analogues did not give statistically significant correlations with log P in the P388 leukemia mouse assay.[6,7]

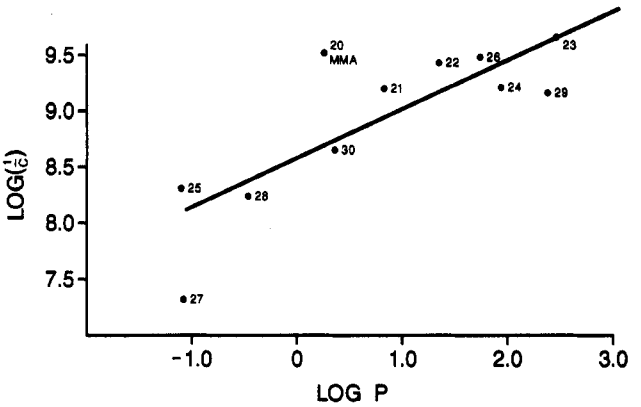


Figure 2. Mitomycin A analogues: log $(1/C)$ vs log P. Data from Table I.

(17) In this calculation, the number of compounds is small for multiparameter analysis; however, because only one variable is significant, the equation reduces to that of a straight line. The number of points is adequate for this equation.

Table III. Activity of Mitomycin A Analogues against P388 Leukemia in Mice[a]

no.	MED (mol/kg)	log (1/C)	log P[b]
20	0.14	6.85	0.26
21	0.13	6.89	0.83
22	1.03	5.97	1.35
23	0.99	6.00	2.47
24	0.24	6.67	1.94
25	0.017	7.78	−1.10
26	3.5	5.45	1.74
27	0.30	7.53	−1.08
28	0.11	6.94	−0.46
29	6.73	5.17	2.38
30	0.026	7.59	0.36

[a] Values taken from Sami, S. M.; Iyengar, B. S.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1987**, *30*, 168. Sami, S. M.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1989**, *32*, 703. [b] Values calculated using the PCLOGP program.

Nevertheless, we examined the earlier antitumor potencies in a correlation with a better estimate of log P values of compounds **20–30** as listed in Table III. This time, a statistically significant correlation was obtained: $N = 11$, $R = 0.84$, $R^2 = 0.71$, $S = 0.50$, $F = 22.0$, sig $F = 0.0011$. The equation was log $(1/C) = 7.07 - 0.56 \log P$,[17] indicating that the least lipophilic compounds have the highest potency. This result is the opposite of that obtained in the human tumor cells.

At this point in the study, it seemed important to establish whether the surprising differences in mitomycin QSAR between human solid tumor cells in culture and P388 leukemia in mice are caused by the nature of the tumors (solid versus hematological) or the test systems (cell culture versus animal). Consequently, we tested the mitomycin A analogues **20–30** and most of the mitomycin C analogues (**1–14**, **18**, and **19**) against P388 leukemia cells in the MTT assay. The results were closely parallel to those obtained with the human solid tumor cells, as shown in Table IV. Thus, for all analogues the equation log $(1/C) = 10.84 + 8.63E_{1/2} + 0.32 \log P$ was obtained: $N = 27$, $R = 0.87$, $R^2 = 0.76$, $S = 0.46$, $F = 36.2$, sig $F = 0.0000$. Separation into the subsets gave log $(1/C) = 13.3 + 14.4E_{1/2}$ for the mitomycin C analogues ($N = 16$, $R = 0.91$, $R^2 = 0.83$, $S = 0.36$, $F = 63.7$, sig $F = 0.0000$) and log $(1/C) = 8.88 + 0.32 \log P$ ($N = 11$, $R = 0.62$, $R^2 = 0.38$, $S = 0.55$, $F = 5.52$, sig $F = 0.04$) for the mitomycin A analogues. Application of the σ, π analysis to the N^7-phenyl-substituted mitomycin C derivatives gave log $(1/C) = 8.50 + 1.07\sigma$: $N = 8$, $R = 0.96$, $R^2 = 0.91$, $S = 0.15$, $F = 64.4$, sig $F = 0.002$.[17]

Those compounds for which net binding energies to GC10 had been calculated (**1–6**, **12**, **13**, **16**, **17**, **20**, **25**, **27**, and **28** in Table II) were subjected to multiple linear regression analysis with log P, $E_{1/2}$, and net binding energy as the independent variables. The results showed that there was no statistically significant correlation (95% confidence limit) with any variable.

Summary and Conclusions

In contrast to earlier studies, statistically significant correlations were made between antitumor potencies and physicochemical properties for a variety of mitomycin C and mitomycin A analogues. The property that dominated the correlation depended on the type of mitomycin analogue (C or A) and the tumor system. Thus, the potencies of mitomycin A analogues, which are readily susceptible to bioreductive activation, were determined by log P. This suggests that uptake by the tumor cells probably was the controlling event and that there is a striking difference between partitioning of the drug in cultured cells and in mice. The determining factor for potencies of the less readily reduced mitomycin C analogues also depended on the assay system. Against tumor cells in culture, the quinone reduction potential was determining,[18] whereas log P was determining against leukemia cells in mice. The effects of the test system on QSAR should be studied further using other antitumor drugs and other test systems, such as human solid tumor xenografts in mice.

Table IV. Activity of Mitomycin Analogues against P388 Leukemia Cells in Culture[a]

no.	IC_{50} (nm)	log (1/C)
	Mit C Analogues	
1	50.8	7.29
2	459	6.34
3	9.7	8.01
4	34.6	7.46
5	26.6	7.58
6	53.5	7.27
7	1.71	8.77
8	18.8	7.73
9	3.03	8.52
10	1.92	8.72
11	1.42	8.85
12	1.62	8.79
13	5.86	8.23
14	0.44	9.36
18	0.50	9.30
19	15.5	7.81
	Mit A Analogues	
20	0.24	9.61
21	0.63	9.20
22	0.31	9.51
23	0.41	9.39
24	0.018	9.75
25	0.71	9.15
26	0.082	10.08
27	18.7	7.73
28	4.1	8.39
29	1.21	8.92
30	1.87	8.73

[a] Activities determined by the MTT assay according to the procedure of Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55.

A possible explanation for these results may lie in the inherent differences of the test systems. In the P388 murine leukemia model, the tumor cells are suspended in ascities, which is an aqueous environment confined to the abdominal cavity. Considering that the drugs are administered intraperitoneally, the more hydrophilic mitomycins would have a tendency to remain in this compartment longer, whereas the concentrations of the lipophilic derivatives may fall rapidly as these analogues penetrate, redistribute, and are subsequently metabolized by the surrounding solid tissues. This supposition implies that bioavailability of the mitomycins may supersede both the $E_{1/2}$ and log P in determining the activity of a particular compound. In the MTT assay system where drug exposure parameters do not vary, the ease of reduction appears to emerge as the dominant factor. However, when both the bioavailability and $E_{1/2}$ are held essentially constant, as for the MMA analogues in culture, the relative lipophilicity or ease of cell penetration then determines activity. As previously mentioned, the calculated DNA binding energies of the mitomycins are favorable enough to suggest that once a compound enters a cell, its ability to alkylate DNA is not a limiting factor to its cytotoxicity.

Our observation that the QSAR of the mitomycins appears to vary with the tumor system is consistent with the

(18) A relationship between the reduction potentials of mitomycins and their cytotoxicity to HCT 116 human colon carcinoma cells was recently reported: Pan, S.-S.; Gonzalez, H. *Molec. Pharmacol.* **1990**, *37*, 966.

extensive evidence recently reviewed by Phillips and coworkers that additional factors, besides the inherent chemosensitivity of tumor cells, may significantly influence the outcome of in vivo chemotherapy.[19] Thus, in order to extract the maximum therapeutic advantage of a drug or class of drugs, it may be important to consider their pertinent physicochemical properties as they relate to both the in vivo pharmacokinetics and the biology of the tumor system. For example, selection of a very hydrophilic mitomycin for the intracavitary therapy of aqueous tumor systems such as malignant abdominal or pleural effusions may maximize drug bioavailability thereby enhancing the overall clinical response. A recent study involving intraperitoneal mitomycin C (10 mg/m^2 every 4 weeks) for persistent or recurrent peritoneal carcinomatosis secondary to ovarian cancer revealed an objective tumor response rate of 86%. The toxicities included mild myelosuppression (suggesting that a fraction of the dose was absorbed) and abdominal discomfort that was dose limiting after approximately four courses.[20] The choice of a more hydrophilic derivative may allow dose reductions that may ameliorate these side effects to some degree. On the other hand, selection of a lipophilic derivative with a low reduction potential for the continuous regional infusion of localized neoplasia may enhance tissue extraction of an active analogue while potentially minimizing the systemic toxicities. It is therefore unlikely that any single mitomycin derivative would be sufficient to treat all varieties of neoplasia.

It is becoming apparent that the appropriate choice of test systems in a stepwise protocol for selecting antitumor drugs is extremely important. Furthermore, the test systems should closely resemble the disease to be treated. For example, if a protocol for selecting mitomycin A analogues were based on optimizing activity against cultured human tumor cells and/or P388 leukemia cells, and then confirming activities of the "best" analogues in the P388 murine leukemia system, it would be found that they were poor choices and, possibly, the most active compounds would have been rejected prematurely. Of course, P388 murine leukemia does not need to be the secondary screen, but the concept of compatibility between in vitro and in vivo screens still is critical. It appears that if laboratories have drug development protocols based on cell culture assay followed by "confirmation" in mouse systems, and they will need to carefully validate and compare these assays.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX90Q (90 MHz) spectrometer using tetramethylsilane as the standard. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Analytical results were within ±0.4% of theoretical values unless specified otherwise.

Preparation of New Mitomycin C Analogues (General Method). A solution of mitomycin A (23–52 mg, 0.07–0.15 mmol) in anhydrous methanol (3–10 mL) was stirred with about 1.25–2.25 equiv of amine at room temperature.[2] For the preparation of **10** this ratio was 5.7. Progress of the reaction was followed by thin-layer chromatography on silica gel with $CHCl_3$–MeOH (9:1 v/v) as solvent. The preparation of **16** was conducted in triethylamine–water (1:2 v/v) because it was too slow in methanol. When the conversion was complete, the mixture was filtered, the filtrate was concentrated under reduced pressure, and the crude product was purified by preparative thin-layer chromatography using precoated silica gel plates (20 × 20 cm and 2-mm thickness) and the solvent system specified in Table I. The appropriate bands were scraped from the plates and extracted with solvents given in Table I. This table also gives the yields and properties of the products.

Preparation of 4-Amino-2-iodophenol.[16] To a yellow suspension of 2,6-diiodo-4-nitrophenol (1.26 g) in 25 mL of 25% NaOH was added sodium dithionite (18 g), and the mixture was heated with stirring at 75 °C until it turned white. It was filtered and the solids were washed with deionized water and dried in air to give 0.2 g (30%) of 4-amino-2-iodophenol as white solid with mp 157–158 °C: ^{1}H NMR (DMSO-d_6 + D_2O + TMS) δ 7.02 (d, 1, J = 2.5 Hz), 6.65 (d, 1, J = 12.9 Hz), 6.55 (dd, 1, J = 2.5, 12.9 Hz); ^{13}C NMR (DMSO-d_6, decoupled) δ 148 (C1), 142 (C4), 124 (C3), 116.2 (C5), 115.5 (C6). Anal. (C_6H_6INO) C, H; N: calcd 5.96, found 4.83.

Antitumor Assays. The MTT tetrazolium dye reduction assay[21] was used to determine antitumor activities of the mitomycins in three adherent human tumor cell lines: WiDr colon (R. Wallace, American Cyanamid Co., Pearl River, NY), 2780 ovarian (R. Ozols, Fox Chase Cancer Center, Philadelpha, PA), and MCF-7 breast (American Type Culture Collection, Rockville, MD). These compounds were further screened against P388 murine leukemia cells (American Type Culture Collection, CCL-46, Rockville, MD).

The human tumor cells were grown in RPMI-1640 culture medium (Grand Island Biologicals, NY) supplemented with 5% (v/v) fetal bovine serum, L-glutamine 292 μg/mL) and 1% (v/v) each of penicillin G (100 μg/mL) and streptomycin (100 μg/mL). For the antitumor assays, single cell suspensions of WiDr colon,[12] 2780 ovarian,[13] MCF-7 breast,[14] and P388 leukemia cells were plated at concentrations of 500, 750, 2000, and 300 cells per well, respectively, onto 96-well microtiter plates (Costar, Cambridge, MA). On day two (day one for P388), drugs dissolved initially in DMSO (J. T. Baker, analytical grade) and then diluted serially with phosphate buffered saline (pH 7.4) were added at concentrations of 10^1–10^{-5} μg/mL in half-log gradations. Final concentrations of DMSO did not exceed 0.1%. The plates were incubated at 37 °C with 5% CO_2, 95% air, and 100% relative humidity for 6 days.

After the 6-days exposure period, 50 μL of a 2 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Chemicals, St. Louis, MO] dye solution was added to each of the wells and the plates were incubated an additional 4 h. This dye forms a colored formazan product under the action of mitochondrial reductases in viable cells. The medium was then aspirated and the formazan product was solubilized by DMSO (100 μL/well). The intensity of the color, which is proportional to viable cell numbers, was quantitated by absorbance at 570 nm on an automated microculture plate reader (Biomek 1000, Beckman Instruments, Fullerton, CA). Test results were calibrated in percent control absorbance from untreated tumor cells. Each drug concentration was tested in six wells and each mitomycin assay was conducted at least twice. The IC_{50} values were then averaged. These results are given in Table II.

Solutions of mitomycins in DMSO could be stored at −5 °C without any dimunition in potency; however, certain compounds lost potency when their aqueous solutions were frozen and thawed.

Statistical Analysis. Multiple linear regression analysis was performed using SPSS-X release 3.1 for VAX/VMS.[22] The NEW REGRESSION program was used with stepwise entry of variables and an entry criterion of PIN = 0.050 (95% confidence limit). The octanol–water partition coefficients were calculated with the aid of Leo's CLOGP program[12] on a microcomputer, using the experimentally determined log P values for mitomycins A and C as starting points. For example, in the calculation of log P for compound **2**, the value for 1-propanol, minus the contribution made by one hydrogen on C-3, was determined by CLOGP to be 0.294 − 0.227 = 0.067. The experimentally determined value for mitomycin C was corrected for removal of one hydrogen from the 7-NH_2 group to give −0.380 − (−0.030) = −0.350. Combination

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(20) Monk, B. J.; Surwit, E. A.; Alberts, D. S.; Graham, V. *Semin. Oncol.* **1988**, *15*, 27.
(21) Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55.
(22) SPSS Inc., Suite 3300, 444 N. Michigan Ave., Chicago, IL 60611.

of the corrected 1-propanol and mitomycin C fragments then gave a net log P of -0.283 for **2**. The contribution of one hydrogen to the 7-NH_2 group was determined by a calculation for aniline using CLOGP. For the substituted N^7-phenylmitomycin C analogues, the experimentally determined log P for N^7-phenylmitomycin C (**7**) was used and π values for the substituents were taken from the literature. The $E_{1/2}$ values used were previously published, except for those of **10**, **11**, **15**, **16**, and **17**, which were unknown. For the first four of these compounds, $E_{1/2}$ was calculated using an equation based on the linear relationship between $E_{1/2}$ and σ values for substituents on the benzene rings of N^7-arylmitomycin C analogues previously published.[5] This equation, derived by statistical analysis of the earlier data using SPSSX, was $E_{1/2} = -0.34 + 0.072\sigma$. In the calculations for σ in compounds with disubstituted N^7-phenyl groups, σ_p and σ_m values for each substituent are taken from the literature and it is assumed that they can be added together to obtain a combined σ for all substituents. Using compound **15** as an example, $E_{1/2} = -0.34 + 0.072(\sigma_{p\text{-OH}} + \sigma_{m\text{-I}}) = -0.34 + 0.072(-0.37 + 0.35) = -0.34$ V. Following this procedure, the $E_{1/2}$ value for **16** is -0.32 V. The $E_{1/2}$ for N^7-(5-indolyl) derivative **17** was estimated to be -0.36 V by comparing the nearly equal calculated electron densities (partial atomic charges) on 5-aminoindole (-0.436) and 4-aminophenol (-0.474), as determined by quantum mechanics using GAUSSIAN-80 UCSF.[23]

Molecular Modeling. New mitomycin analogues were obtained by displaying the mitosene forms of mitomycin C and mitomycin A with no substituent on C1, which has been previously subjected to energy refinement with the molecular mechanics program of AMBER,[24] and docking the new substituent groups onto them using MIDAS.[25] These substituent groups were constructed using CHEMLAB II, and their partial atomic charges (ESP) were calculated with GAUSSIAN-80 UCSF.[23] The resulting structures were then brought to minimum energy conformations using AMBER. Parameters previously outlined were used for the mitomycin part of the structure. Parameters for the new substituents were based on those already in AMBER.[26] For example, the indole substituent and the *p*-hydroxyphenyl substituent were taken from tryptophan and tyrosine, respectively. The united atom force field of AMBER 2.0 was used and all structures were refined until the root mean square gradient was less than 0.1 kcal/Å. A distance-dependent dielectric constant was used, and all nonbonded pairs were included in the calculations. These same parameters and conditions were used for the decanucleotide duplexes and their covalent complexes with the mitomycins described below.

The decanucleotide d(GCGCGCGCGC)$_2$ (referred to henceforth as GC10) was generated in the B form with Arnott's geometry[27] and brought to a minimum-energy conformation. Mitomycin A and mitomycin C were docked onto it near the 2-amino group of the fifth guanine residue in the first strand using MIDAS. The coordinates were captured, and the structures of the resulting complexes, made covalent by defining a 1.47 Å bond between C1 of the mitomycin and N2 of the guanine, were subjected to energy refinement using AMBER. Models for other covalently bound mitomycin analogues were derived from those of mitomycin A and mitomycin C by docking the new substituent onto the mitomycin A 7-CH_3O or mitomycin C 7-NH_2, removing hydrogens, and carrying out energy minimization on the resulting structures. Using the ANALYSIS mode of AMBER, the energies for interactions between the mitomycin analogues and GC10, as well as the internal energies of each, were calculated. Distortion energies in the GC10, resulting from induced fits with the mitomycins, were calculated by subtracting energies of GC10 minimized alone from those of GC10 in the covalent complex. In the same manner, distortion energies in the mitomycins were obtained by subtracting energies of the mitomycins minimized alone from their energies in the covalent complex. Net binding energies, which are used in the comparison of relative binding strengths of mitomycins to GC10, were obtained by adding the calculated intermolecular binding energies (electrostatic + van der Walls) and the two distortion energies. These data are given in Table II.

Concerning the helix distortion energies, their absolute values cannot be used to compare the distortion in a complexed polynucleotide relative to uncomplexed polynucleotide because they are chemically different molecules. It is, however, meaningful to compare the distortion energies among various complexes based on the same drug and polynucleotide and to draw inferences on their relative stabilities. The dominant components in the relative distortions are van der Waals and electrostatic interactions. Bond length and bond angle contributions make little difference. Previous publications discuss the scope of application of AMBER to drug–macromolecule complexes and the validity of parameters in its force fields.

Acknowledgment. We gratefully acknowledge the Medical Research Council of Canada for a fellowship to K.R.K. Dr. S. M. Sami (University of Arizona) generously provided some of the mitomycins used in this project. The CLOGP program was made available by Dr. S. H. Yalkowsky (University of Arizona). AMBER was a gift of Professor Peter A. Kollman (University of California, San Francisco).

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A New Class of Acyclic Phosphonate Nucleotide Analogues: Phosphonate Isosteres of Acyclovir and Ganciclovir Monophosphates as Antiviral Agents[1]

Choung Un Kim,* Peter F. Misco, Bing Yu Luh, Michael J. M. Hitchcock, Ismail Ghazzouli, and John C. Martin[2]

Bristol-Myers Squibb Company, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492.
Received February 12, 1991

Novel phosphonate isosteres of acyclovir (ACV) and ganciclovir (DHPG) monophosphates (**20** and **32**) were found to be potent and selective antiherpesvirus agents. In the series of phosphonate analogues of ACV monophosphate, only the guanine analogue **20** exhibited activity against herpesviruses, similar to the structure–activity relationship observed for base modification of ACV analogues. The phosphonate isostere of ACV monophosphate (**20**) was more effective than ACV in the HSV-1 infected mouse model. The 3′-carba analogues of 9-[3-hydroxy-2-(phosphonomethoxy)propyl]purines/pyrimidines (adenine, HPMPA; guanine, HPMPG; cytosine, HPMPC) are devoid of antiherpesvirus activity. This result confirms that the β-oxygen atom of the phosphonomethyl ether functionality in HPMP-purines/pyrimidines plays a critical role for activity against herpesviruses.

Some acyclic nucleoside analogues have achieved considerable success as antiviral agents.[3] Acyclovir (ACV, **1**)[4] and ganciclovir (DHPG, **2**)[5] exhibit potent and selective activity against herpesviruses including herpes simplex

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