Potential Antitumor Agents. 53. Synthesis, DNA Binding Properties, and Biological Activity of Perimidines Designed as "Minimal" DNA-Intercalating Agents

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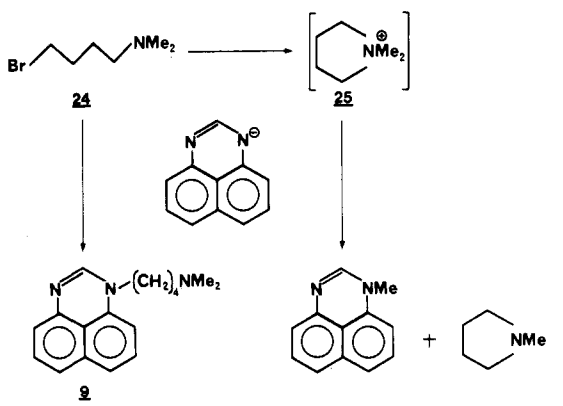
A series of compounds based on perimidine have been synthesized and evaluated for their DNA-binding properties and antitumor activity. The fused tricyclic perimidine chromophore appears to be the minimal structural requirement for intercalative binding to DNA since the mode of binding could be dictated by the position of attachment of the side chain. The intercalating compounds have DNA association constants (log K = 5.8–6.5) and cytotoxic potencies (IC_{50} = 500–1500 nM) comparable to those shown by other classes of linear, tricyclic DNA-intercalating antitumor agents (acridinecarboxamides, phenazinecarboxamides), but none of the compounds showed in vivo activity.

A small number of DNA-binding ligands (e.g. Adriamycin, actinomycin, and more recently amsacrine and mitoxantrone) have assumed an important role in cancer chemotherapy. A large number of studies on the structure–activity relationships of these compounds have demonstrated the requirement for intercalative binding to DNA for biological activity[1,2] and shown positive correlations between cytotoxic potency and both strength of binding[3] and the kinetics of binding.[4] As a consequence, considerable effort has been devoted to designing compounds where these aspects of DNA interaction are maximized. A great deal of recent work has focused on new classes of linear chromophores bearing either one or two cationic side chains.[5-8] These side chains not only increase the strength of binding by simple electrostatic effects but also have important effects on binding kinetics.[9] In order to ensure both intercalative binding and high binding constants, the chromophores have generally been tricyclic or tetracyclic moieties.

Although maximizing the cytotoxicity and maximizing in vivo potency of antitumor drugs are important aims, they are not the main design goal. The imperative need is for compounds with broad-spectrum activity, particularly against remotely sited solid tumors. This requires the optimization of the distributive properties of the drugs also, in order that they can penetrate to remote tumor sites in sufficient concentration to be effective. For DNA-intercalating agents, compounds that will distribute most efficiently are liable to be those with the lowest DNA association constants, since a higher proportion of unbound drug will be available at equilibrium. One way of lowering DNA binding strength is to reduce the size of the chromophore, consonant with retaining intercalative binding.

The naphthalimide 1 is one of the few examples of a condensed chromophore with only two aromatic rings that has been shown[10] to be a DNA-intercalating antitumor agent. In this paper we report the synthesis, DNA-binding properties, and biological activities of molecules of similar topology to 1, based on the perimidine skeleton. Perimidine is a condensed three-ring system with unusual electronic properties, being one of the few azines where the lone pair of a pyrrole-like nitrogen atom participates in the π system of the molecule. The charge-distribution pattern shows a transfer of electron density from the heterocyclic ring to the naphthalene moiety. Thus perimidine shows simultaneously the characteristics of a π-deficient and a π-excessive system, and it was of interest to explore the suitability of some derivatives of this heterocycle as potential DNA-intercalating ligands and antitumor drugs. While perimidine itself (4) and a series of 2-phenylperimidines (e.g. 5) have been reported to have activity against mouse adenocarcinoma AC-755 in vivo,[11-13] this tricyclic system has not been investigated as a possible DNA-intercalating chromophore. *N,N*-Diethylperimidine-1-propanamine (6) and related piperidino and morpholino derivatives have been shown to have tuberculostatic and fungistatic effects,[14] but have not been investigated for antitumor activity. In the present study a total of 16 compounds (7–22), including examples of five

Scheme I



† Department of Chemistry.
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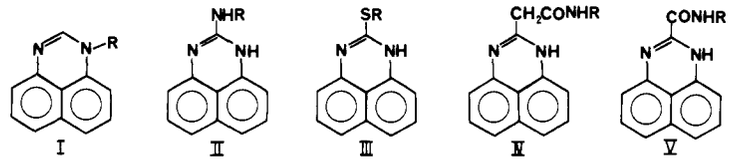
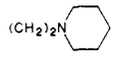
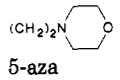
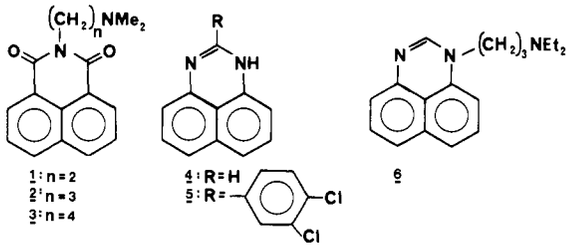
Table I. Physicochemical and Biological Properties of Naphthalimides 1–3 and Perimidines 7–22



					DNA binding		biological activity		
					log K^d			P388	
no.	class	R	Rm[a]	pK_a[b]	ϕ[c]	AT	GC	IC_{50}[e]	OD[f]	ILS[g]
1		$(CH_2)_2NMe_2$	−0.44		13	5.46	5.87	1.6	150	31
2		$(CH_2)_3NMe_2$	−0.17							
3		$(CH_2)_4NMe_2$	−0.13					1.9		
7	I	$(CH_2)_2NMe_2$	−0.72	4.64	0	5.17	5.31	20	150	NA[h]
8	I	$(CH_2)_3NMe_2$	−0.71	5.10				15.3	150	NA
9	I	$(CH_2)_4NMe_2$	0.73	5.30				2.2		
10	II	$(CH_2)_2NMe_2$	−0.42	ca. 6[i]	10	5.77	5.90	2.5	65	NA
11	II	$(CH_2)_3NMe_2$	0.34					4.0		
12	III	$(CH_2)_2NMe_2$	−0.03	2.66	11	5.78	6.10	1.8	150	NA
13	III	$(CH_2)_3NMe_2$	−0.07					5.7		
14	IV	$(CH_2)_2NMe_2$	−0.58	*j*	14	5.74	5.88	16.7	65	NA
15	IV	$(CH_2)_3NMe_2$	−0.75					>21		
16	V	$(CH_2)_2NMe_2$	−0.17	1.74	10	6.51	6.51	0.44	150	NA
17	V	$(CH_2)_3NMe_2$	−0.11					0.87		
18	V	$(CH_2)_4NMe_2$	−0.12					1.5		
19	V	$(CH_2)_2NEt_2$	0.04					1.1		
20	V		0.10					1.3		
21	V		−0.18					14.1		
22	V	5-aza	−1.53			5.71	5.88	21.7	65	NA

[a] Rm values determined by liquid–liquid chromatography as detailed in ref 33 with 4′-(9-acridinylamino)methanesulfonanilide (AMSA) as internal standard. [b] pK_a values were determined spectrophotometrically in aqueous solution (see ref 33). [c] ϕ = unwinding angle (in degrees) for closed circular supercoiled DNA from *E. coli* plasmid pNZ 116, relative to ethidium bromide as 26°, measured as described in ref 28. [d] K = binding constant to poly[d(A-T)] and poly[d(G-C)], determined by ethidium bromide displacement (see ref 25). [e] IC_{50} = concentration of drug (in μM) to inhibit growth of murine leukemia L1210 cells in culture by 50%, following a 40-h exposure. See ref 25. [f] OD = optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol–water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukemia cells. [g] ILS = percentage increase in lifespan of drug-treated tumor-bearing animal compared to that of untreated tumor-bearing controls when treated at the optimal dose. Values above 20% are considered statistically significant. [h] Compound ianctive at all dose levels up to toxic ones. [i] pK_a approximate due to compound insolubility. [j] pK_a unable to be determined; see text.

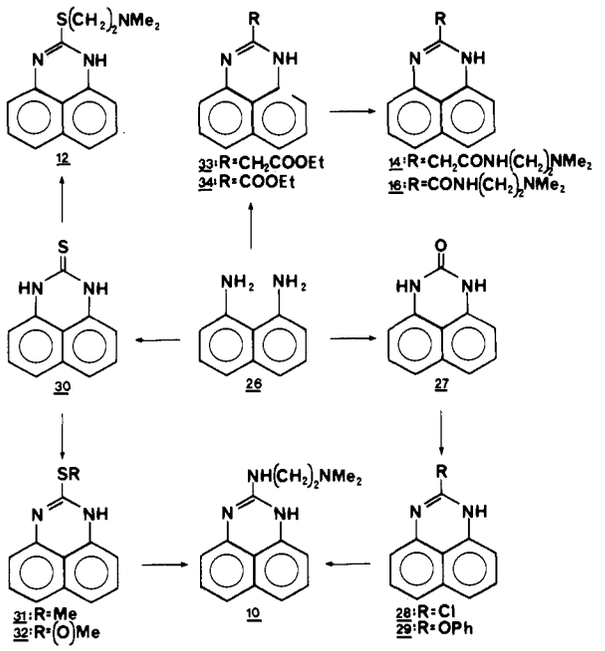
classes of 1- and 2-substituted perimidines, were prepared and investigated (Table I).



Chemistry

In general, the perimidine derivatives were difficult to work with. The π-excessive nature of the naphthalene ring made the compounds susceptible to aerial oxidation, and the yields in many reactions were low. N-Alkylation of perimidine with (dimethylamino)alkyl halides is best carried out in a mixture of toluene and chlorobenzene containing powdered KOH. This method has been used previously[15] for the synthesis of *N,N*-dimethylperimidine-1-propanamine (**8**) and provided the compounds (**7–9**) of class I in 30–50% yields. When (4-bromobutyl)dimethylammonium bromide (**24**) was employed in this reaction to prepare **9**, a significant quantity (12%) of 1-methylperimidine (**23**) was also isolated. This presumably arose by attack of the perimidinyl anion on 1,1-dimethyltetrahydropyrrolium bromide (**25**), which is known[16] to be formed readily from **24** (Scheme I). A

Scheme II



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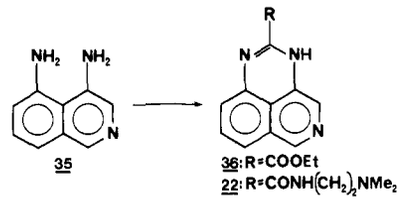
Scheme III



similar cyclization appears to be possible with **9** itself, which decomposes on heating above its melting point to give perimidine.

The compounds **10** and **11** of class II were obtained as shown in Scheme II from naphthalene-1,8-diamine (**26**). This was reacted[17] with formic acid to give 2-perimidinone (**27**). Treatment of this urea with $POCl_3$ gave 2-chloroperimidine (**28**) in variable yield, and this was reacted with phenol to give the intermediate 2-phenoxyperimidine (**29**) and then with the appropriate (dimethylamino)alkylamine. Since this sequence also suffered from unpredictable yields, an alternative synthesis was developed via 2-(methylsulfinyl)perimidine (**32**), which was expected to be as reactive as 2-chloroperimidine toward nucleophilic substitution (Scheme II) by analogy with the corresponding pyrimidines.[18] Reaction of naphthalene-1,8-diamine (**26**) with CS_2 gave perimidine-2-thione (**30**) in good yield, and this was alkylated with methyl iodide in refluxing MeOH and the reaction mixture basified to give 2-(methylthio)-perimidine (**31**). Oxidation of the sulfide **31** with 3-chloroperbenzoic acid gave a moderate yield of 2-(methylsulfinyl)perimidine (**32**), which reacted reproducibly with *N,N*-dimethylalkylamines to give the compounds of class II.

The thio derivatives of class III were also prepared from perimidine-2-thione (**30**), which could be readily alkylated with (dimethylamino)alkyl halides to give the desired products (**12** and **13**) (Scheme II).

The acetamide derivatives **14** and **15** of class IV were synthesized in good yield by reaction of ethyl perimidine-2-acetate[17] (**33**) with the desired (dimethylamino)-alkylamines (Scheme II); the carboxamides **16–21** of class V were prepared similarly with ethyl perimidine-2-carboxylate[19] (**34**). The pyridoquinazoline (**22**) was also made by the latter route from the 1,8-diamine **35** (Scheme III), but the yield of product was lower, due to the increased tendency of the electron-deficient azaperimidine system to undergo hydrolysis and decarboxylation.

Results and Discussion

Physicochemical and biological data for [(dialkylamino)alkyl]perimidines of the five different classes are recorded in Table I. Compounds **7–9** have the cationic side chain attached at the 1-position (class I), whereas classes II–V have the side chain attached at the 2-position through different linking functions. Comparative data for three [(dialkylamino)alkyl]naphthalimides (**1–3**) has also been included.

Physicochemical Properties. The pK_a values for the perimidine chromophore of the dimethylaminoethyl derivatives in each series were measured spectrophotometrically in water at 25 °C, as previously described.[20] The value of 4.64 obtained for the N-substituted compound **7** compares with values of 5.8–5.9 recorded for simple *N*-alkylperimidines.[21] The lower value is due primarily to the proximity of the side-chain cationic center, and as this is moved further away by extending the side chain (compounds **8** and **9**), the pK_a values show a trend toward those for the simple alkyl derivatives (Table I).

As expected, compound **10** of class II shows a higher pK_a, since the NH link group forms a cyclic guanidine system. The pK_a of the acetamide derivative **14** was not able to be determined by UV due to lack of an isosbestic point (possibly because of the formation of additional cyclic H-bonded structures). When the side chain is attached via a sulfide as in compound **12**, the pK_a drops to 2.66, and the carboxamide derivative **16** has an even lower perimidine pK_a of 1.74. However, even compounds **7** and **10** will exist primarily as monocations at physiological pH.

The relative lipophilicity of the compounds was determined as previously[22] by liquid–liquid chromatography in the presence of 0.3% methanesulfonic acid, where all the compounds will run as dications. The *N*-alkyl derivatives **7–9** proved considerably more lipophilic than the other classes, but the values were of the same order as those found for other DNA-intercalating antitumor drugs and are in the permissible range.

The strength of DNA binding of the perimidines was estimated as before[23] by displacement of the fluorophore ethidium bromide. Association constants were determined for the binding to the synthetic homopolymers poly[d(A-T)] and poly[d(G-C)], assuming competitive binding.[24] The log *K* values of ca. 5.0–5.5 found for the perimidines are lower than those measured for analogous tricyclic derivatives, but comparable to those of the naphthalimides **1–3** (Table I). The highest binding constant was shown by the carboxamide derivative **16**, but making this compound even more electron-deficient by introduction of a 5-aza atom to give **22** greatly lowered DNA binding. None of the compounds showed significant base-sequence selectivity.

The mode of binding was determined by measuring the ability (compared to ethidium bromide) of the compounds to unwind and rewind closed circular supercoiled DNA (*E. coli* plasmid pNZ 116), by using viscometric techniques described previously.[1] The naphthalimide derivative **1** had an unwinding angle of 11° (Figure 1), and since this series of compounds has been independently shown to intercalate DNA,[10] the behavior of the perimidines was compared to this. Derivatives of classes II–V, where the side chain is attached to the 2-position, clearly intercalate, with unwinding angles close to that of the naphthalimide (Figure 1). However, the *N*-alkyl compound **7** did not rewind the supercoils (as evidenced by a drop in reduced viscosity at higher D/P ratios), showing only a slow increase in reduced viscosity consistent with nonintercalative binding (Figure 1).

Biological Activity. In vitro cytotoxicity was determined against exponentially growing L1210 leukemia cells in culture by using published protocols.[25] The cells were exposed to drug for 70 h in microtitre trays, and the micromolar drug concentration (IC_{50}) to reduce cell growth to 50% of that of control cultures was calculated. As

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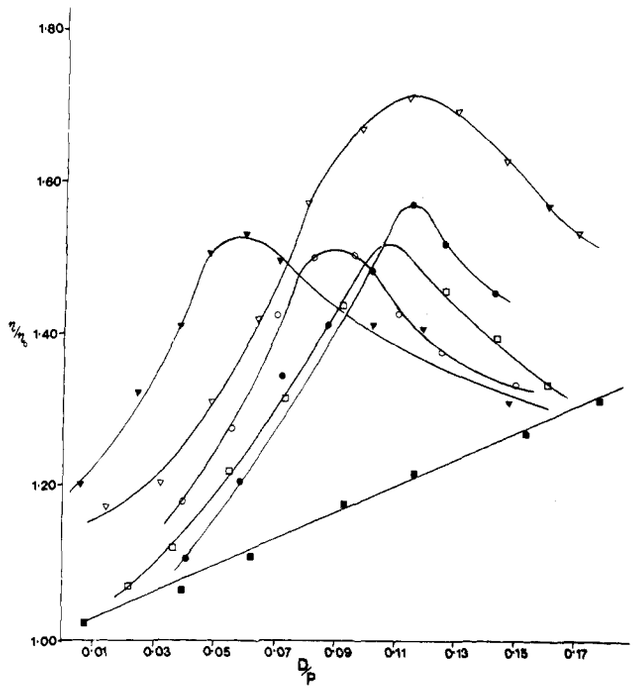
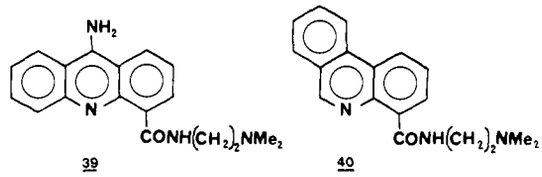


Figure 1. The effect of selected compounds of Table I on the reduced viscosity of covalently closed circular DNA. The ordinate represents the reduced viscosity in deciliters/gram, and the abscissa shows the molar binding ratio (drug molecules per phosphate). Unwinding angles are relative to ethidium, which has an equivalence binding ratio under these conditions of 0.036 (data not shown) and an unwinding angle of 26°. (○) compound 1, naphthalimide; (■) compound 7, class I; (▽) compound 10, class II; (□) compound 12, class III; (▼) compound 14, class IV; (●) compound 16, class V.

expected for nonintercalating agents, the *N*-alkylperimidines **7–9** showed very low cytotoxicity, with IC_{50} values in the 10–20 μM range. The amine- and sulfide-linked compounds of classes II and III were more potent, with cytotoxicities in the 2–6 μM range. However, the carboxamides of class V were the most cytotoxic compounds, with IC_{50} values from 0.4 to 1.5 μM, superior to those of the naphthalimides **1–3** (Table I).

Previous studies with various tricyclic DNA-binding compounds have shown that IC_{50} values broadly correlate with DNA association constants, both within and between different series. Thus the 9-aminoacridine-4-carboxamides such as **39** that bind tightly have high cytotoxicity (0.015 μM),[7] whereas the phenanthridine-4-carboxamide (**40**), whose binding to DNA is weaker by a factor of 50, has an IC_{50} of 2.1 μM, yet shows broad-spectrum in vivo activity.[26] On the basis of this criterion, the relatively low IC_{50} values of the weakly binding perimidinecarboxamides of class V were encouraging, and the loss in potency of the aza derivative **22** was disappointing.



39 40

Preliminary in vivo activity was measured for the (dimethylamino)ethyl derivatives of each class of perimidine in mice inoculated intraperitoneally with 10^6 P388 leukemia cells, in a standard protocol.[27] Drug was given by intraperitoneal injection of solutions on days 1, 5, and 9 after tumor cell inoculation, with doses spaced at 1.5-fold intervals from inactive to acutely toxic. The control naphthalimide compound (**1**) showed low but reproducible in vivo activity (ILS of 31%) at a rather high optimal dose of 150 mg/kg. However, none of the parent perimidines of classes I–V, nor the aza derivative **22**, showed in vivo activity against the leukemia and proved relatively nontoxic with optimal doses of 150 mg/kg. Thus testing against solid tumors was not carried out.

Conclusions

The perimidine compounds discussed here fulfill many of the criteria for which they were designed. The fact that the mode of DNA binding can be dictated by the position of the side chain on the chromophore suggests that the fused, tricyclic perimidine system approaches the minimal structural requirement for intercalative binding. The compounds also show desirably low levels of binding, with the (dimethylamino)ethyl derivatives (**10**, **12**, and **14**) of three out of the four classes of intercalating agents have log *K* values below 5.80, significantly lower than the binding constants of similar compounds in the acridine-,[28] phenazine-,[8] and phenanthridinecarboxamide[26] series of solid tumor active compounds. As expected, the nonintercalating 1-substituted compounds **7–9** of class I showed very weak cytotoxicity. Among the 2-substituted compounds, cytotoxicity appeared to be related to the electron-withdrawing properties of the side-chain linker group, which in turn is reflected by the perimidine pK_a. Thus the relatively high pK_a compounds of classes II and IV are the least cytotoxic, whereas the very weakly basic 2-carboxamides of class V are the most potent, with IC_{50} values (500–1500 nM) well within the levels observed for active compounds in the acridine,[28] phenazine,[7] and phenanthridine[26] series. However, attempts to provide an even more electron deficient system by using the azaperimidine (pyridoquinazoline) chromophore led to an enormous loss in cytotoxic potency.

Despite the favorable set of physicochemical properties of the perimidines generally and the high cytotoxicities of the 2-carboxamides **16–20** of class V, none of the compounds showed in vivo antileukemic activity. An important factor here may be very rapid in vivo metabolism of the compounds that, as noted above, showed considerable sensitivity toward oxidative degradation. However, this result serves to emphasize the difficulties of designing DNA-affinic compounds as antitumor drugs. The declared target of such compounds (DNA) is almost certainly the correct one, and its well-known architecture makes it possible to design for a desired set of ligand–DNA interactions, as we have done. Until the steps by which such ligand–DNA interactions lead to cell death are elucidated at the molecular level, there can be no certainty that any particular DNA-affinic compound will be an effective antitumor drug.

Experimental Section

Elemental analyses were carried out in the Microchemical Laboratory, University of Otago. Where analyses are indicated by the symbols of the elements, results obtained were within ±0.4% of theoretical values. Melting points were determined on a Reichert-Kofler block and are uncorrected. High-resolution mass spectra were recorded on an AEI MS-30 spectrometer at nominal

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Table II. Analytical Data for the New Compounds of Table I

no.	mp, °C	formula	anal.
7	137–138.5	$C_{15}H_{17}N_3 \cdot 2CF_3COOH$	C, H, N, F
8	245–246	$C_{16}H_{19}N_3 \cdot 2HCl$	mp 244–245[a]
9	152.5–155	$C_{17}H_{21}N_3 \cdot 2HCl$	HRMS[b]
10	269–270	$C_{15}H_{18}N_4 \cdot 2HCl$	HRMS
11	261 dec	$C_{16}H_{20}N_4 \cdot 2HCl$	HRMS
12	218.5–219.5	$C_{15}H_{17}N_3S \cdot 2HCl$	HRMS
13	212–213	$C_{16}H_{19}N_3S \cdot 2HCl$	HRMS
14	177–180	$C_{17}H_{20}N_4O$	C, H, N
15	240–244	$C_{18}H_{22}N_4O \cdot HCl \cdot H_2O$	C, H, N, Cl
16	179.5–181.5 (2HCl, 259–261)	$C_{16}H_{18}N_4O$	C, H, N
17	167.5–168.5 (2HCl, 228–238)	$C_{17}H_{20}N_4O$	C, H, N
18	170–171 (2 HCl, 224–235)	$C_{18}H_{22}N_4O$	C, H, N
19	141.5–142.5 (2HCl, 243–245)	$C_{18}H_{22}N_4O$	C, H, N
20	200.5–202 (2HCl, 243–245)	$C_{19}H_{22}N_4O \cdot H_2O$	C, H, N
21	185.5–188.5 (2HCl, 248–251)	$C_{18}H_{20}N_4O_2$	C, H, N
22	198–200	$C_{15}H_{17}N_5O \cdot 2HCl \cdot H_2O$	C, H, N

[a] Reference 15. [b] Satisfactory high-resolution mass spectral analyses were obtained for the free bases of the compounds.

3000 resolution. NMR spectra were recorded with a Varian Associates T60 spectrometer and are reported as chemical shifts in ppm downfield from Me_4Si.

Perimidine (4). This was prepared according to Sachs.[17] A solution of naphthalene-1,8-diamine (**26**) (11.4 g, 70 mmol) and formic acid (16 mL, 0.4 mol) in EtOH (30 mL) was heated under reflux in an atmosphere of N_2 for 40 min and then diluted with water. The mixture was basified with 2 N NH_4OH, and the precipitate of perimidine was collected and dried (11.5 g, 95%). Recrystallization from aqueous EtOH gave yellow crystals, mp 225–228 °C (lit.[17] mp 222 °C).

***N,N*-Dimethylperimidine-1-ethanamine (7).** Powdered KOH (1.9 g, 34 mmol) was added to a suspension of perimidine (**4**) (1.58 g, 10 mmol) and (2-chloroethyl)dimethylammonium chloride (2.62 g, 20 mmol) in toluene–chlorobenzene (38 mL:12 mL) under N_2. The mixture was heated under reflux for 4 h, cooled, and poured into water (40 mL). The organic layer was immediately separated, and the aqueous phase was extracted rapidly with $CHCl_3$. The combined organic fractions were dried and evaporated, and the residue was chromatographed on alumina to give *N,N*-dimethylperimidine-1-ethanamine (**7**) as a yellow oil (0.76 g, 32%): bp 160 °C (1 mmHg); IR ν_{max} (neat) 2950, 1615, 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24 (s, 3 H, NMe_2), 2.47 (t, J = 7 Hz, 2 H, H2′), 3.45 (t, J = 7 Hz, 2 H, H1′), 6.04 (t, J = 4.5 Hz, 1 H, H9), 6.75 (dd, J_o = 6.5 Hz, J_m = 3 Hz, 1 H, H4), 6.98–7.34 (m, 5 H, H2, H6, H7, H8); ^{13}C NMR δ 45.6 ($N(CH_3)_2$), 46.9 (C2′), 55.1 (C1′), 100.3 (C9), 115.0 (C4), 119.3 (C7), 120.3 (C6), 123.0 (C9b), 127.3 (C8), 128.7 (C5), 135.5 (C6a), 137.3 (C9a), 143.2 (C3a), 148.6 (C2); MS, m/z 239 (M, 12), 182 (M – C_3H_7N, 21), 168 (M – C_4H_9N, 11), 167 (M – $C_4H_{10}N$, 16), 140 (167 – HCN, 15), 58 (C_3H_8N, 100). The ditrifluoroacetate salt crystallized from EtOAc–heptane as yellow needles, mp 137–138.5 °C. Anal. (Table II).

Compound **8** was prepared similarly, with (3-chloropropyl)-dimethylammonium chloride, as a yellow-green oil (49%): IR ν_{max} (neat) 1630, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45–2.03 (m, 2 H, H2′), 2.23 (s, 6 H, NMe_2), 2.33 (t, J = 7 Hz, 2 H, H3′), 3.60 (t, J = 6.5 Hz, 2 H, H1′), 6.20 (dd, J_o = 8.5 Hz, J_m = 4.5 Hz, 1 H, H9), 6.80 (dd, J_o = 6 Hz, J_m = 3 Hz, 1 H, H4), 7.08–7.33 (m, 5 H, H2, H5, H6, H7, H8); MS, m/z 253 (M, 12), 182 (M – C_4H_9N, 21), 167 (M – $C_5H_{12}N$, 16), 140 (167 – HCN, 15), 58 (C_3H_8N, 100). The dihydrochloride salt crystallized from EtOH as yellow needles, mp 245–246 °C (lit.[15] mp 244–245 °C).

Compound **9** was prepared from (4-bromobutyl)dimethylammonium bromide (**24**), which was synthesized by the method of ref 16. The product was chromatographed on silica gel to give (i) 1-methylperimidine (**23**) (12% yield) as yellow crystals [mp (heptane) 119.5–120.5 °C (lit.[29] mp 120–121 °C); ^1H NMR ($CDCl_3$) δ 3.07 (s, 3 H, Me), 6.00 (t, J = 4 Hz, 1 H, H9), 6.74 (dd, J_o = 6 Hz, J_m = 5 Hz, 1 H, H4), 6.98–7.25 (m, 4 H, H5, H6, H7, H8)] and (ii) *N,N*-dimethylperimidine-1-butanamine (29% yield) as a yellow oil [bp 86 °C (0.03 mmHg); ^1H NMR ($CDCl_3$) δ 1.47–1.93 (m, 4 H, H2′, H3′), 2.15–2.50 (m, 8 H, H4′, NMe_2 (the latter a singlet at 2.23)), 3.38 (t, J = 8 Hz, 2 H, H1′), 6.14 (t, J = 4.5 Hz, 1 H, H9), 6.80 (dd, J_o = 6.5 Hz, J_m = 2.5 Hz, 1 H, H4), 6.94–7.40 (m, 5 H, H2, H5, H6, H7, H8); MS, m/z 267 (M, 11), 100 ($C_6H_{14}N$, 100), 58 (C_3H_8N, 61). The dihydrochloride crystallized from EtOAc–hexane as yellow needles, mp 152.5–155 °C, and then resolidified to needles melting at ca. 220 °C (cf. mp of perimidine, 225–228 °C).

2-Perimidinone (27). A solution of sodium cyanate (6.5 g, 0.1 mol) was added to a solution of naphthalene-1,8-diamine (15.8 g, 0.1 mol) in hot dilute HCl (300 mL). After cooling, the white precipitate was collected and washed with water to give 2-perimidine (**27**) (16.7 g, 91%), mp 304–305 °C (lit.[17] mp 304–305 °C).

2-Chloroperimidine (28). A solution of 2-perimidinone (**23**) (16.75 g, 83 mmol) in $POCl_3$ (100 mL) was heated under reflux for 3 h. Excess reagent was removed under reduced pressure, and the residue was dissolved in water and basified with 2 N NH_4OH to precipitate crude 2-chloroperimidine (16.4 g, 98%). Crystallization from Me_2CO gave yellow needles: mp 202–203.5 °C (lit.[30] mp 194 °C); ^1H NMR (CD_3SOCD_3) δ 6.51 (dd, J_o = 5.5 Hz, J_m = 3.5 Hz, 2 H, H4, H9), 7.02–7.42 (m, 4 H, H5, H6, H7, H8).

2-[[2-(Dimethylamino)ethyl]amino]perimidine (10). A solution of 2-chloroperimidine (202 mg, 1 mmol) in phenol (4 g) was heated to 110 °C for 10 min and then cooled to 50 °C. 2-(Dimethylamino)ethylamine (90 mg, 1 mmol) was added, and the mixture was heated at 110 °C for a further 30 min. After cooling, the mixture was partitioned between CH_2Cl_2 and 2 N aqueous NaOH. The organic layer was washed with water and evaporated to give crude **10** (200 g, 83%). Crystallization from MeOH–EtOAc–HCl gave the dihydrochloride salt as green-yellow needles: mp 269–270 °C; IR ν_{max} 3200, 2950, 2750, 2600, 2475, 1670, 1590 cm^{-1}; ^1H NMR (D_2O) δ 3.02 (s, 6 H, NMe_2), 3.30–3.83 (m, 4 H, H1′, H2′), 6.35 (dd, J_o = 5.5 Hz, J_m = 3 Hz, 2 H, H4, H9), 6.94–7.32 (m, 4 H, H5, H6, H7, H8); MS, m/z 24H(M – 2HCl, 21), 196 (M – NMe_2, 31), 183 (M – C_4H_9N, 76). Anal. (Table II).

Perimidine-2-thione (30). Solid KOH (50 mg) was added to a solution of naphthalene-1,8-diamine (21 g, 0.125 mol) and CS_2 (9 mL, 0.15 mol) in 95% aqueous EtOH (100 mL). Precipitation of the product began almost immediately, and after 15 min the mixture was filtered to give perimidine-2-thione (**30**) (24.8 g, 99%) as gray leaflets, mp >300 °C (lit.[17] mp >300 °C).

2-(Methylthio)perimidine (31). A suspension of perimidine-2-thione (**30**) (0.7 g, 3.5 mmol) and MeI (0.44 mL, 7 mmol) in MeOH (25 mL) was heated under reflux with stirring for 2 h. After cooling, the mixture was filtered to give 2-(methylthio)-perimidinium iodide (1.09 g, 91%), as yellow crystals, mp >300 °C. (lit.[31] mp >300 °C). The free base crystallized from Me_2CO as yellow prisms: mp 199–200.5 °C; ^1H NMR (CD_3COCD_3) δ 2.50 (s, 3 H, Me), 6.47 (dd, J_o = 6 Hz, J_m = 3 Hz, 2 H, H4, H9), 6.90–7.27 (m, 4 H, H5, H6, H7, H8); MS, m/z 214 (M, 100), 168 (M – CH_2S, 60), 166 (M – MeSH, 42). Anal. ($C_{12}H_{10}N_2S$) C, H, N, S.

2-(Methylsulfinyl)perimidine (32). A solution of 3-chloroperbenzoic acid (446 mg, 2.2 mmol) in $CHCl_3$ (5 mL) was added over 5 min to a stirred suspension of 2-(methylthio)perimidine (**31**) (430 mg, 2 mmol) in $CHCl_3$ (10 mL). The mixture was stirred for 20 min at 20 °C, solvent was evaporated, and the residue was purified by PLC on silica gel in Me_2CO–hexane (1:2) to give 2-(methylsulfinyl)perimidine (**32**) (265 mg, 58%). This was sublimed (136 °C/0.15 mmHg) to give yellow needles: mp 181–182 °C; ^1H NMR ($CDCl_3$) δ 3.00 (s, 3 H, Me), 6.47–6.71 (m, 2 H, H4, H9), 6.98–7.35 (m, 4 H, H5, H6, H7, H8); MS, m/z 230 (M, 77). Anal. ($C_{12}H_{10}N_2OS$) C, H, N, S.

2-[[3-(Dimethylamino)propyl]amino]perimidine (11). A mixture of 2-(methylsulfinyl)perimidine (115 mg, 0.5 mmol) and excess 3-(dimethylamino)propan-1-amine was heated at 100 °C

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for 20 min. Excess reagent was removed under reduced pressure, and the residue was recrystallized from MeOH–EtOAc–HCl to give the dihydrochloride salt of 11 as a yellow-green solid (82 mg, 48%): mp 261 °C dec; IR ν_{max} 2650, 1670; ^{1}H NMR (D_2O) δ 1.91–2.30 (m, 2 H, H2′), 2.96 (s, 6 H, NMe_2), 3.10–3.45 (m, 4 H, H1′, H3′), 6.28–6.50 (m, 2 H, H4, H9), 7.08–7.32 (m, 4 H, H5, H6, H7, H8); MS, m/z 268 (M – 2HCl, 100), 223 (268 – $(CH_3)_2NH$, 79), 197 (268 – C_4H_9N, 74), 168 (268 – $C_5H_{12}N_2$, 57), 58 (C_3H_8N, 86). Anal. (Table II).

2-[[2-(Dimethylamino)ethyl]thio]perimidine (12). A solution of NaOH (0.52 g, 11 mmol) in water (5 mL) was added under N_2 to a stirred suspension of perimidine-2-thione (1.0 g, 5 mmol) and (2-chloroethyl)dimethylammonium chloride (0.79 g, 5.5 mmol) in EtOH (50 mL). The mixture was stirred under reflux for 30 min and then poured into water. The mixture was extracted with CH_2Cl_2 (4 × 30 mL), and the combined extracts were washed with water and evaporated. The residue was chromatographed on silica gel, with CH_2Cl_2–MeOH (95:5) eluting 2-[[2-(dimethylamino)ethyl]thio]perimidine as a yellow solid (0.56 g, 47%): mp 118–120 °C; IR ν_{max} 1590, 670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6 H, NMe_2), 2.57–3.12 (m, 4 H, H1′, H2′), 6.30 (dd, J_o = 6 Hz, J_m = 3 Hz, 2 H, H4, H9), 6.80–7.11 (m, 4 H, H5, H6, H7, H8); ^{13}C NMR ($CDCl_3$) δ 29.3 (C1′), 45.0 ($N(CH_3)_2$), 61.5 (C2′), 107.7 (C4, C9), 118.9 (C6, C7), 121.2 (C9b), 128.1 (C5, C8), 135.4 (C6a), 141.6 (C3a, C9a), 156.6 (C2); MS, m/z 271 (M, 19), 226 (M – Me_2NH, 23), 200 (M – C_4H_9N, 81), 181 (M – C_3H_8NS, 37), 72 ($C_4H_{10}N$, 100), 71 (C_4H_9N, 76), 58 (C_3H_8N, 93). The dihydrochloride salt crystallized from BuOH–MeOH as yellow needles, mp 218.5–219.5 °C. Anal. (Table II).

Ethyl Perimidine-2-acetate (33). A mixture of naphthalene-1,8-diamine (0.38 g, 2.4 mmol) and diethyl malonate (10 mL) was heated to reflux, when the diamine dissolved and a vigorous reaction ensued. After being heated for a further 1 min, the mixture was cooled to 20 °C and filtered to give bis(2-perimidinyl)methane (65 mg, 16%): mp >300 °C (lit.[17] mp >300 °C); MS, m/z 348 (M, 100). The filtrate was diluted with excess hexane and the resulting suspension was filtered to give ethyl perimidine-2-acetate (33) (0.6 g, 80%) as cream crystals: mp 152–153 °C (lit.[17] mp 152 °C); ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 3 H, Me), 3.45 (br s, 2 H, CH_2COO), 4.18 (q, J = 7 Hz, 2 H, OCH_2), 6.47 (dd, J_o = 5.5 Hz, J_m = 3 Hz, 2 H, H4, H9), 6.90–7.27 (m, 4 H, H5, H6, H7, H8), 8.08 (br s, 1 H, NH).

***N*-[2-(Dimethylamino)ethyl]perimidine-2-acetamide (14).** A mixture of ethyl perimidine-2-acetate (33) (125 mg, 0.49 mmol) and *N*,*N*-dimethylethylenediamine (60 mg, 0.6 mmol) was heated at 110 °C for 1 h. $CHCl_3$ (2 mL) was added to the cooled mixture, and the resulting suspension was filtered to give *N*-[2-(dimethylamino)ethyl]perimidine-2-acetamide (14) (71 mg, 49%); more product (68 mg, 96% total) was recovered by PLC of the filtrate residue. Crystallization from $CHCl_3$–heptane gave yellow needles: mp 177–180 °C; IR ν_{max} 3200, 1645, 1615, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 6 H, NMe_2), 2.42 (t, J = 6 Hz, 2 H, CH_2NMe_2), 3.20–3.52 (m, 4 H, $CH_2CONHCH_2$), ca. 6.2 (br s, 1 H, NH), 6.34–6.55 (m, 2 H, H4, H9), 6.90–7.15 (m, 4 H, H5, H6, H7, H8); ^{13}C NMR ($CDCl_3$) δ 36.8 (CH_2NMe_2), 41.8, 41.9 (CH_2CO), 44.8 ($N(CH_3)_2$), 57.3 ($CONHCH_2$), 108.0 (C4, C9), 119.3 (C6, C7), 121.7 (C9b), 128.2 (C5, C8), 135.1 (C6a), 140.3 (C3a, C9a), 152.0 (C2), 168.0 (C=O); MS, m/z 296 (M, 12), 208 (M – $C_4H_{12}N_2$, 20), 181 ($C_{12}H_9N_2$, 11), 58 (C_3H_8N, 100). Anal. (Table II).

Ethyl Perimidine-2-carboxylate (34). A mixture of naphthalene-1,8-diamine (0.9 g, 5.7 mmol) and diethyl oxalate (0.93 g, 6.8 mmol) was heated under argon at 135–140 °C for 90 min. Excess reagent was removed under reduced pressure to leave ethyl perimidine-2-carboxylate (34) as scarlet needles (1.37 g, 100%): mp 200.5–202.5 °C (lit.[19] mp 195 °C); ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7 Hz, 3 H, Me), 4.45 (q, J = 7 Hz, 2 H, CH_2), 6.44–6.76 (m, 2 H, H4, H9), 7.01–7.23 (m, 4 H, H5, H6, H7, H8).

***N*-[2-(Dimethylamino)ethyl]perimidine-2-carboxamide (16).** A mixture of ethyl perimidine-2-carboxylate (34) (120 mg, 0.5 mmol) and *N*,*N*-dimethylethylenediamine (53 mg, 0.6 mmol) was heated at 120–130 °C for 90 min. Excess amine was removed under reduced pressure, and the resulting red solid was purified by PLC. Sublimation (112 °C, 0.03 mmHg) gave *N*-[2-(dimethylamino)ethyl]perimidine-2-carboxamide (16) as orange needles (135 mg, 95%): mp 179.5–181.5 °C; IR ν_{max} 3250, 1685, 1630, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 6 H, NMe_2), 2.50 (t, J = 6 Hz, 2 H, H2′), 3.48 (q, J = 6 Hz, 2 H, H1′), 6.17–6.52 (m, 1 H, H9), 6.58–6.83 (m, 1 H, H4), 6.93–7.35 (m, 4 H, H5, H6, H7, H8); ^{13}C NMR ($CDCl_3$) δ 37.5 (C1′), 45.3 ($N(CH_3)_2$), 57.8 (C2′), 13.1 (C9), 115.5 (C4), 119.2 (C7), 121.5 (C6), 123.3 (C9b), 127.7 (C8), 128.8 (C5), 135.6 (C6a), 136.7 (C9a), 143.4 (C3a), 145.6 (C2), 160.0 (CONH); m/z 282 (M, 7), 211 (M – C_4H_9N, 4), 140 ($C_{10}H_6N$, 10), 58 (C_3H_8N, 100). Anal. (Table II). The dihydrochloride crystallized from MeOH–EtOAc as scarlet prisms, mp 259–261 °C. Compounds 17–21 of Table II were prepared similarly.

***N*-[2-(Dimethylamino)ethyl]pyrido[3,4,5-*de*]quinazoline-2-carboxamide (22).** Isoquinoline-4,5-diamine[32] (35) (0.61 g, 3.8 mmol) and diethyl oxalate (0.63 mL, 4.6 mmol) were heated together at 135 °C under N_2 for 60 min. The resulting brown solid was purified by chromatography on alumina, with $CHCl_3$ eluting ethyl pyrido[3,4,5-*de*]quinazoline-2-carboxylate (36) as a red solid (0.51 g, 56%). Crystallization from benzene gave needles: mp 196–198 °C; IR ν_{max} 3450, 1720, 1630, 1600 cm^{-1}; ^{1}H NMR (CD_3SOCD_3) δ 1.35 (t, J = 7 Hz, 3 H, Me), 4.37 (q, J = 7 Hz, 2 H, CH_2), 6.78–6.98 (m, 1 H, H9), 7.20–7.41 (m, 2 H, H7, H8), 7.82 (s, 1 H, H4), 8.53 (s, 1 H, H6); MS, m/z 241 (M, 33), 169 (M – $COOC_2H_4$, 49), 168 (M – $COOC_2H_5$, 21), 167 (M – $HCOOC_2H_5$, 100), 141 (167 – CN, 20), 140 (167 – HCN, 21). Anal. ($C_{13}H_{11}N_3O_2$) C, H, N. A mixture of the above compound (36) (58 mg, 0.24 mmol) and (dimethylamino)ethylenediamine (40 mg, 0.36 mmol) was heated for 90 min at 100 °C. Excess reagent was removed under reduced pressure, and the residue was purified by chromatography on silica gel to give *N*-[2-(dimethylamino)ethyl]-pyrido[3,4,5-*de*]quinazoline-2-carboxamide (22) (42 mg, 62%), which sublimed as orange needles, mp 217.5–219.5 °C. The dihydrochloride was crystallized from MeOH–EtOAc: mp 198–200 °C; IR ν_{max} 3200–2600, 1695, 1610 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.03 (s, 6 H, NMe_2), 3.46 (t, J = 5 Hz, 2 H, H2′), 3.80 (q, J = 5.5 Hz, 2 H, H1′), 7.30 (d, J_o = 5.5 Hz, J_m = 4 Hz, 1 H, H9), 7.58–7.79 (m, 3 H, H4, H7, H8), 8.80 (s, 1 H, H6); MS, m/z 283 (M, 4), 167 ($C_{10}H_5N_3$, 3), 141 (167 – CN, 4), 114 (141 – HCN, 3), 58 (C_3H_8N, 100). Anal. (Table II).

Acknowledgment. This work was supported by the Auckland Division of the Cancer Society of New Zealand (through a Warner-Lambert Fellowship to J.M.H.) by the Universities Research Committee and by the Medical Research Council of New Zealand. We thank Dr. B. C. Baguley for assistance with animal testing.

Registry No. 4, 204-02-4; 7, 110191-61-2; 7·2CF_3COOH, 110191-62-3; 7·HCl, 110191-63-4; 8, 59283-17-9; 8·2HCl, 27066-85-9; 9, 110191-64-5; 9·2HCl, 110191-65-6; 10, 110191-66-7; 10·2HCl, 110191-67-8; 11, 110191-68-9; 11·2HCl, 110191-69-0; 12, 110191-70-3; 12·2HCl, 110191-71-4; 13, 110191-72-5; 13·2HCl, 110191-73-6; 14, 110191-74-7; 15, 110191-75-8; 16, 110191-76-9; 16·2HCl, 110191-77-0; 17, 110191-78-1; 17·2HCl, 110191-79-2; 18, 110191-80-5; 18·2HCl, 110191-81-6; 19, 110191-82-7; 19·2HCl, 110191-83-8; 20, 110191-84-9; 20·2HCl, 110191-85-0; 21, 110191-86-1; 22, 110191-87-2; 26, 479-27-6; 27, 5157-11-9; 28, 30837-50-4; 30, 30837-62-8; 31, 92972-05-9; 31·HI, 89473-00-7; 32, 110191-88-3; 33, 43183-27-3; 34, 109735-80-0; 35, 110191-89-4; 36, 110191-90-7; (2-chloroethyl)dimethylammonium chloride, 4584-46-7; (3-chloropropyl)dimethylammonium chloride, 5407-04-5; (4-bromobutyl)dimethylammonium bromide, 17375-58-5; 2-(dimethylamino)ethylamine, 108-00-9; *N*,*N*-dimethylpropane-1,3-diamine, 109-55-7; diethyl malonate, 105-53-3; bis(2-perimidinyl)methane, 110191-91-8; diethyl oxalate, 95-92-1.

Supplementary Material Available: Details of IR and NMR spectra of all the compounds of Table I (13 pages). Ordering information is given on any current masthead page.

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