doses with the test compound suspended in CMC (1%) or dissolved in water, respectively. Male Swiss mice weighing 30 g were treated intravenously, and male New Zealand rabbits weighing about 10 kg received the drug by oral administration. The number of dead animals was counted after 14 days, and the LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[28]

Pharmacokinetic Tests. Groups of fasted male Wistar rats weighing 180–230 g received the drug by oral and ip routes. Four male beagle dogs and two monkeys (*Macaca fascicularis*) received the drug by oral route. Four healthy volunteers of both sexes received the drug in capsules of 100 mg. Plasma and urine samples in the animals and in humans and tissue homogenates in rat were assayed by an HPLC method. Some sample of rat urine was also assayed by a microbiological method.

Microbiological Assay of the Urine. Compounds **10c**, **30**, and **31** were orally administered to rats (50 mg/kg), and urinary recoveries were evaluated within 24 h after dosing. Quantitative determinations of microbiologically active compounds in the urine were made according to Bennet,[29] with *K. pneumoniae* 4 as bioassay organism.[21]

HPLC Assay in the Plasma and Organs. Compound **31** was administered to rats by oral and ip routes at various doses, and the heparinized plasma was withdrawn at various times after drug administration. The unmodified compound was determined in plasma, urine, and homogenized organs according to the following procedure. *N*-Dimethyldiazepam (2 μg; internal standard), 1 mL of citrate buffer (0.1 M, pH 6.2), and 6 mL of $CHCl_3$ were added to 1 mL of plasma or homogenized organs. After mixing for 20 min and centrifugation at 3000 rpm for 5 min, the organic layer was separated and dried in a 70 °C water bath. The residue was recovered with 50 μL of MeOH, 20 μL of which were then injected into a HPLC μ-Bondapak C-18 (Waters) column: eluent AcOH (0.1%) (A)–MeOH (B); gradient outline 60%, 70%, and 60% of B at 1, 10, and 18 min, respectively; flow rate 0.7 mL/min; effluent monitored at 245 nm (UV detector). The retention times were 7.7 min for compound **31** and 13.8 min for the internal standard. The coefficients of interassay and intraassay variability were about 10%.

Registry No. **1**, 106016-79-9; **2**, 106016-80-2; **3**, 100638-20-8; **4**, 101337-93-3; **5**, 101337-92-2; **6**, 101337-95-5; **7**, 106016-81-3; **8a**, 106016-82-4; **8b**, 106016-85-7; **8c**, 101337-96-6; **8d**, 106016-83-5; **8e**, 106016-84-6; **8f**, 3080-99-7; **8g**, 6431-65-8; **9a**, 106016-86-8; **9b**, 106016-87-9; **9c**, 101337-97-7; **9d**, 106016-88-0; **9e**, 106039-46-7; **9f**, 106016-89-1; **9g**, 106016-90-4; **10a**, 106016-91-5; **10b**, 106016-92-6; **10c**, 101337-81-9; **10d**, 106016-93-7; **10e**, 106016-94-8; **10f**, 106016-95-9; **10g**, 106016-96-0; **11b**, 106017-12-3; **11c**, 101337-82-0; **11f**, 106017-13-4; **12**, 106016-97-1; **13**, 106016-98-2; **14**, 106016-99-3; **14** (free base), 106017-14-5; **15**, 106017-00-9; **16**, 106017-01-0; **17**, 106017-02-1; **18**, 106017-03-2; **19**, 106017-04-3; **20**, 106017-05-4; **21**, 106017-06-5; **22**, 101337-84-2; **23**, 106017-07-6; **24**, 106017-15-6; **25**, 106017-16-7; **26**, 106039-47-8; **27**, 85741-48-6; **28**, 101337-85-3; **29**, 101337-83-1; **29**·C_4H_9N, 102052-49-3; **30**, 102052-48-2; **30** (free base), 101337-87-5; **31**, 106017-08-7; **31** (free base), 101363-10-4; **32**, 106017-09-8; **33**, 101337-99-9; **34**, 101337-88-6; **35**, 106017-10-1; **36**, 106017-11-2; EMME, 87-13-8; 2,3,4-trichloronitrobenzene, 17700-09-3; thioglycolic acid, 68-11-1; 3-chloro-4-fluoroaniline, 367-21-5; 2-amino-6-fluorobenzothiazole, 348-40-3; 7-chloro-3,4-dihydro-3-oxo-2*H*-[1,4]benzothiazine, 5333-05-1; 1-bromo-2-chloroethane, 107-04-0; pyrrolidine, 123-75-1; morpholine, 110-91-8; piperazine, 110-85-0; 1-methylpiperazine, 109-01-3; 1-piperazineethanol, 103-76-4; 1-benzylpiperazine, 2759-28-6; *N*-(1,1,1-trifluoroethan-2-yl)piperazine, 13349-90-1; 1-(ethoxycarbonyl)piperazine, 120-43-4; 2,6-dimethylpiperazine, 108-49-6; tetrahydrothiazine, 123-90-0.

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Redox Chemistry of the 9-Anilinoacridine Class of Antitumor Agents

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9-Anilinoacridines bearing a 1′-NHR substituent on the anilino ring undergo facile, chemically reversible, two-electron oxidation to quinone diimines. The chemical and electrochemical oxidation of three groups of 9-anilinoacridines (1′-substituted derivatives, together with 3′-substituted analogues and acridine-substituted analogues of the clinical antileukemic drug amsacrine) have been studied and their redox potentials determined. For aniline-substituted derivatives, redox potentials ($E_{1/2}$) correlate well with substituent electronic properties, with electron-donating substituents facilitating oxidation. Substituents in the acridine ring have little effect on redox potentials, indicating minimal transmission of electronic effects from the acridine to the aniline rings. Although the broad class of 9-anilinoacridines show biological activity over a very wide range of structural variations, a 1′-NHR substituent is a common feature of the most active derivatives. Nevertheless, no clear quantitative relationships between redox potential and biological activity could be discerned, and the relevance of this redox chemistry to the mode of action of amsacrine and other 9-anilinoacridines remains unclear.

The 9-anilinoacridines have been extensively investigated as antitumor agents.[1-3] One derivative, amsacrine (**18**), has become a valuable clinical drug for the treatment of leukemia (reviewed in ref 4 and 5), and a second analogue (CI-921; **39**) has recently begun clinical trials.[6,7] The 9-anilinoacridines belong to the broad class of compounds known as DNA-intercalating agents, whose biological activity is probably due to their causation of double-strand DNA breaks;[8,9] amsacrine in particular is known to be a very potent inhibitor of the DNA nicking–closing enzyme topoisomerase II.[10,11] The aniline ring of amsacrine is readily and reversibly oxidized either chemically[12] or microsomally[13] to give the quinone diimine **40** (Scheme I), and this redox chemistry has been shown to play a major part in both its mammalian metabolism[12,13] and its ability

† Department of Chemistry, University of Auckland.

(1) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. in *Molecular Aspects of Anticancer Drug Action*; Neidle, S., Waring, M. J., Eds.; MacMillan: London, 1983; pp 1–34.
(2) Denny, W. A.; Cain, B. F.; Atwell, G. J.; Hansch, C.; Panthananickal, A.; Leo, A. *J. Med. Chem.* **1982**, *25*, 276.
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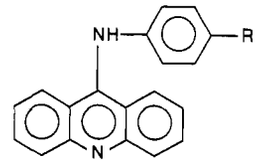
0022-2623/87/1830-0473$01.50/0

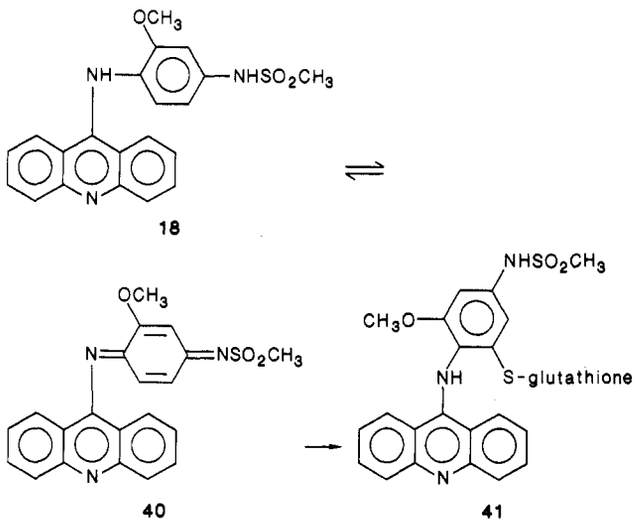
Table I. Electrochemistry of 1′-Substituted 9-Anilinoacridines



no.	R	$E_{1/2}$,[a] mV	peak separation,[a] mV	σ_p[b]	IC_{50},[c] nM	log $(1/D_{50})$[d]
1	H	(625)[e]		0.00		
2	CH_3	(750)		−0.17		
3	OCH_3	(635)		−0.27		
4	Cl	(765)		0.23		
5	OH	215	50	−0.37	250	3.81
6	NH_2	263	25	−0.66	350	4.09
7	$NHCH_3$	235	30	−0.84	180	3.79
8	$NHCH_2CH_3$	230	20	−0.61	250	3.59
9	$N(CH_3)_2$	275	30	−0.83	600	3.64
10	$NHCOCH_3$	460	40	0.00	100	4.20
11	NHCOPh	383	100	−0.19	20	3.45
13	$NHCOOCH_3$	404	78	−0.15	60	4.08
13	$NHP(O)(OCH_3)_2$	340	20		15	4.46
14	$NHSO_2CH_3$	330	35	0.03	35	4.27
14a	$N(CH_3)SO_2CH_3$	(660)				
15	$NHSO_2Ph$	355	30	0.01	3.5	4.62
16	$NHSO_2PhpNH_2$	378	25	0.01		5.83

[a] Determined at a scan rate of 20 mV/s. [b] σ_p values for 1′-substituent: from ref 28. [c] IC_{50}: nanomolar concentration of drug required to reduce the growth of L1210 cells by 50% after 70 h. [d] D_{50}: dose of drug (mol/kg) providing a 50% life extension in L1210 assays when given a qd 1–5 schedule; see ref 2. [e] Figures in parentheses: not chemically reversible reactions.

Scheme I

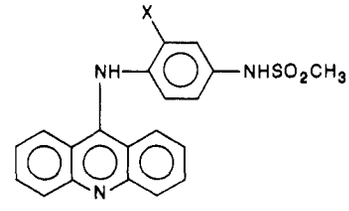


to cut DNA.[14,15] It is not yet clear if such oxidation is obligatory for biological activity or if it is merely a detoxification pathway via rapid reaction of the quinone diimine with water-soluble thiols such as glutathione (Scheme I). However, these redox reactions represent an important facet of the chemistry of 9-anilinoacridines and are similar to the redox reactions known to be undergone by the ellipticine family of antitumor antibiotics.[16]

This paper details a preliminary investigation of the electrochemical oxidation of 9-anilinoacridines, including a study of the nature of the redox processes, the products of electrochemical and chemical oxidation, and the effects of substituents at various positions on redox potentials.

Chemistry. All the compounds listed in Tables I–III have been reported previously.[2,3,17,18] The quinoneimines **42** and **49** and the quinone diimines **50** and **54** required

Table II. Electrochemistry of 3′-Substituted Amsacrine Analogues



no.	X	$E_{1/2}$,[a] mV	peak separation,[a] mV	σ_p[b]
14	H	330	35	0
17	OH	180	50	−0.37
18	OCH_3	280	30	−0.27
19	NH_2	180	30	−0.66
20	$NHCH_3$	145	*c*	−0.84
21	$N(CH_3)$	195	30	−0.83
22	CH_3	289	25	−0.17
23	F	340	20	0.06
24	Cl	335	10	0.23
25	3′,5′-$(OCH_3)_2$	240	40	−0.54[d]

[a] See footnote *a*, Table I. [b] σ_p values for 3′-substituents from ref 28. [c] Unstable quinone diimine: see text. [d] Sum of σ_p values for both substituents.

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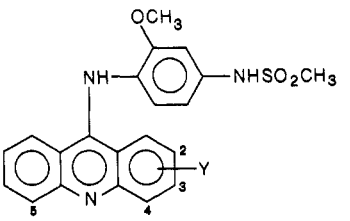
(17) Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1984**, *27*, 1053.
(18) Atwell, G. J.; Rewcastle, G. W.; Denny, W. A.; Cain, B. F.; Baguley, B. C. *J. Med. Chem.* **1984**, *27*, 367.

Table III. Electrochemistry of Acridine-Substituted Amsacrine Analogues



no.	Y	$E_{1/2}$,[a] mV	ΔE[b]	σ[c]
18	H	280		0.00
26	2-NH_2	260	-20	-0.16
27	2-CH_3	273	-7	-0.07
28	3-$NHCH_3$	263	-17	-0.84
29	3-$NHCOCH_3$	278	-2	0.00
30	3-NO_2	253	-27	0.78
31	3-CH_3	278	-2	-0.17
32	3-OCH_3	278	-2	-0.27
33	3-Br	265	-15	0.23
34	3-$CONHCH_3$	268	-12	0.36
35	4-CH_3	263	-17	-0.07
36	4-OCH_3	278	-2	0.12
37	4-Br	248	-12	0.39
38	4-$CONHCH_3$	243	-37	0.35
39	4-CH_3, 5-$CONHCH_3$	240	-40	0.28[d]

[a] See footnote *a*, Table I. [b] ΔE: difference (mV) in $E_{1/2}$ values between substituted and parent (18) compounds. [c] σ: σ_p values for 3-substituents, σ_m values for 2- and 4-substituents; see text. [d] Sum of σ values for both substituents.

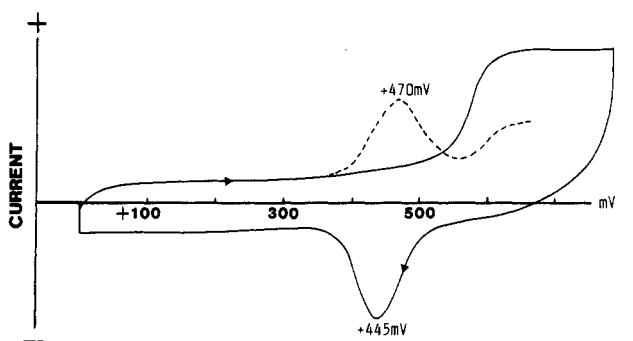


Figure 1. Cyclic voltammogram for 9-anilinoacridine (1).

were prepared by published methods[12] with activated MnO_2.

Cyclic Voltammetry. Cyclic voltammograms were carried out with 0.1 mM solutions of the compounds in acetate buffer containing 40% acetonitrile to ensure solubility of all compounds, at pH 4.5. An anodic sweep was carried out up to +1.0 V, with any oxidative processes occurring above this range considered to be unimportant for the present study. A variety of scan rates was used, from the maximum of 200 mV/s permitted by the recorder down to 20 mV/s. $E_{1/2}$ values were obtained from the slowest scan rate used (normally 20 mV/s but occasionally 50 mV/s). Coulometry was carried out on 0.1 mM solutions in 10% acetonitrile/acetate buffer at pH 4.5.

Results

The oxidation potentials for a series of 1′-substituted 9-anilinoacridines are given in Table I. At the buffer pH of 4.5 used, all the compounds will be essentially fully charged, and the oxidation potentials should be affected only by the acridine ring substituents. The weakest base is the 1′-Cl derivative 4 with a pK_a of 7.65 and will thus be less than 0.1% in the free base form. The first four compounds (1–4) did not show reversible redox couples. The voltammogram of the 1′-H derivative (1) is shown in

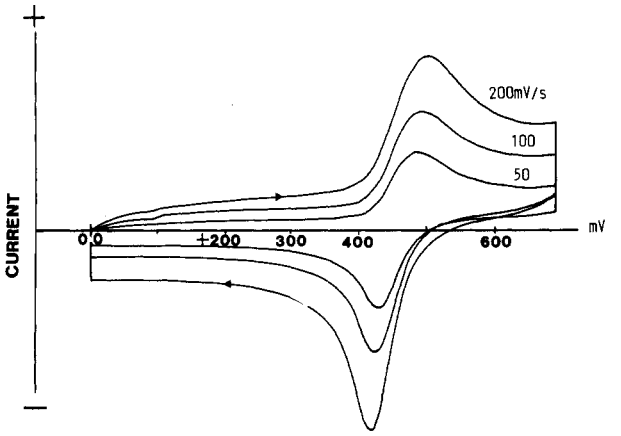


Figure 2. Cyclic voltammogram for compound 5, showing reversibility of the reaction.

Table IV. Cyclic Voltammetry Data for Selected 9-Anilinoacridines

no.	scan rate, mV/s	$E_{1/2}$, mV	peak separation, mV	$i_{pa}/V^{1/2}$
14	200	310	50	0.47
	100	318	35	0.42
	50	328	25	0.38
	20	330	35	0.36
18	200	273	35	0.47
	100	278	35	0.35
	50	278	25	0.31
	20	280	30	0.30
21	200	198	35	0.52
	100	195	30	0.47
	50	195	30	0.44
	20	195	30	0.38
40	200	283	55	
	100	283	45	
	50	278	45	
	20	278	35	

Figure 1. At a potential greater than 550 mV, oxidation occurred to give a species that exhibited a reduction peak following reversal of the scan in the cathodic direction. If the scan is then repeated, a redox couple of $E_{1/2}$ = 458 mV is produced, with a peak separation of 25 mV, suggesting a two-electron reaction. If the first anodic sweep is terminated below 500 mV, this species is not seen. The nature of the product produced by this reaction is not known; a comparison with the 1′-OH compound 5 shows that it is not this species. Qualitatively similar chemically irreversible oxidations at above 600 mV were seen for compounds 2–4 also.

However, cyclic voltammograms for all the other derivatives show chemically reversible redox couples (Figure 2). For such reactions, cyclic voltammetry can be used to directly measure the potential of the half-reaction ($E_{1/2}$). Application of the diagnostic criteria[19] for electrochemical reversibility indicates that quasi-reversible, two-electron charge transfer occurs with those derivatives showing chemically reversible redox couples. Thus the peak separation, where Epa and Epc are the peak anodic and cathodic potentials, respectively (see Figure 2), approaches

$$dE = \text{Epa} - \text{Epc} \quad (1)$$

30 mV at low scan rates but increases as the scan rate increases[19] (see Table IV). In addition, the ratio of the anodic peak current (i_{pa}) to the square root of the scan rate

(19) Adams, R. N. *Electrochemistry at Solid Electrodes*: Dekker: New York, 1969.

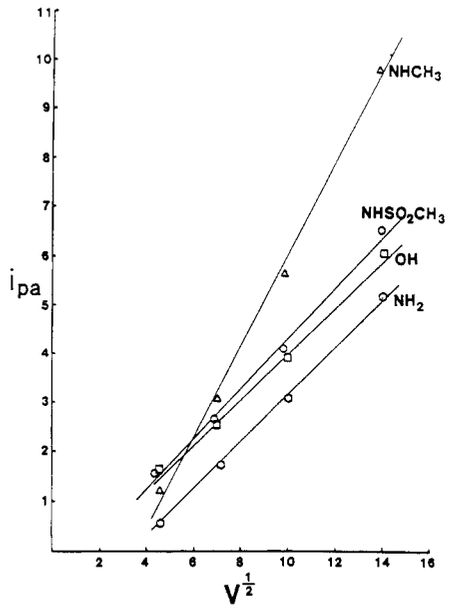


Figure 3. Plots of anodic peak current (i_{pa}) vs. the square root of the scan rate ($V^{1/2}$) for selected compounds as indicated: △, 7; ○, 14; □, 5; ○, 6.

Table V. Number of Electrons Transferred in Redox Reactions of Selected 9-Anilinoacridines

	electrons transferred[a]	
redox couple	oxidation	reduction
5:42	1.88	
6	1.78	
9	3.77	
10	2.24	
12	2.22	
14	2.40	
18:44	1.97	1.52
21:50	1.80	1.90

[a] Determined by coulometry; see text.

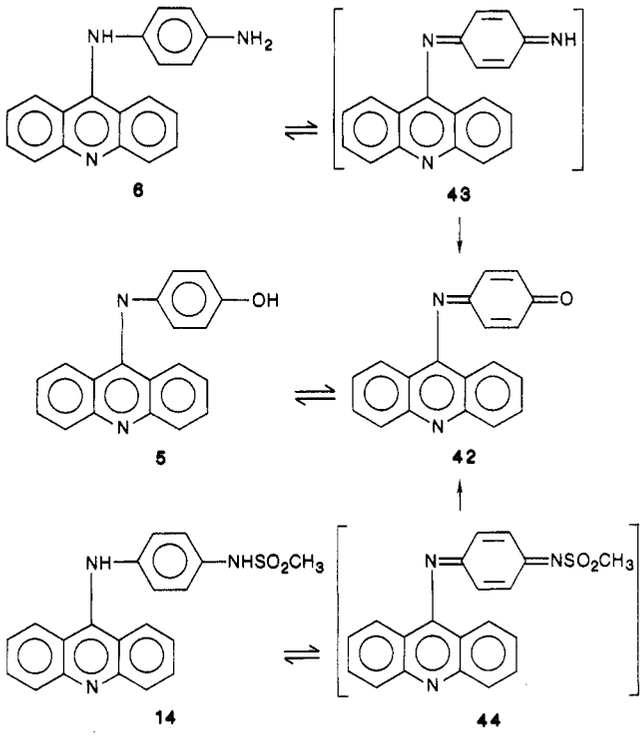
(V) is virtually independent of scan rate (see Table IV). Plots of i_{pa} vs. the square root of V (Figure 3) indicate a linear relationship between the two variables, with a nonzero intercept, indicative of quasi-reversible charge transfer.[19] As a general visual observation, the cathodic peak was always sharper than the anodic peak, which again is consistent with quasi-reversible charge transfer.[19] Such electrochemical irreversibility is caused by slow electron exchange of the electroactive species with the working electrode.

Cyclic voltammetry suggested a two-electron oxidation was occurring for those compounds that showed chemical reversibility, by application of the equation

$$\text{Epa} - \text{Epc} = 58/n \text{ (mV)} \quad (2)$$

where n is the number of electrons transferred (Table I). However, the quasi-reversible nature of the electrochemical process did not permit an accurate determination of the number of electrons involved. Coulometry using a platinum electrode was used to determine the value of n for a number of 1′-substituted compounds (Table V). The potential of the platinum electrode was set at 650 mV (vs. SCE), which was found to give an acceptable rate of oxidation (complete within 90 min). As seen in Table I, with the exception of the 1′-$N(CH_3)_2$ derivative **9**, all oxidations involve loss of two electrons. For the 1′-$N(CH_3)_2$ derivative, coulometry indicates the loss of four electrons. This is not consistent with the cyclic voltammetry data (Table I), since at low scan rates the peak separation was 30–35 mV. The identity of the oxidation product of this compound is unknown. Chemical oxidation with MnO_2 gave a number of products that were not characterized.

Scheme II



Thus all of the 1′-substituted 9-anilinoacridines in Table I that possess a β-hydrogen on the heteroatom (O or N) show chemically reversible redox couples undergoing a two-electron, quasi-reversible charge transfer. The product of oxidation (either chemical or electrochemical) of the 1′-OH compound **5** is the quinone imine **42**. This compound was prepared by MnO_2 oxidation of **5** and found to have an identical cyclic voltammogram ($E_{1/2}$ = 218 mV, ΔE = 55 mV) compared to that of the phenol ($E_{1/2}$ = 215 mV, ΔE = 50 mV). As expected from work with *p*-aminophenol and *p*-phenylenediamine,[19,20] chemical oxidation of the 1′-NH_2 derivative **6** also gave the quinone imine **42** as the only isolable product, due to hydrolysis of the very unstable unsubstituted quinone diimine **43** (Scheme II). Electrochemical oxidation of **6** also gave only the quinone imine **42**, as judged by an identical cyclic voltammogram with that of **5** and by UV and TLC.

The quinone diimine oxidation product **44** of the 1′-$NHSO_2CH_3$ compound **14** was somewhat more stable. Chemical oxidation of **14** under anhydrous conditions (MnO_2 in anhydrous EtOAc) gave the pure quinone diimine **44**, although this compound was still unstable to hydrolysis, and any manipulations resulted in formation of significant quantities of the quinone imine **42** (Scheme II). However, the cyclic voltammogram of compound **14** showed chemical reversibility (Figure 4a), indicating the quinone diimine **44** is sufficiently stable to survive on the electrode surface. If the β-hydrogen of compound **14** was replaced by a methyl group (compound **14a**), a quite different cyclic voltammogram is seen (Figure 4b). A chemically irreversible oxidation at 660 mV is observed following the first scan in the anodic direction. A small return peak (with a peak separation of 35 mV) is seen following scan reversal, followed by an additional cathodic peak at 185 mV. If the scan cycle is repeated without polishing the electrode surface, an anodic peak corresponding to the

(20) Mark, H. B.; Anson, E. C. *Anal. Chem.* **1963**, *35*, 722.

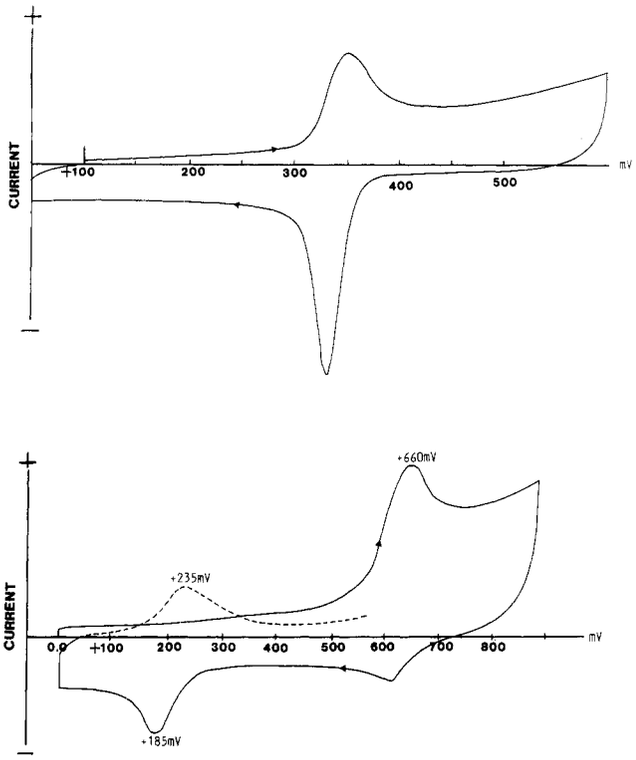
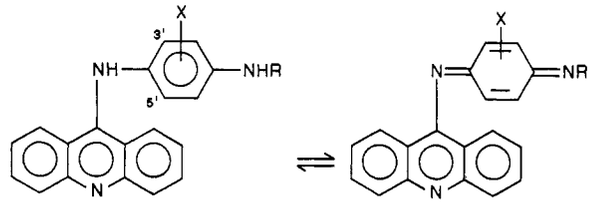


Figure 4. (a) Cyclic voltammogram for AMSA (14). (b) Cyclic voltammogram for *N*-methyl-AMSA (14a), showing irreversible reaction.

cathodic peak at 185 mV appears, with a peak separation for this new redox couple of 40 mV. This $E_{1/2}$ at 210 mV is consistent with that of the quinone/phenol **42**/**5** redox couple, suggesting that the initial product of two-electron oxidation is rapidly hydrolyzed to the quinone imine **42**.

The quinone diimines **45**–**47** corresponding to the 1′-$NHCOCH_3$, 1′-$NHCOOCH_3$, and 1′-$NHP(O)(OCH_3)_2$ compounds **10**, **12**, and **13** were less stable than **44**. The chemical oxidation of these compounds (MnO_2 in anhydrous EtOAc) was much slower, as expected from their higher redox potentials (Table I), but did give products with UV spectra consistent with the quinone diimines **45**–**47**. However, analysis by TLC showed mainly the quinone imine **42**, indicating very ready hydrolysis.



10:	R = $COCH_3$, X = H	**45**
12:	R = $COOCH_3$, X = H	**46**
13:	R = $P(O)(OCH_3)_2$, X = H	**47**
31:	R = SO_2CH_3, X = 3′-$N(CH_3)_2$	**50**
25:	R = SO_2CH_3, X = 3′,5′-$(OCH_3)_2$	**53**

Since the reactions discussed here are electron-transfer processes, they should be dependent on substituent electronic parameters, as are the redox potentials of other quinone systems.[21] The system studied here (stable quinone diimine) is unusual, and the substituents that are compatible with this have only a limited range of electronic parameter values. Nevertheless, statistically significant relationships of redox potentials ($E_{1/2}$) with a variety of

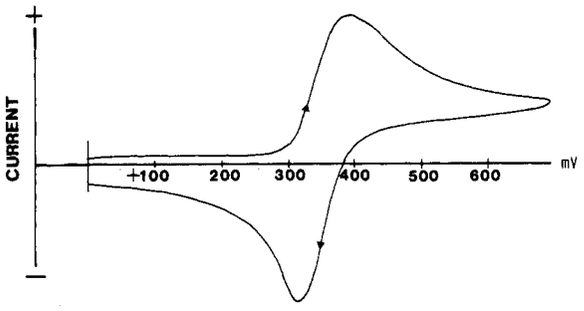


Figure 5. Cyclic voltammogram for amsacrine (18).

substituent electronic properties could be derived. The best fit to the $E_{1/2}$ values of the 1′-NHR derivatives **6**–**12**, **14**, and **15** was found for σ_p values

$$E_{1/2} = 180\ (\pm 47)\sigma_p + 391\ (\pm 23) \qquad (3)$$

$$n = 9,\ r = 0.82,\ s = 49$$

The single example of a 1′-oxygen substituent, the 1′-OH derivative **5**, fitted the equation poorly and was not included in its derivation. No σ_p values were available for the substituents of compounds **13** and **16**.

It is clear from the results that oxidation of the 1′-NHR compounds to form the quinone diimines is facilitated by electron-donating substituents R, as expected.

The next group of compounds studied (Table II) all possessed a 1′-$NHSO_2CH_3$ group, with varying substituents (X) at the 3′-position. These are of particular interest, since compound **18** (X = OCH_3) is the clinical drug amsacrine, and quantitative structure–activity relationship (QSAR) studies[2,22] suggest that high biological activity in this series is correlated with the electron-donating ability of groups in this position. The 3′-$NHCH_3$ and 3′-$N(CH_3)_2$ derivatives **20** and **21** have been shown[3,18] to have superior antitumor activity to amsacrine and are currently under advanced evaluation.

The oxidation potentials for a series of these compounds are given in Table II. Except for the 3′-NH_2 and 3′-$NHCH_3$ derivatives **19** and **20**, all reactions were chemically reversible, with quasi-reversible charge transfer being observed in all cases (see Figure 5 and Table IV). Analysis of peak separation data (Table II) showed that two-electron oxidation was occurring in all cases except possibly for the 3′-Cl derivative **24**. Here the peak separation of 10 mV suggests a six-electron process, which is not compatible with a quinone diimine intermediate; it is possible that further reaction takes place to displace the halogen as a radical.[23]

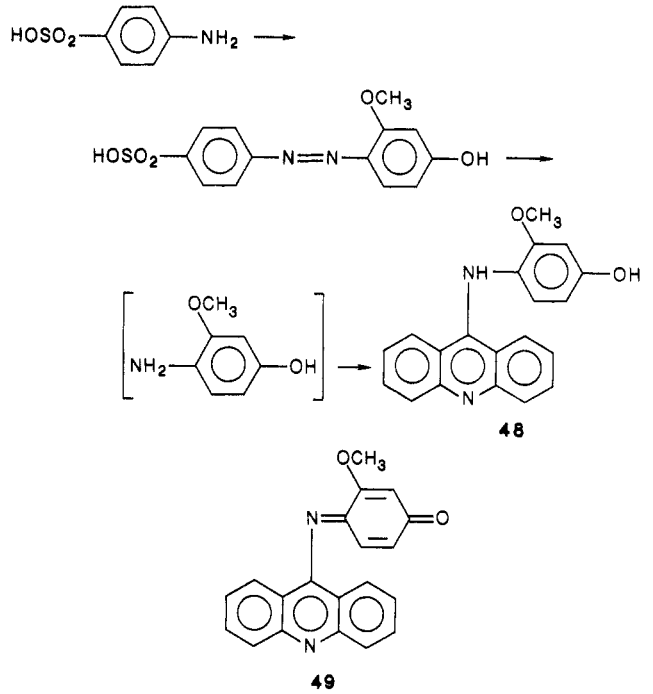
The redox reactions of amsacrine (**18**) and its 3′-$NHCH_3$ and 3′-$N(CH_3)_2$ analogues **20** and **21** were of particular interest. Amsacrine (**18**) was more easily oxidized than the corresponding unsubstituted 1′-$NHSO_2CH_3$ derivative **14** ($E_{1/2}$ of 280 mV compared to 330 mV), due to the electron-donating influence of the 3′-OCH_3 group. The observed peak separation of 30 mV suggests two-electron oxidation, and independent coulometry measurements gave a value of 1.97 ± 0.03 electrons lost. The two-electron oxidation product of amsacrine, the quinone diimine **40**, has already been described.[12] This compound is quite stable to hydrolysis (more so than the corresponding compound **44**; see above), and its electrochemical behavior was also studied (Table IV). A chemically reversible redox

(21) Shaika, I. A.; Johnson, F.; Grullman, A. P. *J. Med. Chem.* **1986**, *29*, 1329.

(22) Denny, W. A.; Atwell, G. J.; Baguley, B. C.; Rewcastle, G. W. In *QSAR in the Design of Bioactive Compounds*; Kuchar, M., Ed.; Prous: Barcelona, 1984; pp 97–114.

(23) Mason, R. P. *Rev. Biochem. Toxicol.* **1979**, 151.

Scheme III



couple with an $E_{1/2}$ of 280 mV was found, with no other detectable electroactive species. Coulometry gave a value of 1.52 electrons gained during reduction at 200 mV. The low number is probably due to incomplete reduction at this potential, but use of lower potentials is liable to result in competing reduction of the solvent.[19] This work taken together establishes the quinone diimine **40** as the product of reversible electrochemical oxidation as well as of chemical[12] and microsomal[13] oxidation.

In spite of the stability of the quinone diimine **40**, it might be expected to hydrolyze to some extent under biological conditions to the corresponding quinone **49**, and this has been suggested.[13] Thus the quinone **49** and the corresponding phenol **48** were prepared (Scheme III), and their redox properties were also examined. Cyclic voltammetry indicated a common redox couple for both compounds, with an $E_{1/2}$ of 170 mV.

The 3′-dimethylamino analogue **21** had a significantly lower redox potential than amsacrine (195 vs. 280 mV), due to the very powerful electron-donating properties of the dimethylamino group. However, in all other respects the reaction was identical with that of amsacrine. Chemically reversible two-electron oxidation to the stable, isolable quinone diimine **50** was confirmed by the identical cyclic voltammograms of both compounds and by separate coulometry measurements, which gave values of 1.80 and 1.90 electrons transferred for oxidation and reduction, respectively.

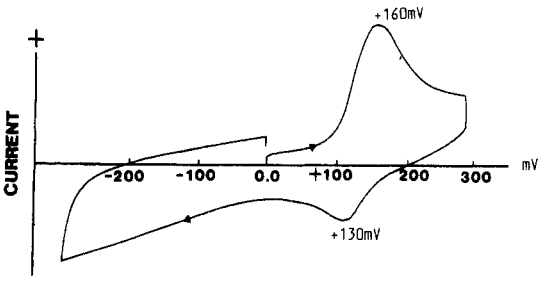


Figure 6. Cyclic voltammogram for 3′-$NHCH_3$ derivative **20**, showing irreversible reaction.

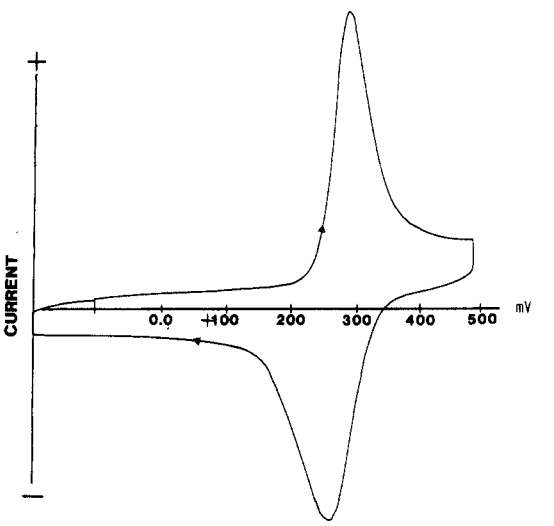
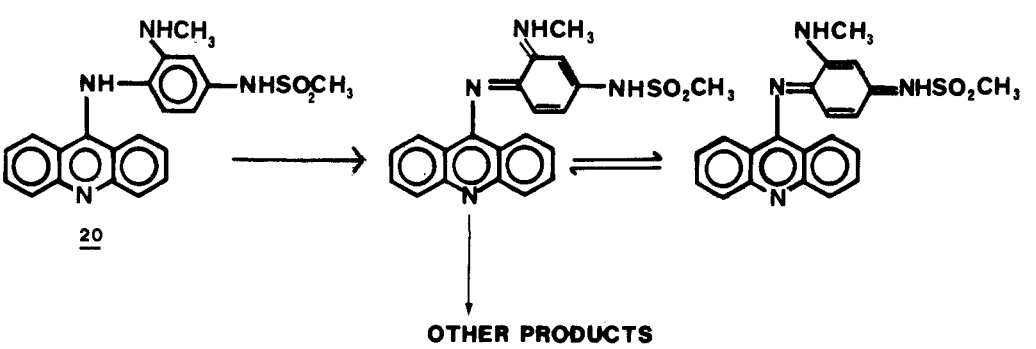


Figure 7. Cyclic voltammogram for CI-921 (**39**).

The 3′-$NHCH_3$ compound **20** underwent oxidation to give an unstable intermediate. If the anodic scan was terminated at +300 mV (Figure 6), a single oxidation with an $E_{1/2}$ of 145 mV was seen, with a peak separation of 30 mV, indicating a two-electron process. However, as the scan rate was increased, the ratio of anodic to cathodic peak currents decreased, indicating the occurrence of a chemical reaction after oxidation. An alternative oxidation pathway is possible for **20** (Scheme IV), which has a β-hydrogen on the 3′-substituent, leading to the *o*-quinone diimine **51**, which is likely to be much more unstable than the usual *p*-quinone diimine **52**, and significant formation of this product might account for the chemical irreversibility of this reaction. Chemical oxidation of **20** gave a mixture of unstable products, some of which had quinone diimine like properties, but no pure compounds could be isolated. The redox potentials of the 3′-substituted amsacrine analogues of Table II were closely correlated with the electronic properties of the substituent groups, as was shown for the 1′-substituted derivatives. A number of

Scheme IV



different electronic parameters were evaluated, but the best fit was again shown by substituent σ_p values:

$$E_{1/2} = 177\ (\pm 30)\sigma_p + 306\ (\pm 16) \quad (4)$$

$$n = 9,\ r = 0.92,\ s = 32$$

One disubstituted analogue, the 3′,5′-dimethoxy derivative **25**, was also studied. This and related disubstituted compounds still appear to intercalate DNA, although with greatly reduced insertion into the intercalation site,[24] and are completely inactive biologically. The second methoxy substituent appears to limit the conformational freedom of the side chain of **25**,[24] and it was of interest to see whether there might be severe steric hindrance to formation of the corresponding quinone diimine **53**, which must force the side chain toward even greater coplanarity with the acridine ring. However, the redox properties of **25** were unexceptional, with chemically reversible two-electron oxidation to a stable product (presumably the quinone diimine **53**) being observed. The $E_{1/2}$ of 240 mV is predictable entirely from the electronic properties of the substituents, without any modifications for steric effects; thus addition of one OMe group to **14** to give amsacrine (**18**) lowers $E_{1/2}$ by 50 mV, and addition of the second to give **25** lowers it by a further 40 mV. Inclusion of compound **25** in eq 4 had no effect on the goodness-of-fit statistics:

$$E_{1/2} = 173\ (\pm 29)\sigma_p + 308\ (\pm 15) \quad (5)$$

$$n = 10,\ r = 0.91,\ s = 31$$

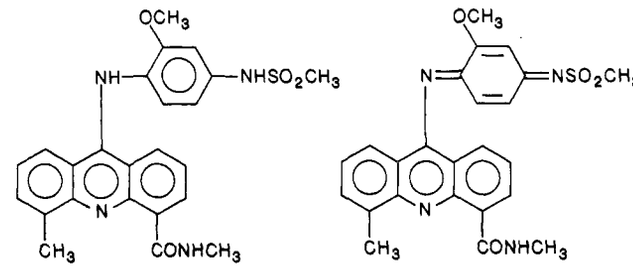
Finally, the redox properties of a number of acridine-substituted derivatives of amsacrine were examined (Table III). Peak separations of approximately 30 mV at low scan rates indicate a two-electron oxidation process (presumably to the quinone diimines) in all cases. A feature of the results is the very small effects the acridine substituents have on the redox potentials of the anilino ring system. All substituents lower $E_{1/2}$ from that of the parent compound, but only by a few tens of millivolts, even for the most powerful electron-withdrawing groups, and no clear pattern is seen (Table III). Thus the 3-$NHCH_3$ and 3-NO_2 compounds **28** and **30** have very similar redox potentials. This is in contrast to substituents in the anilino ring, where the effects of substituents at both the 1′- and 3′-positions can be related to their electronic properties by a single equation:

$$E_{1/2} = 233\ (\pm 41)\sigma_p[3'] + 115\ (\pm 41)\sigma_p[1'] + 347 \quad (6)$$

$$n = 18,\ r = 0.83,\ s = 51$$

and suggests there is little transmission of electronic effects from the acridine to anilino rings.

One acridine disubstituted compound, the 4-methyl-5-methyl carboxamide derivative **39**, was also studied. This compound (CI-921) has been selected as a "second-generation" amsacrine analogue due to its broad spectrum of activity in solid tumor systems[7] and has recently entered clinical trials. Cyclic voltammetry of **39** at low scan rates showed the usual chemically reversible redox couple, with an $E_{1/2}$ of 240 mV (Figure 7). Although this is a lower $E_{1/2}$ than for any of the monosubstituted acridine derivatives (a ΔE of −40 mV over amsacrine), it is not as low as that predicted by addition of the individual effects for the methyl and methyl carboxamide substituents, confirming that there is no simple relationship between $E_{1/2}$ and



39 (c_I-921) **54**

acridine ring substituents. A peak separation of 35 mV was consistent with two-electron oxidation, and this was confirmed by coulometry. Final confirmation of the participation of the quinone diimine **54** was provided by its chemical synthesis and demonstration of an identical cyclic voltammogram.

Relationship of Redox Potentials to Biological Activity

The broad class of 9-anilinoacridine antitumor agents is remarkable for the wide range of structural variations that can be tolerated while retaining biological activity.[1] However, a common feature of the most active compounds is a 1′-NHR substituent, with the consequent capacity for facile and chemically reversible oxidation to a quinone diimine. Detailed studies of the mode of action of amsacrine have concluded that the drug binds to DNA and then interferes with the action of the replication enzyme topoisomerase II, resulting in both poisoning of the enzyme and double-stranded DNA breaks.[10,11] Although the drug acts only when bound to DNA, the structure of the anilino side chain is clearly important because the isomeric compound *o*-AMSA (**55**) has little enzyme-inhibiting activity[10] and is biologically inactive, in spite of tight DNA binding and a similar redox potential (288 mV). Amsacrine and derivatives can thus be considered as possessing two distinct domains that are both essential for high antitumor activity: the acridine DNA-binding domain and the aniline protein-binding domain. For acridine-substituted amsacrine analogues, where the structure of the anilino ring is held constant and acridine substituents have little effect on redox potentials, biological activity does correlate well with DNA binding. Thus, QSAR studies with acridine-substituted amsacrine derivatives have been quite successful in correlating measures of biological potency (both in vitro IC_{50} values[25] and in vivo D40 values[26]) with DNA binding strength, and it seems firmly established that the influence of acridine substituents on biological potency is mediated through their effects on DNA binding levels.

For variations in the anilino ring, structure–activity relationships are more complex due to the ready transmission of electronic effects from the anilino to the acridine rings.[27] While the electronic properties of the 1′-substituents in the compounds of Table I influence redox potentials (eq 3), they also affect the pK_a values of the acridine ring and through this the proportions of ionized species at physiological pH. For the compounds of Table I, there is no significant correlation between redox potentials and either in vitro IC_{50} values against L1210 leu-

(24) Denny, W. A.; Atwell, G. J.; Baguley, B. C. *J. Med. Chem.* **1983**, *26*, 1625.
(25) Baguley, B. C.; Cain, B. F. *Mol. Pharmacol.* **1982**, *22*, 486.
(26) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 520.
(27) Denny, W. A ; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1978**, *21*, 5.
(28) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

kemia or drug potency in vivo.

Conclusions

The most active subgroup of the very large family of 9-anilinoacridine antitumor agents are those bearing 1'-NHR substituents. These compounds are capable of facile oxidation to reactive quinone diimines. The measured redox potentials (150–350 mV approximately) are sufficiently low to ensure ready oxidation in vivo, and therefore the consequence of this chemistry on the antitumor activity of these compounds has to be considered. Although there are no convincing quantitative correlations between biological activity and ease of oxidation, that is the general trend observed. For the amsacrine analogues of Tables II and III, which might be expected to act similarly to amsacrine itself, it is tempting to conclude that there are two necessary conditions for biological activity: high levels of DNA binding influenced primarily by acridine ring substituents (but see compound **25**) and a sufficiently low anilino ring redox potential to allow ready quinone diimine formation. The surprising inactivity of analogues such as **23**, with small and only mildly electron-withdrawing groups at the 3'-position (σ_p of F is 0.06), could then be attributed to their relatively high redox potentials (above 330 mV) and consequent slow rate of oxidation to some active form. However, related compounds such as the 1'-$NHCOOCH_3$ derivative **12** show moderate in vivo activity in spite of a much higher redox potential (404 mV). Additionally, the inactivity of *o*-AMSA (**55**) in spite of DNA binding levels and a redox potential similar to that of amsacrine argue for a more subtle structural requirement. While the redox chemistry of 9-anilinoacridines will have to be considered in studies of the metabolism of future clinical examples of this class (such as CI-921; **39**), the importance of its role in the mode of action of such compounds remains uncertain.

Experimental Section

Electrochemistry. Cyclic voltammetry was carried out on a Princeton Applied Research M173 instrument (voltage capability ±100 V, accuracy ±1 mV). Solutions of the samples were prepared at 0.1 mM in 40% acetonitrile/acetate buffer at pH 4.5. Approximately 30 mL of solution was placed in the cell and purged for 5 min with oxygen-free nitrogen before running the cyclic voltammogram. Each sample was evaluated twice at each of a series of scan rates from 200 to 20 mV/s, with the glassy carbon electrode being repolished between each different scan rate. For coulometry, a Princeton Applied Research M179 plug-in unit was used to provide current integration. Samples for coulometry were made up at 0.1 mM in 10% acetonitrile/acetate buffer at pH 4.5 and purged with oxygen-free nitrogen as above.

Chemistry. Where analyses are indicated by the symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus with the manufacturer's stem-corrected thermometer and are as read.

***N*-9-Acridinyl-1,4-benzoquinone Imine (42).** A solution of 4-(9-acridinylamino)phenol (**5**) (1 g, 3.5 mmol) in dry EtOAc (300 mL) was treated at 20 °C with activated MnO_2 (5g), and the mixture was stirred vigorously for 30 min. Filtration gave a purple-black solution, which was evaporated to dryness at 30 °C under vacuum. Recrystallization of the black residue from EtOAc/Et_2O by vapor diffusion gave the quinone imine as violet-black plates (0.95 g, 95%), mp 215–217 °C. Anal. ($C_{19}H_{12}N_2O$) C, H, N.

4-(9-Acridinylamino)-3-methoxyphenol (48). Sulfanilic acid (19 g, 11 mmol) was dissolved in water (400 mL) containing NaOH (4.4 g 11 mmol). A solution of $NaNO_2$ (7.5 g, 11 mmol) in water was added slowly at 10 °C, followed by a mixture of concentrated HCl and water (1:1, 60 mL). The resulting suspension was added slowly to a solution of resorcinol monomethyl ether (12.4 g, 10 mmol) in excess dilute NaOH, the pH was adjusted to 8, and the mixture was kept at 20 °C for 1 h. The pH was then adjusted to 2 with concentrated HCl, and the orange-red precipitate was collected and washed well with water. Crystallization from water gave 4-[*N*-(4-hydroxy-2-methoxyphenyl)azo]benzenesulfonic acid as glittering red-bronze platelets (21 g, 68%), mp >350 °C. Anal. ($C_{13}H_{12}N_2O_5S\cdot H_2O$) C, H, N.

The above azo compound (2 g, 6.5 mmol) was dissolved in water (100 mL) containing $NaHCO_3$ (0.6 g, 7 mmol) and hydrogenated over Pd/C until the color was completely discharged. The resulting unstable green-yellow precipitate of 4-amino-3-methoxyphenol was collected and washed with water. While still damp, the precipitate was added to a solution of 9-chloroacridine (0.95 equiv) in MeOH, a drop of HCl was added, and the mixture was heated briefly to reflux to complete the coupling reaction. The mixture was filtered hot to remove the hydrogenation catalyst, and the filtrate was diluted with EtOAc to give the crude hydrochloride to **48**. Recrystallization from MeOH/EtOAc gave bronze crystals (1.8 g, 78%), mp 285–288 °C. Anal. ($C_{20}H_{16}N_2O_2\cdot HCl$) C, H, N, Cl.

The above hydrochloride (1 g) was dissolved in 50% aqueous EtOH (200 mL) and treated with $NaHCO_3$ to precipitate the free base. This was dried, dissolved in EtOAc (300 mL), and treated with MnO_2 as above. Recrystallization of the resulting product from EtOAc/Et_2O gave N^4-9-acridinyl-3-methoxy-1,4-benzoquinone imine (**49**) as black crystals (0.83 g, 93% overall), mp 256–259 °C. Anal. ($C_{20}H_{14}N_2O_2$) C, H, N.

***N*$^{1'}$-(Methylsulfonyl)-*N*$^{4'}$-9-acridinyl-3'-(dimethylamino)-2',5'-cyclohexadiene-1',4'-diimine (50).** A solution of the free base of **21** (1 g, 2.46 mmol) was suspended in dry EtOAc (150 mL) and treated with activated MnO_2 (4 g) as above. The purple solution was filtered through Celite and evaporated to dryness at low temperature, and the residue was crystallized from EtOAc/Et_2O by vapor diffusion to give the quinone diimine **50** as black plates (0.92 g, 92%), mp 260–280 °C dec. Anal. ($C_{22}H_{20}N_4O_2S$) C, H, N.

Similar oxidation of the free base of the 3'-$NHCH_3$ derivative **20** gave a purple solution containing several products of varying polarity by TLC, and no pure compound corresponding to the quinone diimine **52** could be isolated.

***N*$^{1'}$-(Methylsulfonyl)-*N*$^{4'}$-[4-(methylcarbamoyl)-5-methyl-9-acridinyl]-3'-methoxy-2',5'-cyclohexadiene-1',4'-diimine (54).** The free base of CI-921 (**39**) was similarly oxidized by MnO_2, in this case with Me_2CO as solvent. The crude residue from the reaction was crystallized from $CHCl_3$/hexane by vapor diffusion to give the quinone diimine **54** in good yield, mp 225–228 °C. Anal. ($C_{24}H_{22}N_4O_4S$) C, H, N, S.

Acknowledgment. We thank Dr. G. Bowmaker for his advice on cyclic voltammetry. This work was supported by the Auckland Division of the Cancer Society of New Zealand, the Medical Research Council of New Zealand, and, in part, a fellowship provided by the Warner-Lambert Co. A.L. thanks the Auckland Medical Research Foundation for a summer research student grant.

Registry No. 1, 3340-22-5; 2, 73655-57-9; 3, 61421-82-7; 4, 61462-75-7; 5, 61421-83-8; 6, 58658-11-0; 7, 75776-00-0; 8, 75775-99-4; 9, 13365-38-3; 10, 64990-15-4; 11, 76015-18-4; 12, 64990-16-5; 13, 82720-32-9; 14, 53478-38-9; 14a, 64894-82-2; 15, 57164-59-7; 16, 57164-91-7; 17, 64894-86-6; 18, 51264-14-3; 19, 61417-10-5; 20, 88412-78-6; 21, 88412-94-6; 22, 57164-89-3; 23, 61417-09-2; 24, 72738-96-6; 25, 86955-71-7; 26, 76708-43-5; 27, 79453-36-4; 28, 66147-74-8; 29, 76708-33-3; 30, 64895-35-8; 31, 53478-40-3; 32, 79453-41-1; 33, 57164-79-1; 34, 89267-50-5; 35, 51963-57-6; 36, 76708-48-0; 37, 79453-51-3; 38, 76708-55-9; 39, 80841-47-0; 40, 87764-57-6; 41, 87764-58-7; 42, 106063-36-9; 43, 106063-37-0; 44, 106063-38-1; 45, 106063-39-2; 46, 106063-40-5; 47, 106063-41-6; 48, 106063-42-7; 48·HCl, 90625-54-0; 49, 90625-53-9; 50, 106063-43-8; 51, 106063-44-9; 52, 106063-45-0; 53, 106063-46-1; 54, 106063-47-2; *p*-$HO_3SC_6H_4NH_2$, 121-57-3; resorcinol monomethyl ether, 150-19-6; 4-[*N*-(4-hydroxy-2-methoxyphenylazo]benzenesulfonic acid, 106063-48-3; 4-amino-3-methoxyphenol, 61638-01-5; 9-chloroacridine, 1207-69-8.