Mitomycin C Analogues with Aryl Substituents on the 7-Amino Group

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A series of 30 different N^7-phenyl-substituted mitomycin C analogues, including 25 new compounds, was prepared from mitomycin A. Seven of these compounds were clearly superior to mitomycin C in activity against P-388 murine leukemia. The para- and the meta-substituted derivatives were subjected to Hansch analysis, which revealed that the lipid–water distribution coefficient π was the only significant factor in determining antitumor potency (MED). The substituent electronegativity factor σ was statistically insignificant in determining potency, despite the good correlation of σ_p with the polarographic quinone-reduction potential. These results suggest that diffusion into the tumor cell or access to the receptor is more important than bioreductive activation in determining antitumor potency for this particular group of mitosanes. Fifteen new mitomycin C analogues with heterocycles on the 7-amino group also were prepared. Two of them, containing pyrazolyl and aminopyridyl substituents, were more active than mitomycin C against P-388 murine leukemia. No broad correlations could be made among the antitumor potencies and physicochemical properties for this type of analogue.

A preceding article in this series described mitomycin C analogues with substituted ethylamines at position 7.[1] These compounds were chosen in part to permit the study of substituent effects at a site removed from the quinone ring. This study revealed no statistically significant correlation between physicochemical properties, such as hydrophilicity or substituent size, and activity against P-388 leukemia in mice.

We had previously noted, in a rough correlation among a variety of 7-substituted mitosanes, that increasing the ease of quinone reduction enhanced potency against P-388 leukemia.[2] This correlation was not completely satisfactory because other factors, such as hydrophilicity, were ignored. Furthermore, other studies of mitomycin structure–activity relationships, wherein substituents varied at a number of positions on the molecule, had suggested an inverse correlation between antitumor activity and quinone reduction potential[3] or the presumably related electron-withdrawing power of substituents.[4] In view of these factors, it seemed important to conduct a study in which the number and nature of structural changes were strictly limited. This approach would permit a Hansch-type analysis to be made with more confidence than when many structure factors were varied. The N^7 para-substituted phenyl analogues of mitomycin C appeared especially valuable for this purpose. Substituent variation would be at a single position, yet it would influence both hydrophilicity and quinone reduction potential. Furthermore, suitable substituent constants are available from the investigations of Hansch. A final reason was that a previous, limited study on anilino analogues of mitomycin C revealed substantial activity against P-388 leukemia for certain compounds of this type, especially the *p*-hydroxyphenyl analogue.[5]

Preparation of N^7-Phenyl Analogues. A series of 14 para-substituted analogues was prepared by treating a solution of mitomycin A in methanol with an excess of the aniline. The reaction progress was followed by thin-layer chromatography, and it was observed that the more nucleophilic anilines, such as *p*-phenylenediamine and *p*-aminophenol, reacted at a moderate rate, whereas the less nucleophilic anilines, such as *p*-chloroaniline and *p*-aminobenzonitrile, reacted so slowly that solid potassium carbonate had to be added to promote the conversion. Even with this promoter, *p*-nitroaniline reacted very slowly and the yield was poor. The bifunctionality of *p*-phenylenediamine presented a special case in that formation of the dimeric product **30** was possible. We found that when excess *p*-phenylenediamine was used, the product was the expected monomer **21**. However, with 0.5 equiv of *p*-phenylenediamine, the dimer **30** could be isolated in good yield. The N^7-phenyl-substituted mitomycin C analogues are listed, along with their physical properties, in Table I. As in our previous preparations of mitomycin analogues, it was necessary to carefully purify the products by preparative-layer chromatography; otherwise, traces of the highly potent and toxic mitomycin A could influence the testing results.[1] Certain of the products adhered tenaciously to solvent, even under high vacuum, and they could not be heated because of instability. Thus, some of the elemental analyses must be corrected for the presence of solvent. Evidence for the solvates is given in Table I.

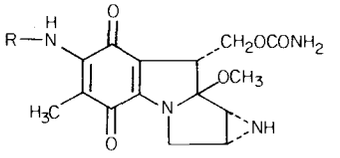
A series of seven meta-substituted analogues also was prepared, along with five analogues that were disubstituted in the meta and para positions. Only two compounds with ortho substituents were prepared because substitution at this position appeared unfavorable in the previous study.[5] The preparation of these compounds closely resembled that of the corresponding para analogues. It was necessary to promote the reaction of anilines having electron-withdrawing substituents, and certain of the carefully chromatographed products retained solvents. These analogues also are listed in Table I.

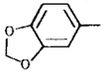
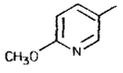
Antitumor Activity. The activities of the analogues against P-388 lymphocytic leukemia in mice are given in Table II. The assays were not all run concurrently, but a mitomycin C standard was run in each assay. Therefore, compounds should not be compared directly with each other but compared on the basis of how each one relates to mitomycin C. This procedure is necessary because of the substantial variation in the maximum therapeutic effect to mitomycin C from one experiment to another. From Table II it is evident that seven compounds, **10–12**, **15**, **16**, **21**, and **25**, are clearly superior to mitomycin C. One of them, the *p*-hydroxy derivative **10**, was previously found to have superior activity in this assay,[5] and it is presently undergoing clinical trials in Japan. It also appears to be nonleukopenic.[6] In contrast to the previous report,[5] we

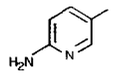
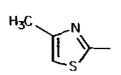
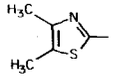
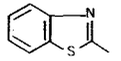
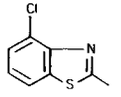
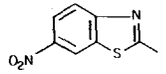
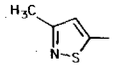
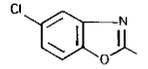
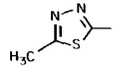
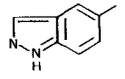
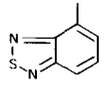
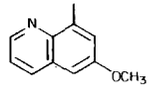
(1) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16.
(2) Iyengar, B. S.; Lin, H. J.; Cheng, L.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1981**, *24*, 975.
(3) Matsui, M.; Yamada, Y.; Uzu, K.; Hirata, T. *J. Antibiot.* **1968**, *21*, 189.
(4) Moriguchi, I.; Komatsu, K. *Chem. Pharm. Bull.* **1977**, *25*, 2800.
(5) Imai, R.; Ashizawa, T.; Urakawa, C.; Morimoto, M.; Nakamura, N. *Gann* **1980**, *71*, 560.
(6) Kobayashi, T.; Inaba, M.; Tsukagoshi, S.; Sakurai, Y.; Imai, R.; Morimoto, M. *Gann* **1981**, *72*, 950.

0022-2623/84/1827-0701$01.50/0

Table I. Preparation and Properties of New N^7-Aryl-Substituted Mitomycin C Analogues[a]



no.	R	method	yield, %	solvents for recrystn or chromatogr	solvent impurity	mp, °C	^{1}H NMR signals for the 7-substituent; signals for the solvent impurity, δ [b]
1	C_6H_5	ref 10 and 11					
2[f]	4-CH_3-C_6H_4	A	86	$CHCl_3$-CH_3OH	1.5H_2O	113-115 dec	2.3 (s, 3), 6.5-7.3 (br s, 4), 7.55 (br s, 1)
3	3-CH_3-C_6H_4	A	78	$CHCl_3$-CH_3OH	1H_2O	89-91 dec	2.37 (s, 3), 6.65-7.5 (m, 4), 7.75 (s, 1)
4[g]	4-CN-C_6H_4	C	24	$CHCl_3$-CH_3OH		124-126 dec	6.60 (d, 2), 7.40 (d, 2), 7.0-7.30 (br s, 1)
5[h]	3-CN-C_6H_4	C	34	$CHCl_3$-CH_3OH	1.5H_2O	97-98 dec	6.87-8.20 (m, 5)
6[i]	4-F-C_6H_4	A	85	$CHCl_3$-CH_3OH		109-112 dec	6.6-6.97 (br s, 2), 7.03 (s, 1), 7.1-7.3 (br s, 2)
7	4-Cl-C_6H_4	ref 5					
8	3-Cl-C_6H_4	A	38	$CHCl_3$-CH_3OH		112-114 dec	6.65-7.45 (m, 4), 7.67 (s, 1)
9	4-I-C_6H_4	A	97	$CHCl_3$-CH_3OH	1H_2O	135-137 dec	6.70 (d, 2), 7.37-7.97 (m, 3)
10	4-OH-C_6H_4	ref 5					
11	3-OH-C_6H_4	ref 5					
12	4-CH_3O-C_6H_4	A	72	$CHCl_3$-CH_3OH	0.5H_2O	103-108 dec	3.77 (s, 3), 6.60-7.2 (br s, 4), 7.70 (s, 1)
13[j]	3-CH_3O-C_6H_4	A	69	$CHCl_3$-CH_3OH	1H_2O	112-115 dec	3.77 (s, 3), 6.37-7.50 (m, 4), 7.70 (s, 1)
14	2-CH_3O-C_6H_4	A	79	$CHCl_3$-CH_3OH		115-117 dec	3.37 (s, 3), 6.53-7.37 (m, 4), 7.53-7.77 (br s, 1)[c]
15[k]	2-CH_3-4-OH-C_6H_3	A	49	$CHCl_3$-CH_3OH	2H_2O	118-122 dec	2.06 (s, 3), 6.37-6.63 (br s, 3)
16[l]	3,4-$(CH_3O)_2$-C_6H_3	A	91	$CHCl_3$-CH_3OH		114-116 dec	3.38 (s, 6), 6.3-6.9 (m, 3), 7.7 (s, 1)
17	3,5-$(CH_3O)_2$-C_6H_3	A	88	$CHCl_3$-CH_3OH		98-100 dec	3.77 (s, 6), 5.90-6.35 (br s, 3), 7.6 (s, 1)
18[m]		A	48	CH_3OH-CH_2Cl_2	0.4CH_2Cl_2	86-88 dec	5.97 (s, 2), 6.0-6.7 (m, 3), 7.27 (s, 1); 5.33 (s)
19	4-SH-C_6H_4	A	47	CH_3OH-CH_2Cl_2		99-97 dec	6.53 (d, 2), 7.0-7.7 (m, 3)
20[n]	4-NH_2SO_2-C_6H_4	C	26	$CHCl_3$-acetone	3.5H_2O	113-115 dec	7.00 (d, 2), 7.47 (s, 2), 7.87 (d, 2)[c]
21	4-NH_2-C_6H_4	ref 5					
22	3-NH_2-C_6H_4	A	68	$CHCl_3$-CH_3OH	1.5H_2O	110-112.5 dec	3.80-3.90 (br s, 1), 6.10-7.30 (m, 3), 7.60 (s, 1)
23[o]	3-CH_3O-4-NH_2-C_6H_3		54	CH_3OH-CH_2Cl_2		83-87 dec	3.88 (s, 3), 6.25 (br s, 1), 6.5 (br s, 3), 7.26 (s, 1), 7.75 (s, 1); 5.33 (s)
24[p]	3-NH_2-4-CH_3O-C_6H_3		76	CH_3OH-CH_2Cl_2		107-110 dec	3.77 (s, 3), 6.05 (s, 1), 6.34 (s, 2), 6.50-6.67 (br s, 2), 7.62 (s, 1); 5.33 (s)
25[q]	4-CH_3CONH-C_6H_4	A	76	$CHCl_3$-CH_3OH	1H_2O	143-145 dec	2.10 (s, 3), 6.97 (d, 2), 7.4 (d, 2), 7.60 (s, 1), 8.98-9.27 (s, 1)
26[r]	3-CH_3CONH-C_6H_4	C	72	$CHCl_3$-CH_3OH	2H_2O	140-143 dec	2.10 (s, 3), 6.67-7.5 (m, 4), 8.03 (br s, 1), 9.30 (s, 1)[d]
27[s]	4-$COOHCH_2NH$-C_6H_4	A	90	EtOAc-CH_3OH	2H_2O	83-85 dec	3.10 (s, 2), 6.30-6.57 (br s, 2), 6.60-7.10 (m, 4)[e]
28[t]	4-NH_2CONH-C_6H_4	A	67	$CHCl_3$-CH_3OH	1.5H_2O	93-95 dec	5.03 (s, 3), 6.87 (d, 2), 7.33 (d, 2), 8.0 (s, 1), 8.43 (s, 1)[c]
29[u]	4-NO_2-C_6H_4	C	9	$CHCl_3$-CH_3OH		132-134 dec	6.9-7.3 (d, s), 7.4-7.9 (d, 2), 7.9-8.35 (br s, 1)
30[v]	-C_6H_4- (dimer)		41	$CHCl_3$-CH_3OH	2.5H_2O	340 dec	6.46-7.3 (br s, 4), 7.7-8.3 (br s, 2)[c]
31	3-pyridyl	ref 1					
32		A	76	CH_3OH-CH_2Cl_2		260-262 dec	3.93 (s, 3), 6.77 (s, 1), 7.26 (d, 1), 7.60 (d, 1), 7.87 (s, 1)

33		B	49	CH_3OH–CH_2Cl_2	$0.25CH_2Cl_2$	200 dec	6.03–7.89 (m, 6); 5.91 (s)
34		C	45	CH_2Cl_2–CH_3OH	$0.5H_2O$	85–87 dec	6.46 (s, 1), 7.36 (d, 1), 7.80 (d, 1)
35[w]		C	59	CH_3OH–CH_2Cl_2	$0.5CH_3OH$	116–118 dec	2.23 (s, 3), 6.30–6.60 (br s, 1), 7.30 (s, 1); 3.3–3.4 (br s)
36		B	49	CH_3OH–CH_2Cl_2		100 dec	2.50 (br s, 6); 7.33 (s, 1)
37		C	18	acetone	$1CH_3COCH_3$	82–85 dec	7.06–8.03 (m, 5); 2.03 (s)
38[x]		C	14	acetone		89–91 dec	7.06–8.03 (br s, 4)
39[y]		C	27	acetone	$1.5CH_3COCH_3$	86–89 dec	(Me_2SO-d_6) 6.87–8.3 (m, 4); 2.02 (s)
40		C	8.5			87–90 dec	2.30 (s, 3), 6.1 (s, 1), 6.40 (s, 1)
41		C	25	acetone	$1.75CH_3COCH_3$	118–120 dec	6.70–7.63 (m, 4); 2.03 (s)
42		C	25	CH_3OH–CH_2Cl_2	$0.25CH_2Cl_2$	91–94 dec	2.68 (s, 3), 7.47–7.63 (br s, 1); 5.9 (s)
43		A	44	CH_3OH–CH_2Cl_2	$0.25CH_2Cl_2$	142–145 dec	6.50 (d, 2), 6.67–6.83 (br s, 1), 8.07 (s, 1); 5.9 (s)
44		C	75	$CHCl_3$–acetone	$2.5H_2O$	340 dec	6.8–7.65 (m, 3), 8.0 (s, 1)
45[z]		C	48	$CHCl_3$–acetone	$3.5H_2O$	139–140	6.6 (m, 1), 7.6 (m, 2), 8.25 (br s, 1)
46[aa]		C	32	$CHCl_3$–acetone	$1.5H_2O$	135–145 dec	6.4 (d, 1), 6.67 (d, 1), 7.30 (dd, 1), 8.0 (dd, 1), 8.90 (dd, 1)

[a] Analytical results were within ±0.40% of theoretical value for all elements (C, H, and N), except as shown in subsequent footnotes. In some examples the solvent impurities indicated in the table had to be added to reconcile the calculated and found values for these elements. NMR signals for the solvent impurities are given in the table. It was not possible to obtain exact ratios for protons in the solvent impurities with respect to those in the compound by integrating the spectra. [b] The solvent was $CDCl_3$ unless specified otherwise. [c] Spectrum determined in $CDCl_3$ plus CD_3OD. [d] Spectrum determined in acetone-d_6. [e] Spectrum determined in Me_2SO-d_6. [f] H: calcd, 5.98; found, 5.46. N: calcd, 12.41; found, 11.98. [g] C: calcd, 58.26; found, 57.81. N: calcd, 15.44; found, 13.67. [h] N: calcd, 15.15; found, 14.46. [i] N: calcd, 11.20; found, 10.15. [j] N: calcd, 12.22; found, 11.78. [k] C: calcd, 52.67; found, 52.24. [l] N: calcd, 11.06; found, 10.03. [m] N: calcd, 10.94; found, 11.46. [n] H: calcd, 5.43; found, 4.45. [o] H: calcd, 5.39; found, 5.88. [p] C: calcd, 55.97; found, 55.48. [q] C: calcd, 56.91; found, 57.41. [r] N: calcd, 13.91; found, 13.19. [s] N: calcd, 13.48; found, 12.95. [t] N: calcd, 16.96; found, 15.74. [u] N: calcd, 14.83; found, 13.63. [v] N: calcd, 14.23; found, 13.60. [w] N: calcd, 15.04; found, 14.59. [x] H: calcd, 4.02; found, 4.59. [y] N: calcd, 14.01; found, 13.40. [z] N: calcd, 15.81; found, 15.04. [aa] N: calcd, 15.31; found, 12.94.

Table II. Antitumor Activity and Leukopenia of N^7-Aryl-Substituted Mitomycin C Analogues[a]

	P388 Leukemia					leukopenia[b]
	max effect, % T/C					% change in
compd	compd	Mit C	opt dose, mg/kg	MED, mg/kg	TR OD/MED	WBC at opt dose on day 3
1	161	200	12.8	1.6	8	
2	206	200	25.6	0.8	32	-37
3	181	319	12.8	0.4	32	
4	150	233	3.2	0.8	4	
5	172	156	12.8	0.2	64	
6	167	278	12.8	0.4	32	
7	144	250 (1)	25.6	6.4	4	
8	139	156	12.8	6.4	2	
9	128	200	25.6	25.6	1	
10	>300	144	25.6	0.2	128	-27
11	356	211	12.8	<0.2	>64	
12	316 (3)	268 (1)	12.8	0.8	16	-76
13	181	319	12.8	0.2	64	
14	200	206	25.6	0.4	128	
15	244	200	25.6	0.4	64	-34
16	>333 (3)	278	12.8	0.2	64	
17	161	278	6.4	0.4	16	
18	167	211	3.2	<0.2	>16	
19	131	250	25.6	1.6	16	
20	225	213	25.6	0.2	128	
21	>337 (3)	250 (1)	12.8	<0.2	>64	-64
22	222	200	1.6	<0.2	>8	-17
23	239	311	25.6	1.6	16	
24	228	311	6.4	0.2	32	
25	>333 (4)	200	25.6	0.2	128	-40
26	231	211	25.6	0.2	128	
27	322 (2)	294 (2)	25.6	0.8	32	
28	194	278	25.6	1.6	16	
29	172	233	3.2	<0.2	>16	
30	161	250 (1)	25.6	3.2	8	
31	211	198	3.2	0.2	16	-33
32	211	233	6.4	0.2	32	-45
33	277	206	12.8	0.4	32	
34	194	281	6.4	0.2	32	-22
35	172	224	6.4	0.4	16	0
36	188	219	25.6	0.2	128	
37	150	325	25.6	12.8	2	
38	144	319	25.6	0.4	64	
39	128	156	25.6	25.6	1	
40	169	325	25.6	0.8	32	
41	178	267	25.6	1.6	16	-33
42	222	267	25.6	0.8	32	-32
43	333 (3)	161	25.6	0.8	32	-15
44	206	250	25.6	0.2	128	
45	161	250	12.8	0.8	32	
46	178	178	6.4	0.4	16	
mit C	144-325 (0-2)		3.2	0.2	16	-42

[a] Determined at Bristol Laboratories, Syracuse, NY. A tumor inoculum of 10^6 ascites cells was implanted ip into CDF$_1$ female mice. Six mice were used at each dose of the mitosane, and ten control mice were injected with saline. A control group of six mice at each dose received mitomycin C in the same experiment: MST = median survival time; max effect (% T/C) = MST treated/MST control × 100 at the optimal dose (opt. dose); MED = minimum effective dose (% T/C ≥ 125); TR = therapeutic ratio (opt dose/MED). The number of 30-day survivors at the optimal dose is given in parentheses beside the maximum effect. Complete testing results, including for each dose the therapeutic effect, number of 5- and 30-day survivors, and average weight difference between the test and control group are available as supplementary material (see paragraph at end of paper). [b] The mitosane was given in a single dose, ip, on day 0 to BDG, male mice.

also found superior activity for the *m*-hydroxy derivative **11** and the *p*-amino derivative **21**, although the latter is strongly leukopenic. New compounds in Table II with superior activity are the *p*-methoxy analogue **12**, the *p*-hydroxy-*o*-methyl analogue **15**, the *m*,*p*-dimethoxy analogue **16**, and the *p*-acetylamino analogue **25**. Also showing a substantial prolongation of life with two 30-day survivors was the *p*-*N*-glycyl analogue **27**; however, the mitomycin C control had good activity in this assay. A number of analogues, including *p*-methyl (**2**), *m*-cyano (**5**), *o*-methoxy (**14**), *p*-sulfonamido (**20**), *m*-amino (**22**), and *m*-acetylamino (**26**) had antitumor activity comparable to that of mitomycin C.

QSAR Studies. For the analysis of quantitative structure–activity relationships, we have concentrated on the para-substituted analogues. These compounds are listed in Table III, along with certain physicochemical properties and substituent constants from tables by Hansch and co-workers. We originally thought that the electron-withdrawing power of substituents would be important because it would influence the reduction potential of the quinone system, and reduction of the quinone to the hydroquinone is known to initiate the bioactivation of mitomycins as alkylating agents.[7] Therefore, we sought to establish that a linear correlation would exist between the Hammett σ_p and the polarographic half-wave reduction potential of the compound. It had been previously es-

(7) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55.

Table III. Structure-Activity Relationships among N^7-Phenyl-Substituted Mitomycin C Analogues

compd	substit on Ph	MED,[a] mg/kg	C,[b] mol/kg	log $1/C$	$E_{1/2}$,[c] V	σ[d]	π[e]	log P[d]	E_s[f]
				Para					
21	NH_2[g]	0.1[h]	2×10^{-7}	6.7	−0.38	−0.66	−1.23	0.07	0.63
10	OH	0.1[h]	2×10^{-7}	6.7	−0.37	−0.37	−0.67	0.63	0.69
4	CN	0.4	9×10^{-7}	6.1	−0.30	0.66	−0.57	0.72	0.73
12	CH_3O[i]	0.4	9×10^{-7}	6.1	−0.37	−0.27	−0.02	1.28	0.69
1	H	1.6	3.9×10^{-6}	5.41	−0.33	0	0	1.30*	1.24
6	F	1.6	3.2×10^{-6}	5.49	−0.32	0.06	0.14	1.44	0.78
19	SH	1.6	3.6×10^{-6}	5.44		0.15	0.39	1.69	0.17
2	CH_3	0.8	1.7×10^{-6}	5.8	−0.37	−0.17	0.56	1.86	0
7	Cl	6.4	1.4×10^{-5}	4.85	−0.31	0.23	0.71	2.01	0.27
9	I	25.6	4.6×10^{-5}	4.34	−0.33	0.18	1.12	2.42	−0.16
29	NO_2	0.2	4×10^{-7}	6.3	−0.28	0.78	−0.28	1.02	
25	CH_3CONH	0.2	4×10^{-7}	6.3		0.00	−0.97	0.33	
20	NH_2SO_2	0.1[h]	2×10^{-7}	6.7		0.57	−1.82	−0.52	
28	NH_2CONH	1.6	3.3×10^{-6}	5.52	−0.39		−1.30	0.00	
27	$COOHCH_2NH$	0.8	1.5×10^{-6}	5.8	−0.39	−0.78	−2.08	−0.78	
				Meta					
22	NH_2	0.1[h]	2×10^{-7}	6.7		−0.16	−1.23	0.07	0.63
11	OH	0.1[h]	2×10^{-7}	6.7		0.12	−0.67	0.63	0.69
5	CN	0.2	4×10^{-7}	6.3	−0.35	0.56	−0.57	0.73	0.73
13	CH_3O	0.2	4×10^{-7}	6.3	−0.38	0.12	−0.02	1.28	0.69
1	H	1.6	3.9×10^{-6}	5.41	−0.33	0	0	1.30*	1.24
3	CH_3	0.4	9×10^{-7}	6.1	−0.36	−0.07	0.56	1.86	0
8	Cl	6.4	1.4×10^{-5}	4.85	−0.32	0.37	0.71	2.01	0.27
26	CH_3CONH	0.4	8×10^{-7}	6.1	−0.35	0.21	−0.97	0.33	

[a] Activity against P-388 mouse leukemia from Table II. [b] Obtained by dividing the MED by the molecular weight, including solvent, and by 1000. [c] Determined by differential pulse polarography on a Model 174A EG & G polarographic analyzer. The electrolyte was 1.0 M KCl solution, and the standard was 10^{-3} M $CdCl_2$ in 1.0 M KCl. $E_{1/2}$ values are given relative to saturated calomel electrode. The following conditions were used: potential scan, 0.1 V/in.; potential scan rate, 1 mV/s; voltage range, 1.5 V; initial potential, −0.1 V; rate of mercury drops, 60/min. [d] Values from Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lein, E. J. *J. Med. Chem.* 1973, *16*, 1207. [e] Distribution between 1-octanol and water containing phosphate buffer at pH 7.4. Determination by the method of Hansch, C.; Muir, R. M.; Fujita, T.; Malongy, P. P.; Geiger, F.; Struch, M. J. *J. Am. Chem. Soc.* 1963, *85*, 2817, is indicated by an asterisk. Other values are estimated from log P for 1 and aromatic π values given in the reference in footnote *d*. [f] Values from Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley-Interscience: New York, 1979. [g] Although this is a known compound, it was submitted for combustion analysis, which showed the calculated formula plus H_2O. [h] Estimated value. Screening showed MED < 0.2. [i] The sample sent for screening had $0.5H_2O$. The analytical sample had $1.5H_2O$.

tablished that mitomycin C was reduced polarographically to the hydroquinone in a reversible two-electron, two-proton process,[8] and we followed the previous methodology, except for the use of a 1 M KCl solution as the electrolyte. The results of these measurements are listed in Table III and displayed graphically in Figure 1. It is apparent that there is a linear correlation between $E_{1/2}$ and σ_P. The other substituent effects that we thought might be important are the lipophilicity, given by π values, and the size, for which E_s is used. Simple observation of Table III suggests that the π values are important, with the hydrophilic groups, such as amino and hydroxyl, conferring the greatest potency as measured by the minimum concentration required for a T/C of 125% in the P-388 assay (MED). We had previously reported the importance of hydrophilicity for mitomycin activity.[9] The log P values in Table III were obtained by direct determination of the value for the unsubstituted aniline analogue 1 and estimation of the others by π values for substituents on aromatic rings. It was not obvious from Table III what the relative importance of σ_P and E_s would be to the antitumor potency. This determination required regression analysis.

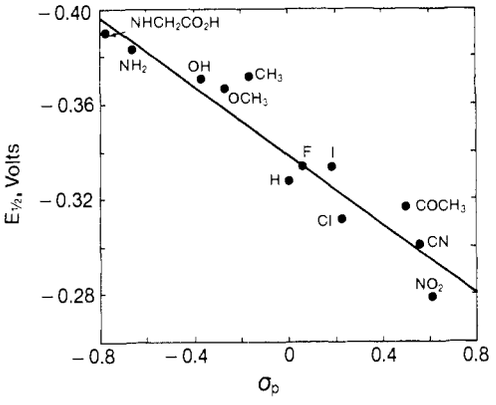


Figure 1. 7-Anilinomitosanes: reduction potential vs. electronegativity.

For the multiple linear regression analysis, we used a standard program (BMDP2R) available from the University of California, Los Angeles. Compounds from Table III were limited to the ten for which the E_s values were available. The analysis showed that π accounted for 84% of the variance in the data set, but the contributions of σ_P and E_s were statistically insignificant. The following equation could be obtained from the results:

$$\log (1/C) = -0.96\pi + 5.69$$

$$n = 10,\ r = 0.92,\ s = 0.33$$

Once it was known that π was the only known physicochemical parameter responsible for variation in log

(8) Rao, G. M.; Begleiter, A.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* 1977, *124*, 199.
(9) Remers, W. A.; Schepman, C. S. *J. Med. Chem.* 1974, *17*, 729.
(10) Kinoshita, S.; Uzu, K.; Nakano, K.; Shimizu, M.; Takahashi, T.; Wakaki, S.; Matsui, M. *Progr. Antimicrob. Agents Chemother.* 1970, 1058.
(11) Cosulich, D. B.; Patrick, J. B.; Williams, R. P. U.S. Patent 3 332 944, 1967.

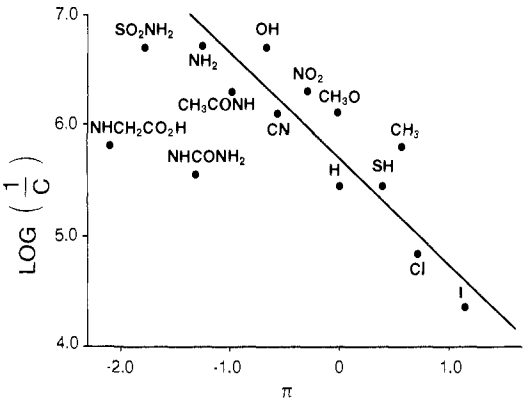


Figure 2. 7-Anilinomitosenes: log $(1/C)$ vs. π para-substituted compounds.

$(1/C)$, it was possible to include in the regression analysis the para-substituted compounds in Table II, for which E_s values were not available. Inclusion of all of the compounds gave a poorer correlation with π, affording the following equation: log $(1/C) = -0.49\pi + 5.63$; $n = 15$, $s = 0.52$, $r = 0.67$. The main contributors to the decrease in correlation are the *N*-glycinyl analogue **27** and the ureido analogue **28**, which are outliers by more than one standard deviation. When these compounds were deleted from the regression analysis, the first equation was regenerated: log $(1/C) = -0.96\pi + 5.69$; $n = 13$, $r = 0.92$, $s = 0.33$. A plot of this equation is given in Figure 2, which clearly shows that **27** and **28** are outliers. The glycinyl analogue probably is ionized at physiological pH; thus, its poorer correlation is not a surprise. However, the anomalous activity of the ureido analogue is more difficult to rationalize.

It was surprisingly to find that σ_P makes an insignificant contribution to the difference in antitumor activity of the ten compounds analyzed, considering that they have a variation in $E_{1/2}$ of 0.08 V, and quinone reduction is essential to bioactivation. The explanation must be that other factors, such as compound penetration of the cell and access to the receptor, dominate the biological variation. This explanation is not unreasonable considering that all of the anilino analogues are easy to reduce by polarography. The most difficult one of them, the *p*-amino derivative, has $E_{1/2} = -0.39$ V, which is smaller in magnitude than the $E_{1/2} = -0.45$ V of mitomycin C.[1] It should be emphasized that the equation derived above and the unimportance of σ_P might be limited to this particular series of aniline derivatives. We have preliminary evidence that for aziridinomitosenes, which have the more difficulty reducible indoloquinone chromophore, σ_P is more important than π in determining antitumor potency.

The potencies and physicochemical properties of some meta-substituted analogues are given in Table III. Hansch analysis was made on this set of compounds, even though the small number makes conclusions unreliable. The result tends to support the conclusions that emerged from the para-substituted compounds in that the only significant independent variable is π. The following regression equation was obtained: log $(1/C) = -1.08\pi + 5.62$; $n = 7$, $r = 0.96$, $s = 0.25$. The methyl-substituted analogue **3** was rejected as an outlier by the statistical program. A plot of the regression equation is given in Figure 3. Thus, it appears that the meta series parallels rather closely the corresponding para series analogues in potencies and that the partition coefficient appears to be the one significant factor in determining these potencies.

Two of the more active compounds described above, **16** and **25**, were evaluated against B-16 melanoma in mice. As shown in Table IV, dimethoxyaniline derivative 16 was

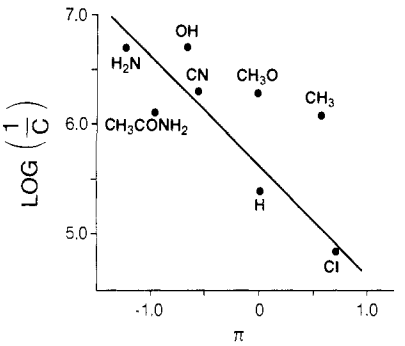


Figure 3. 7-Anilinomitosenes: $(1/C)$ vs. π meta-substituted compounds.

Table IV. Comparative Activities of Compounds 16, 25, and Mitomycin C against B-16 Melanoma in Mice[a]

compd	dose,[b] mg/(kg day)	effect: MST, % T/C	cures[c]	av wt change, g
16	8	227	3/10	0.9
	6	263	4/10	0.6
	4	252	5/10	-0.6
	2	180	2/10	-1.3
25	12	175	2/10	-1.3
	8	207	1/10	0.3
	5	185	0	0.8
	3	155	0	1.4
mit C	4	200	0	-0.1
	3	165	0	-0.1
	2	152	0	0.5
	1	155	0	0.2

[a] Determined according to standard NCI protocols at Bristol Laboratories. [b] Injections given ip on days 1, 4, and 7. The vehicle was Me_2SO + buffered saline. Ten mice were used at each dose. [c] Tumor-free survivors on day 62. No deaths were observed in any group on day 10.

superior to mitomycin C in this assay, particularly in the number of cures (tumor-free survivors on day 62). (Acetylamino)aniline derivative **25** gave more cures than mitomycin C, but it showed little improvement in MST.

Preparation of N^7-Heterocyclic Analogues. Because of the antitumor activity shown by some of the N^7-phenyl derivatives of mitomycin C, we decided to extend this study to related analogues in which N^7 was substituted with aromatic heterocycles. A variety of amino heterocycles are available commercially, and it appeared that some of them would be sufficiently nucleophilic to displace the 7-methoxy group of mitomycin A. Furthermore, only a few mitomycin analogues of this type had been reported in the literature.

Pyridyl substituents were the first group studied. We had previously reported the 3-pyridyl derivative **31** of mitomycin C and noted its comparable activity.[2] New N^7-substituted mitomycin C derivatives that could be prepared from mitomycin A included 6-methoxy-3-pyridyl (**32**) and 6-amino-3-pyridyl (**33**). 2-Aminopyridine, 4-aminopyridine, and 6-chloro-3-aminopyridine failed to react with mitomycin A, probably because of decreased nucleophilicity.

Aminothiazoles reacted slowly with mitomycin A, but potassium carbonate promoted their reactions. The series of N^7-substituted 2-thiazolyl- and 2-benzothiazolyl-mitomycin C analogues **34–39** (Table I) were obtained. A 3-methyl-5-isothiazolyl derivative, **40**, also was prepared. When the thiazole ring was substituted with electron-withdrawing groups, such as chloro and trifluromethyl, there was no reaction with mitomycin A. Aminooxaxoles and aminoisoxazoles are not readily available; however, we

Table V. Structure–Activity Relationships among N^7-Heterocycle-Substituted Mitomycin C Analogues

compd	MED,[a] mg/kg	log P[b]	$E_{1/2}$,[c] V
mit C	0.2	−0.38	−0.45
31	0.2	0.52	−0.31
32	0.2	0.59	−0.31
33	0.4	−0.32	−0.31
34	0.2	0.62	−0.27
35	0.4	1.16	−0.26
36	0.2	1.29	
40	0.8	−0.30	−0.27
43	0.8	0.08	−0.38

[a] Activity against P-388 mouse leukemia from Table II. [b] Distribution between 1-octanol and water containing phosphate buffer at pH 7.4. Determination by the method of Hansch, C.; Muir, R. M.; Fujita, T.; Malongy, P. P.; Geiger, F.; Struch, M. *J. Am. Chem. Soc.* 1963, *85*, 2817. [c] Determined by differential pulse polarography on a Model 174A EG8G polarographic analyzer. The electrolyte was 1.0 M KCl solution, and the standard was 10^{-3} M $CdCl_2$ in 1.0 M KCl. Mitomycin analogues were 10^{-3} M in 1.0 M KCl. $E_{1/2}$ values are given relative to saturated calomel electrode. The following conditions were used: potential scan, 0.1 V/in.; potential scan rate, 1 mV/s; voltage range, 1.5 V; initial potential, −0.1 or 0.2 V; modulation amplitude, 25; rate of mercury drops, 60/min.

were able to prepare the N^7-(5-chloro-2-benzisoxazole) derivative (**41**) of mitomycin C. Two other analogues based on five-membered heterocyclic rings were prepared. They were the N^7-(5-methyl-1,3,4-thiadiazol-2-yl) (**42**) and N^7-(3-pyrazolyl) (**39**) derivatives of mitomycin C. The good antitumor activity of **43** led us to investigate the reactions of other 3-aminopyrazoles with mitomycin A. Unfortunately, the available 4-cyano and 4-amino-5-hydroxy congeners failed to react.

The final group of mitomycin analogues was based on benzo heterocycles in which the benzene ring was attached to N^7. Three new compounds, including the 5-benzopyrazolyl (**44**), 2,1,3-benzothiadiazol-4-yl (**45**), and 6-methoxyquinolin-8-yl (**46**) derivatives, were prepared.

A number of types of amino heterocycles failed to react with mitomycin A in methanol, even in the presence of potassium carbonate. They included 2-aminopyrimidine, 3-amino-1,2,4-triazine, 2-aminoimidazole, and 5-aminotetrazole. Anions derived from pyrrole and indole also failed to react.

Antitumor Activity. The activities of the N^7-heterocyclic derivatives of mitomycin C against P-388 leukemia are given in Table II. It is apparent that only two of these compounds, the 6-amino-3-pyridyl (**33**) and the 3-pyrazolyl (**43**) derivatives, give appreciably greater prolongation of life than mitomycin C. However, they both suffer some loss in potency. Two other pyridyl derivatives, **31** and **32**, and the 6-methoxyquinolin-8-yl (**46**) derivative were about as effective as mitomycin C in prolonging life. None of the thiazoles was as effective as mitomycin C against P-388, but the simpler ones (**34–36**) have good potencies, and they appear to be relatively nonleukopenic. The various benzo heterocycles generally have poor potencies, which might be attributed to their relatively large size and lipophilicity.

Table V gives structure–activity relationships for the simpler heterocyclic derivatives. The limited number of compounds of each type makes multiple linear regression analysis impractical, but simple inspection of the data reveals that the trends stand in contrast to the correlations found for the N^7-phenyl analogues. Thus, the heterocyclic derivatives show no trend between partition coefficient and potency (MED). It also is surprising that substituents on the pyridine ring (**31–33**) have no effect on the polarographic half-wave reduction potentials. The reduction potentials do vary with the type of heterocycle, with the N^7-thiazolyl derivatives being the easiest and the N^7-pyrazolyl derivatives the most difficult to reduce, but all types are easier to reduce than mitomycin C, and no trends are evident for reduction potential and antitumor potency.

Among the various heterocyclic derivatives of mitomycin C, the N^7-(3-pyrazolyl) (**43**) analogue clearly is desirable because of its significant prolongation of life in P-388 leukopenia. We do not know why this particular compound is so good. However, it is an interesting coincidence that it has nearly the same log P and $E_{1/2}$ values as **21**, the most active N^7-phenyl analogue.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM 360 (60 MHz) spectrometer using tetramethylsilane as the standard. Elemental analyses were performed by the Analytical Center, University of Arizona, Tucson, AZ. Analytical results were within ±0.4% of theoretical values unless specified otherwise.

Preparation of Mitomycin C Analogues (General Method). A solution of mitomycin A (100 mg or 0.286 mmol) in 8 mL of anhydrous methanol was stirred with 0.6 mmol of amine or amine hydrohalide (method A). In the case of an amine hydrohalide, 0.5 mL of triethylamine was added (method B). The progress of the reaction was followed by thin-layer chromatography on silica gel with CH_3OH–$CHCl_3$ (2:8 or 1:9, v/v) as the solvent. If the reaction was very slow, a small amount of solid potassium carbonate was added to promote it (method C). The colored solution 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 described above. The products were recrystallized from a mixture of methylene chloride and methanol or other solvents as specified in Table I. The homogeneity of each product was determined by thin-layer chromatography on silica gel with different ratios of CH_3OH to CH_2Cl_2 as solvent (1:9, 2:8, 3:7, 5:5). Some of the N-heterocyclic derivatives were also chromatographed on alumina-coated plates with mixtures of acetone and ether (usually 1:1) as the solvent. Table I gives the yields and properties of the products.

The dimeric product (**30**) formed from two molecules of mitomycin A and one molecule of phenylenediamine was prepared in the same way as those described above, except that the amount of phenylenediamine was reduced to 11.8 mg (0.105 mmol) and a few milligrams of potassium carbonate was added. The product contained a small amount of monomer, but it was purified readily by chromatography.

Acknowledgment. This investigation was supported by Grant CA 21430, awarded by the National Cancer Institute, DHHS, and by funds from Bristol Laboratories. We thank Steven Johnson for performing the multiple linear regression analysis. The polarographic analyzer was made available by the Department of Chemistry.

Registry No. 1, 14896-01-6; 2, 88854-37-9; 3, 88854-38-0; 4, 88854-39-1; 5, 88854-40-4; 6, 88854-41-5; 7, 75491-90-6; 8, 88854-42-6; 9, 88854-43-7; 10, 70343-57-6; 11, 75491-89-3; 12, 84397-46-6; 13, 88854-44-8; 14, 88854-45-9; 15, 88854-46-0; 16, 88854-47-1; 17, 88854-48-2; 18, 84397-38-6; 19, 84397-37-5; 20, 88854-49-3; 21, 70343-58-7; 22, 88854-50-6; 23, 88854-51-7; 24, 88854-52-8; 25, 88854-53-9; 26, 88854-54-0; 27, 88854-55-1; 28, 88854-56-2; 29, 88854-57-3; 30, 88854-58-4; 31, 78142-97-9; 32, 84397-27-3; 33, 88854-59-5; 34, 78327-27-2; 35, 84397-36-4; 36, 88854-60-8; 37, 88854-61-9; 38, 88854-62-0; 39, 88854-63-1; 40, 88854-64-2; 41, 84397-34-2; 42, 84397-35-3; 43, 84397-45-5; 44, 84397-40-0; 45, 84397-41-1; 46, 84397-28-4; 4-CH_3-$C_6H_4NH_2$, 106-49-0; 3-CH_3-$C_6H_4NH_2$, 108-44-1; 4-CN-$C_6H_4NH_2$, 873-74-5; 3-CN-$C_6H_4NH_2$, 2237-30-1; 4-F-$C_6H_4NH_2$, 371-40-4; 3-Cl-$C_6H_4NH_2$, 108-42-9; 4-I-$C_6H_4NH_2$, 540-37-4; 4-CH_3O-$C_6H_4NH_2$, 104-94-9; 3-CH_3O-$C_6H_4NH_2$, 536-90-3; 2-CH_3O-$C_6H_4NH_2$, 90-04-0;

2-CH_3-4-OH-$C_6H_3NH_2$, 2835-99-6; 3,4-$(CH_3O)_2$-$C_6H_3NH_2$, 6315-89-5; 3,5-$(CH_3O)_2$-$C_6H_3NH_2$, 10272-07-8; 4-SH-$C_6H_4NH_2$, 1193-02-8; 4-NH_2SO_2-$C_6H_4NH_2$, 63-74-1; 3-NH_2-$C_6H_4NH_2$, 108-45-2; 3-CH_3O-4-NH_2-$C_6H_3NH_2$, 5307-02-8; 3-NH_2-4-CH_3O-$C_6H_3NH_2$, 615-05-4; 4-CH_3CONH-$C_6H_4NH_2$, 122-80-5; 3-CH_3CONH-$C_6H_4NH_2$, 102-28-3; 4-CO_2HCH_2NH-$C_6H_4NH_2$, 2835-08-7; 4-NH_2CONH-$C_6H_4NH_2$, 21492-80-8; 4-NO_2-$C_6H_4NH_2$, 100-01-6; 4-NH_2-$C_6H_4NH_2$, 106-50-3; 1,3-benzodioxol-5-amine, 14268-66-7; 6-methoxy-3-pyridinamine, 6628-77-9; 2,5-pyridinediamine, 4318-76-7; 2-thiazolamine, 96-50-4; 4-methyl-2-thiazolamine, 1603-91-4; 4,5-dimethyl-2-thiazolamine, 2289-75-0; 2-benzothiazolamine, 136-95-8; 4-chloro-2-benzothiazolamine, 19952-47-7; 6-nitro-2-benzothiazolamine, 6285-57-0; 3-methyl-5-isothiazolamine, 24340-76-9; 5-chloro-2-benzoxazolamine, 61-80-3; 5-methyl-1,3,4-thiadiazol-2-amine, 108-33-8; 1*H*-pyrazol-3-amine, 1820-80-0; 1*H*-indazol-5-amine, 19335-11-6; 2,1,3-benzothiadiazol-4-amine, 767-64-6; 6-methoxy-8-quinolinamine, 90-52-8; mitomycin A, 4055-39-4.

Supplementary Material Available: Full screening data for compounds submitted to the P-388 assay (8 pages). Ordering information is given on any current masthead page.

Book Reviews

Indoles. Part 4: The Monoterpenoid Indole Alkaloids (A Monograph in the Series "The Chemistry of Heterocyclic Compounds"). Edited by J. Edwin Saxton. Wiley-Interscience, New York. 1983. x + 886 pp. 17 × 24 cm. ISBN 0-471-89748-5. $200.

The first known monoterpenoid indole alkaloids, strychnine and quinine, have been focusses of interest among organic chemists during the entire history of organic chemistry. In 1952 the indole alkaloid reserpine was isolated from Indian medicinal preparations that had been used for centuries in ayurvedic medicine. It was immediately applied clinically for the treatment of hypertension and a variety of psychiatric conditions. This stimulated great interest in indole alkaloids as potential chemotherapeutic agents, which was given an added dimension by the discovery of the clinically useful antitumor properties of the bis(indole) alkaloids vinblastine and vincristine in the late 1950's. Moreover, academic interest in the monoterpenoid indole alkaloids was further stimulated by Woodward's standard-setting total synthesis of reserpine in 1958. During this 30 years of recent activity in indole alkaloid chemistry, organic chemists have been able to use the authoritative Manske series of volumes to review the field; in addition, the *Specialist Periodical Reports* of the Royal Society of Chemistry entitled "The Alkaloids" have quickly established themselves as tremendously valuable to all practitioners in the field.

We are now indeed fortunate that a group of chemists distinguished not only for their research in the indole alkaloid field but also for their outstanding contributions to the review literature, including the Manske volumes and the *Specialist Periodical Reports*, have produced a one-volume text on the monoterpenoid indole alkaloids that provides an extremely thorough and well-written summary of the field in less than 1000 pages. It is always a pleasure to read the scientific and literary work of these contributors, and the quality of the contributions is extremely high. The book begins with a chapter on structural and biosynthetic relationships among the alkaloids (R. B. Herbert), which surveys the field and in so doing points clearly to numerous fascinating areas for further exploration. There follow a number of chapters reviewing the alkaloids by structural type; the recently investigated *Aristotelia* alkaloids (J. E. Saxton), the corynantheine–heteroyohimbine alkaloids (R. T. Brown), the yohimbine alkaloids (R. T. Brown), the sarpagine–ajmaline group (J. A. Joule), the uleine–ellipticine–vallesamine alkaloids (J. A. Joule), the *Strychnos* alkaloids (H.-P. Husson), the aspidospermine group (J. E. Saxton), the eburnamine–vincamine group (J. E. Saxton), the ibogamine–catharanthine group (G. A. Cordell), the bis(indole) alkaloids (G. A. Cordell), the *Cinchona* group (G. Grethe and M. R. Uskokovic), and camptothecin (C. R. Hutchinson and Jun-Chao Cai). The book ends with an interesting, critical, and up-to-date study of pharmacology, biochemistry, and clinical applications of the monoterpenoid alkaloids (W. A. Creasey).

The book is very well produced, and the references are copious and accessible. The indexing is also good.

I consider this to be an outstanding one-volume treatment of this important topic by expert writers who have performed a valuable service to the chemical, biological, and pharmaceutical research communities.

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Pharmacochemistry Library. Volume 6. Quantitative Approaches to Drug Design. Edited by John C. Dearden. Elsevier, Amsterdam. 1983. x + 296 pp. 17 × 24.5 cm. ISBN 0-444-42200-5. $57.75.

This book records the proceedings of the Fourth European Symposium on "Chemical Structure–Biological Activity: Quantitative Approaches", held at Bath, United Kingdom, September 6–9, 1982, and constitutes Volume 6 in the *Pharmacochemistry Library* series. The prime aim of the symposium-organizing committee was to provide a forum for the presentation and exchange of new work in the broad field of quantitative structure–activity relationship (QSAR) studies in drug design, reflecting the state of the art in QSAR at the present time. Within the framework of selected important subject areas in the field, key papers were given by 14 researchers, and, in addition, 6 contributed papers and 39 posters were presented, all of which are contained in this volume.

Following the symposium format, the book is nicely organized into six sections, each dealing with a major subject area: "Parameters and Modelling in QSAR", "Enzymes and Receptors", "Molecular Graphics and Conformational Studies", "Pharmacokinetic and Rate Effects in Relation to QSAR", "Series Design for QSAR", and "QSAR in Practice".

Altogether the book provides a wealth of information on current research activities in the broadly defined QSAR field. There is good balance in subject areas covered and between theoretical