when omeprazole inhibits the H^+,K^+-ATPase enzyme. This formation of a disulfide is a fundamentally different mechanism of action of omeprazole than those previously proposed by Im et al.[10] and by Rackur et al.[11]

In vitro the enzyme–adduct complex **6** reacts with β-mercaptoethanol to form the sulfide **8** and the enzyme–β-mercaptoethanol complex enzyme–$SSCH_2CH_2OH$, in which form the enzyme is still blocked. This complex then slowly reacts with β-mercaptoethanol to the free enzyme enzyme–SH.[19] It is not clear whether, under in vivo conditions, glutathione or any other endogenous mercaptan can form a corresponding enzyme–mercaptan complex and furthermore react in a second step to generate the free enzyme and the disulfide of glutathione. Recovery of the enzyme activity might require synthesis of the enzyme de novo.

Since the sulfenamide **4** and the sulfenic acid **3** are permanent cations, neither possible active inhibitors can probably penetrate the secretory membrane of the parietal cell. This fact and maybe also the permanent-cation character of the enzyme–inhibitor complex **6** might be important factors for the in vivo inhibiting effect of omeprazole (**1**).

The sulfide **8** is formed from omeprazole in isolated gastric glands.[9] This is probably also the case in the gastric mucosa in vivo. We have also demonstrated that the sulfide **8** in vivo is oxidized to the sulfoxide omeprazole (**1**), thus closing the cyclic reaction process. However, in the in vivo situation, due to simultaneous metabolic processes, probably only a fraction of the total omeprazole dose may complete this cyclic process.

Factors contributing to the specificity of the inhibition of gastric acid secretion by omeprazole are as follows: (1) It is a well-known fact that weak bases concentrate in acidic compartments. Omeprazole is a weak base and therefore concentrates in acidic compartments.[9] (2) The parietal cell containing the enzyme H^+,K^+-ATPase is the only cell in the body with a low pH value. The low pH value causes the conversion of omeprazole into the active inhibitor close to the enzyme that produces the acid. (3) The active inhibitors, the sulfenamide **4**, and the sulfenic acid **3** are permanent cations with limited possibilities to penetrate membranes.

Registry No. **1**, 73590-58-6; **2**, 102283-09-0; 4·$AuCl_4^-$ (3,9-$(OMe)_2$), 102283-06-7; 4·$AuCl_4^-$ (3,10-$(OMe)_2$), 102283-08-9; **5**, 102283-11-4; **8**, 73590-85-9; **9**, 102260-42-4; **10**, 102260-44-6; HO-$(CH_2)_2SH$, 60-24-2.

Supplementary Material Available: X-ray data on compounds **9** and **10** (7 pages). Ordering information is given on any current masthead page.

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Articles

Streptonigrin. 1. Structure–Activity Relationships among Simple Bicyclic Analogues. Rate Dependence of DNA Degradation on Quinone Reduction Potential

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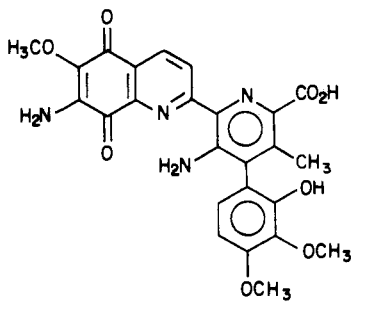
A series of simple aza and diaza bicyclic quinones related to the AB ring system of streptonigrin (**1**) have been synthesized and tested in vitro for their ability to degrade DNA under conditions similar to those used with the parent drug. The results obtained from a study of 22 quinones indicate that there is a quantitative linear relationship between their reduction potentials and the rate at which they degrade DNA under identical conditions in vitro. Almost all of the synthetic substances were superior to **1** in their DNA-degrading ability.

I. Introduction

Streptonigrin (**1**) is an antitumor agent that has seen only limited use as an anticancer agent because of its toxicity[1] but continues to receive attention because of interest in its ability, common to a number of quinone antibiotics, to degrade[2] DNA. This ability has been demonstrated both in vivo and in vitro. The drug is lethal to *Escherichia coli* and to human leucocytes and in both cases DNA degradation[3,4] is observed. These effects seem to be



1

(1) Teller, M. N.; Wagshul, S. F.; Wolley, G. W. *Antibiot. Chemother.* **1961**, *11*, 165. McBride, J. J.; Oleson, T. J.; Wolff, D. *Cancer Res.* **1966**, *26*, 727. Ebert, P. S.; Chirigos, M. A.; Ellsworth, P. A. *Ibid.* **1968**, *28*, 363. Kuang, D. T.; Whittington, R. M.; Spencer, H. H.; Patno, M. E. *Cancer* **1969**, *23*, 597.
(2) For a recent review, see: Lown, J. W. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; Chapter 9, pp 283–314.

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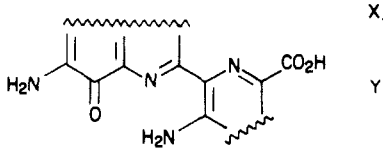
correlated and are associated with interference during the S phase of the cell growth cycle.[5] Single-strand cleavage of ccc-DNA (covalently closed circular DNA) in vitro has been demonstrated and the reaction, which was shown to be oxygen dependent, also requires the presence of a reducing agent.[6] The facts that the free-radical scavengers catalase and superoxide dismutase both inhibit the DNA degradation led Lown[6] to propose that free OH radicals, generated from reduced streptonigrin and oxygen, are the principal species that initiate attack on the DNA. The presence of divalent metals such as Cu^{2+} or Fe^{2+} accelerate[7] the degradation and appear to play an intrinsic role in the bactericidal action of the antibiotic.[8] Although the part played by the metal is not well-understood, it has been suggested[9] that it may aid both in the binding of the streptonigrin to DNA and its ensuing oxidation. The nature of the streptonigrin–metal–DNA complex is not known, but it does not appear to involve intercalation.[6]

II. Biological Rationale

Studies on variations in the structure of 1 and their influence on cytotoxicity or ability to degrade DNA in vivo are not extensive. Modifications of 1 by Rao[10] almost invariably led to compounds of lower activity in an increased life-span test,[11] although the methyl ester 1 and a ring-B tetrahydro derivative showed comparable activity. They proposed that the minimum structural requirements for biological activity are those embodied in partial structure 2. A series of simpler compounds, analogous to 1 in that only the AB ring structure is maintained (formula 3), have also been studied,[11] again using the increased life-span screening procedure as a measure of activity. Most of these substances had at least one amino or oxygen function as the X or Y substituent, but it is evident from the comparative test data[11] that attempts to approximate the antitumor activity of 1 have not been successful.



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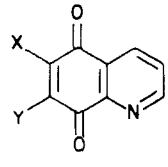

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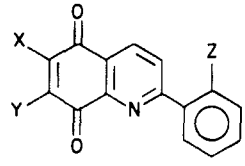

4

On the other hand, in a study of DNA degradation in vitro, Lown[7] has examined a series of synthetic analogues of 1, having the general structure 4. Most of these analogues proved to be more active than 1 in their ability to degrade DNA, the most active invariably having an NO_2 group as the Z substituent and electron-withdrawing groups as X and Y. Inspection of these results and knowing that the DNA-degradation reaction needs,[6] as an absolute requirement for success, the presence of a reducing agent led us to conclude that the reduction potential of the quinone (and the influence of the X, Y, and Z substituents on it) must play an important role in determining the activity of the drug.[12] It is well-established that the presence of electron-withdrawing groups increases the reduction potential of quinones, making their reduction easier, whereas electron-donating agents show an inverse effect. The inference is that, the higher the reduction potential of the quinone, the greater should be its ability (as reflected by a faster rate) to degrade DNA in the test system. Although it might appear more logical to test this idea using solely derivatives of streptonigrin (1), there are no general chemical methods available at present that would allow the substituents of the quinone ring of 1 to be varied selectively. In addition, the number of known derivatives of 1 is small. Thus, we elected to synthesize a series of aza and diaza bicyclic quinones related to 3 and 4 and ostensibly also to 1 and measure their activity in a DNA-degradation assay and subsequently their reduction potentials. The compounds that we have synthesized are assembled in Table II (section IV). The methodology employed together with limited proofs of structure is described in section III below. Our intention in pursuing this line of investigation is to attempt to understand the mechanism by which 1 cleaves DNA and perhaps throw some light on its antitumor activity should these properties prove to be connected. An ultimate objective would be the design of useful drug less toxic than I.

III. Chemical Syntheses

Because a number of the quinoline compounds to be used in the biological studies discussed in section IV were known to the literature,[7] these are dealt with first. The syntheses of the other classes of compounds are presented thereafter generally in the order that they appear in Table II. In each class the key compound is the dichloroquinone since quinones with other substituents are derived from them. Thus much of the reasearch was devoted to obtaining these particular quinones. Even so, low yields in some cases limited subsequent work.

In the case of the napthaquinone class, 7 was prepared exactly according to the literature[13] as was 6.[14] The 2-chloro-3-hydroxy derivative 5 was obtained from 6 according to Conant and Fieser,[15] and an improved method is recorded in this paper.

A. 2-(2-Nitrophenyl)quinoline-5,8-diones. The key compound in this series, namely 18, had been synthesized previously by Lown.[7] Although we could not improve on the Skraup synthesis of 28 (Scheme I), we were able to convert the latter to 18 simply and easily in 75% yield by oxidative chlorination by the use of $HCl/NaClO_3$. Demethylation of 28 followed by chloroxidation of the intermediate hydroquinone according to the published procedure[7] is not only complicated experimentally but

(3) White, H. L.; White, J. R. *Mol. Pharmacol.* **1968**, *4*, 549. Levine, M.; Borthwick, M. *Virology* **1962**, *21*, 568.
(4) Mizuno, N. S. *Biochim. Biophys. Acta* **1965**, *108*, 394.
(5) Mizuno, N. S.; Gulboe D. P. *Biochim. Biophys. Acta* **1970**, *224*, 319.
(6) Cone, R.; Hasan, S. K.; Lown, J. W.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 219.
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(9) White, J. R. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 387. Sinha, B. K. *Chem. Biol. Interact.* **1981**, *36*, 179.
(10) Rao, K. V. *Cancer Chemother. Rep.* **1974**, *4*(4), 11.
(11) Driscoll, J. S.; Hazard, G. F.; Wood, H. B.; Goldin, A. *Cancer Chemother. Rep.* **1974**, *4*(1), 1.
(12) In the case of the saframycins A and C it has been suggested also that the difference in rates of cleavage of DNA by these antibiotics may be due to the difference in their reduction potentials. However, the idea was not explored further: Lown, J. W.; Joshua, A. V.; Lee, J. S. *Biochemistry* **1982**, *21*, 419.
(13) Reynolds, G. A.; Van Allan, J. A. *J. Org. Chem.* **1964**, *29*, 3591.
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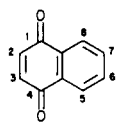
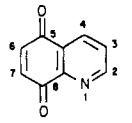
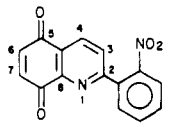
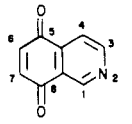
(15) Conant, J. B.; Fieser, L. F. *J. Am. Chem. Soc.* **1924**, *46*, 1858.

Table I. Degradation of DNA by Streptonigrin (SN)[a]

expt no.	SN	NADH	Cu^{2+}	Co^{2+}	other reductants	EDTA	sodium benzoate	CPM[c]
1	+	+	+	−	−	−	−	10000
2	+	+	−	−	−	−	−	2344
3	+	−	+	−	−	−	−	100
4	−	+	+	−	−	−	−	100
5	+	+	−	+	−	−	−	100
6	+	−	+	−	+ (abcd or e)	−	−	100
7	+	+	+	−	−	+	−	100
8	+	+	+	−	−	−	+	100

[a] The standard reaction mixtures were incubated with different components. Signs + and − indicate the presence and absence of that component in the assay. After 18 h the reaction mixtures were processed as described in the assay procedure. The final concentrations of the additional components were Co^{2+} = 100 μM, other reductants = 15 mM, EDTA = 2 mM, sodium benzoate = 50 mM. [b] (a) $NaBH_4$, (b) $Na_2S_2O_4$, (c) Na_2SO_3, (d) $Na_2S_2O_3$, or (e) $NaHSO_3$. [c] CPM = counts per minute (total CPM = 10000).

Table II

no.	compound	$E_{1/2}$	CPM[a,c]	% cleavage[b]	general formula
	naphthalene-1,4-diones				
5	2-chloro-3-hydroxy-	−0.652			
6	2,3-dichloro-	−0.604			
7	2-cyano-3-hydroxy-	−0.586			
1	streptonigrin	−0.560	517	1.0	
	quinoline-5,8-diones				
8	6-hydroxy-7-chloro-	−0.552	1573	3.1	
9	6-chloro-7-hydroxy-	−0.550	1900	3.8	
10	2-methyl-6,7-dichloro-	−0.524	6262	12.5	
11	6,7-dichloro-	−0.510	8072	16.1	
12	6,7-dichloro-,N-oxide-	−0.490	12894	25.8	
13	6-hydroxy-7-cyano-	−0.480	13033	26.0	
14	6-nitro-7-hydroxy-	−0.474	8976	18.0	
15	6-hydroxy-7-nitro-	−0.470	9440	18.8	
	2-(o-nitrophenyl)quinoline-5,8-diones				
16	6-hydroxy-7-chloro-	−0.502	9484	19.0	
17	6-chloro-7-hydroxy-	−0.500	10000	20.0	
18	6,7-dichloro-	−0.456	14999	30.0	
19	6-hydroxy-7-cyano-	−0.432	20000	40.0	
20	6-nitro-7-hydroxy-	−0.426	18100	36.2	
21	6-hydroxy-7-nitro-	−0.424	18376	36.7	
	isoquinoline-5,8-diones				
22	6-chloro-7-hydroxy-	−0.464	14002	28.0	
23	6,7-dichloro-	−0.418	21213	42.4	
24	6-cyano-7-hydroxy-	−0.392	25270	50.5	
	diazanaphthalene-5,8-diones				
25	6,7-dichloroquinoxaline-5,8-dione	−0.404	25150	50.3	see Table III for formulas
26	6,7-dichloroquinazoline-5,8-dione	−0.348	32922	66.0	
27	6,7-dichlorophthalazine-5,8-dione	−0.232	48076	98.0	

[a] Total CPM = 50000. [b] CPM/total CPM × 10^2. [c] The incubations were carried out as described in the assay procedure. Radioactivity was measured by using 250 μL of supernatant rather than the 100 μL used in the case of streptonigrin.

results in markedly reduced yields of 18.

The conversion of 18 to 17 was accomplished by treatment with hot NaOH solution followed by fractional crystallization of the red sodium salts to effect separation of the isomers. Acidification then gave the two acids, mp 241–242 °C (16) and 234–236 °C (17). The usual physical data were not useful in deciding which isomer had which structure. The assignments that we have made basically rely on the relative reactivities expected for the chlorine atoms in 18. These might be expected to parallel those of 6,7-dichloroquinoline-5,8-dione (11), because of the similarity in structure. In the latter case Russian workers[16] found that strong nucleophiles substitute both chlorine atoms, but under mild conditions weak nucleophiles including very dilute aqueous hydroxide ion substitute only at position 6. Their theoretical calculations also indicated that the electron density at C-6 in 18 is slightly lower than

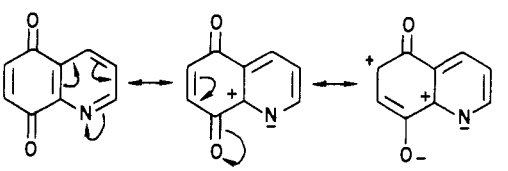


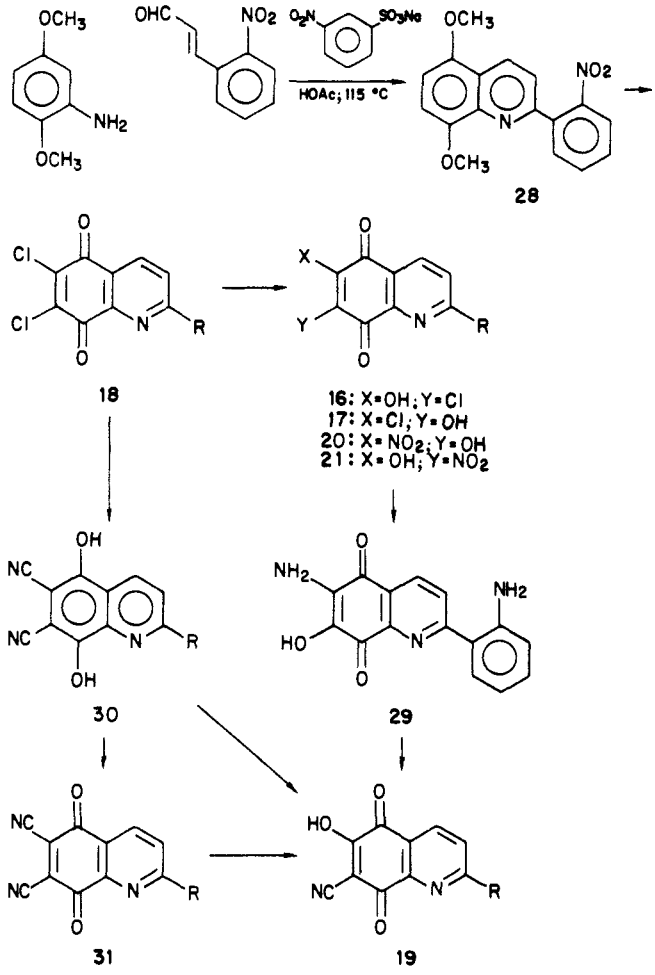
Figure 1.

at position 7. In examining the reactivity of 18, we found that whereas the yield ratio of 7:8 is 4:3 at 60 °C, this changes to 3:1 at room temperature. We therefore assign structure 8 to the isomer with mp 241–242 °C because it is produced in higher yield and structure 9 to the lower melting compound. Finally, although lacking refinement, resonance arguments[17] (Figure 1) in which the carbonyl groups of 18 are regarded as ortho and meta substituents of a pyridine ring favor substitution at C-6. The "ortho"

(16) Klimovich, O. S.; Kolesnikov, V. T.; Sazhnikov, V. A. *Zh. Prickl. Khim (Leningrad)* **1976**, *49*, 1823; *Chem. Abstr.* **1977**, *86*, 29288.

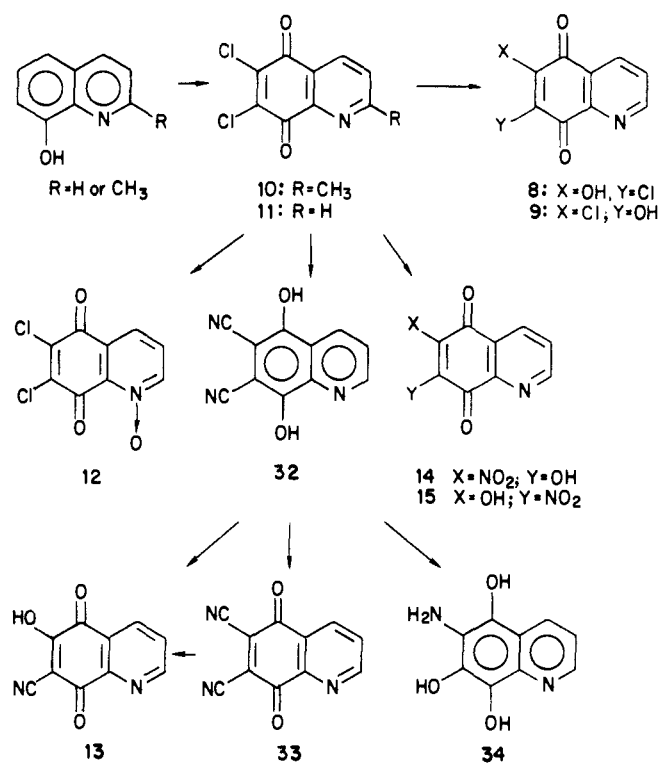
(17) Finley, K. T. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: New York, 1974; Chapter 17, Part 2, pp 909–910, 1077–1079.

Scheme I[a]



[a] R = o-$NO_2C_6H_4$.

Scheme II



carbonyl group should show the greater electron deficiency. This should be reflected in a faster addition–elimination reaction over the unsaturated carbonyl system represented by the assembly at positions 6, 7, and 8 as opposed to that at positions 7, 6, and 5. All other assignments of structure, to related substitution products in the quinoline and isoquinoline series, are based on similar arguments.

Treatment of **18** in boiling aqueous acetone with sodium nitrate led to a 2:1 mixture of the sodium salts of **20** and **21**, respectively. Fractional crystallization of these salts from aqueous methanol followed by acidification gave the free acids **20** and **21**. Because of lack of volatility in the mass spectrometer, one of these compounds (**20**) was characterized as its diamine **29** by reduction with sodium dithionite. When the nitro subsitution reaction was conducted at room temperature the ratio of **20**:**21** became 6.5:1. Thus structure **20** was assigned to the product obtained in higher yield. Reactivity arguments suggest that in this double substitution reaction replacement of chlorine by nitro precedes the substitution of chlorine by hydroxyl. The synthesis of **19** proceeded via the dinitrile **31**, which in turn was synthesized by an adaptation of the two-step method used by Reynolds and van Allen[15] for the preparation of 2,3-dicyanonaphthalene-1,4-dione. In our case it proved necessary to use a nonaqueous reaction medium, namely, THF/MeOH (1:1), in the preparation of **30**, because the presence of water led to severe decomposition. The nitric acid oxidation of **30** to **31** did not pose any problems except that **31** in common with dicyanodichloroquinone (DDQ) is light sensitive and must be stored in the dark. Treatment of **31** with sodium hydroxide solution led after acidification to solely **19** (84% yield), the structure assignment being based on previous argument. In neutral aqueous solution **31** slowly decomposes to **19**, thus precluding its use in the DNA-cleavage study.

B. Quinoline-5,8-diones. Previous methods[18-20] for the synthesis of the key intermediate in this series, namely, 6,7-dichloroquinoline-5,8-dione (**11**), involved multistep procedures. We have found that chloroxidation of 8-hydroxyquinoline allows its preparation in one step, and although the yield is not high (30%), its simplicity and the ease of product isolation made it the preferred method. The higher homologue **10** was prepared similarly in 13% yield from 2-methyl-8-hydroxyquinoline. Apart from the *N*-oxide **12**, the remaining compounds in this series namely, **8**, **9**, **13**, **14**, and **15** were prepared (Scheme II) by methods similar to those used in the 2-nitrophenyl series. Structures were assigned to these products by means of the arguments presented in section IIIA above. Interestingly, and in comparison with the Russian claims,[16] we found that treatment of **11** with hydroxide solution gives both **8** (46%) and **9** (37%) and not solely **8**. The latter, however, is the only product when the milder base NaOCN is used in aqueous methanol. The nitro isomers **14** and **15** were difficult to volatilize in the high-resolution mass spectrometer so one of them (**14**) was reduced to the trihydroxyamine **34**, which was more easily characterized by HRMS.

Brief treatment of **11** with a boiling solution of NaCN in THF/methanol afforded **32** in 82% yield and this on oxidation with nitric acid in acetic acid led directly to **13** in 30% yield. The dinitrile **33** could only be obtained (23%) if enough acetic anhydride were added to the oxidizing mixture to render it anhydrous. Treatment of **33**

(18) Babu, B. H.; Rao, N. V. S. *Symp. Synth. Heterocycl. Compd Physiol. Interest, Proc.* **1964**, 114; *Chem. Abstr.* **1968**, *69* 10339f.
(19) Babu, B. H.; Rao, N. V. S. *Proc. Indian Acad. Sci.* **1968**, *67A*, 31.
(20) Urbanski, T.; Krzyzanowski, S. *Rocz. Chem.* **1953**, *27*, 390.

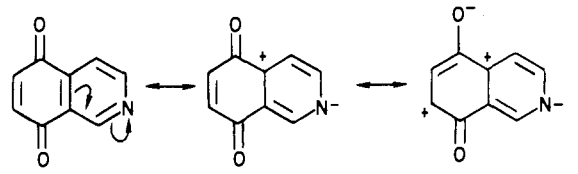
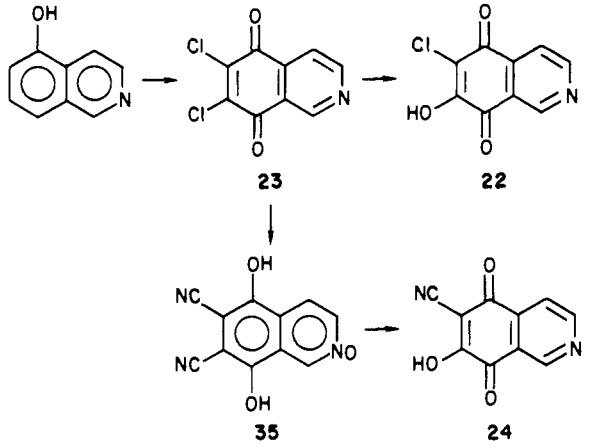


Figure 2.

Scheme III



with water, as expected, afforded **13**.

Finally, the *N*-oxide **12** could be prepared either by the treatment of commercially available 8-hydroxyquinoline *N*-oxide with aqua regia or by peracid (CF_3CO_3H) oxidation of **11**. Neither method gave better than 10% yield.

C. Isoquinoline-5,8-diones. In this series the desired key compound, 6,7-dichloroisoquinoline-5,8-dione (**23**), was unknown to the literature and in fact only one synthetic route to the parent isoquinoline-5,8-dione had been published.[21] Using the $NaClO_3$/HCl variation of the chloroxidation procedure, we were able to obtain only 3% of **23** from 5-hydroxyisoquinoline. However, this yield could be raised to 11% by substituting concentrated nitric acid in place of $NaClO_3$.

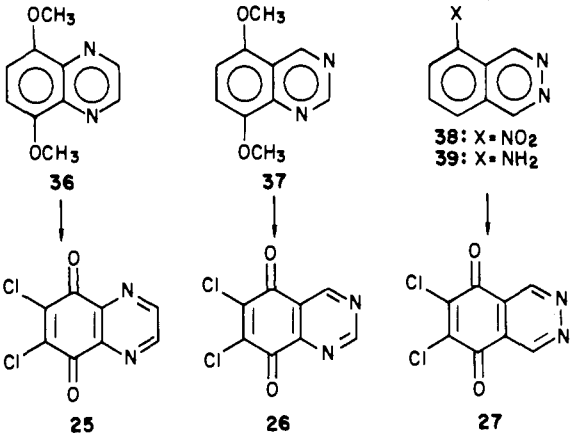
In the substitution reaction (Scheme III) of Cl by hydroxide ion in **23**, greater selectivity was observed than in the cases of **11** or **18**. Only a single compound was obtained (87%), to which we ascribe structure **22**. This again is based mainly on the speculation that the C-5 carbonyl group, which is para to the nitrogen, is more electron deficient than the C-8 carbonyl group (meta to nitrogen) as depicted in the resonance structures (Figure 2). Thus 1,4-addition might be expected to be dominated by the former carbonyl group.

In the synthesis of the cyano hydroxy compound **24**, fearing its likely water sensitivity, no attempt was made to obtain the intermediate 6,7-dicyanoquinone. Instead the product **35** of the action of sodium cyanide on **23** was oxidized with nitric acid in acetic acid and afforded **24** directly in 37% yield.

D. 6,7-Dichlorodiazanaphthalene-5,8-diones. The desired but previously unknown 6,7-dichloroquinone heterocycles **25–27** were all prepared by using chloroxidation reactions, in poor yields however.

In the case of **25** (Scheme IVa) the desired quinoxoline precursor **36** had been synthesized[22] previously. Improvements that were effected in this synthetic route are recorded in the Experimental Section. Unfortunately, the chloroxidation of **36** proceeded poorly, and although we

Scheme IV



were able to obtain **25**, the yield was only 1.1%.

Again in the case of **26** (Scheme IVb) the needed precursor, **37**, was known,[23] and as in the synthesis of **36** we were able to improve the overall methods for its preparation. These are detailed in the Experimental Section and are not discussed here. Although quinazoline **37** was not chlorinated by $NaClO_3$/HCl, it could be converted in 10% yield to **26** by means of aqua regia.

The synthesis of the phthalazine quinone **27** (Scheme IVc) began with phthalazine itself. The latter was prepared by an adaptation of the work of Reynolds[24] and of Carter.[25] Nitration of phthalazine according to Kanahara[26] gave **38**, which was then reduced to the amino compound **39** by sodium dithionite. By contrast with the oxidation of **37**, HCl/HNO_3 did not affect **39**. However, HCl/$NaClO_3$ cleanly converted the latter to the desired quinone **27** but in only 13% yield.

IV. Assay Procedure and Discussion of Results with Streptonigrin

Even before proceeding with the chemical synthesis work it was necessary to develop and optimize a satisfactory in vitro assay system to compare the DNA-degrading ability of the synthetic compounds with that of streptonigrin (**1**). The general procedure employed was patterned on a method developed[27] by one of us previously. The substrate in the assay was a mixture of nonradioactive calf thymus DNA and radioactive Hela cell DNA. At timed intervals, as described in detail in the Experimental Section, uncleaved DNA was precipitated and then separated by centrifugation. The level of radioactivity of the supernatant was measured and taken as a measure of the soluble cleaved DNA and was used as a direct measure of the ability of the test compound (standard assay) or of the system (variations in components) to degrade DNA. From the assays using streptonigrin as the active compound, it is possible to reach the following conclusion:

(1) As shown in Table I, the presence of NADH is essential to the reaction. In our hands, other reducing agents were ineffective although Lown has claimed[7] that $NaBH_4$ can replace NADH with equal success. Consistent with his work, Cu^{2+} has a powerful synergistic effect on the rate of strand scission whereas the DNA was completely pro-

(21) Joseph, P. K.; Joullié, M. M. *J. Med. Chem.* **1964**, *7*, 801.
(22) King, F. E.; Clark, N. G.; Davis, P. M. *J. Chem. Soc.* **1949**, 3012.
(23) Malesano, G.; Pietrogrande, A.; Rodighiero, G. *Farmaco Ed. Sci.* **1968**, *23*, 765.
(24) Reynolds, G. A.; Van Allan, J. A.; Tinker J. F. *J. Org. Chem.* **1959**, *24*, 1205.
(25) Carter, S. D.; Cheeseman, W. H. *Org. Prep. Proc. Int.* **1974**, *6*, 67.
(26) Kanahara, S. *Yakugaku Zasshi* **1964**, *84*, 433.
(27) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983.

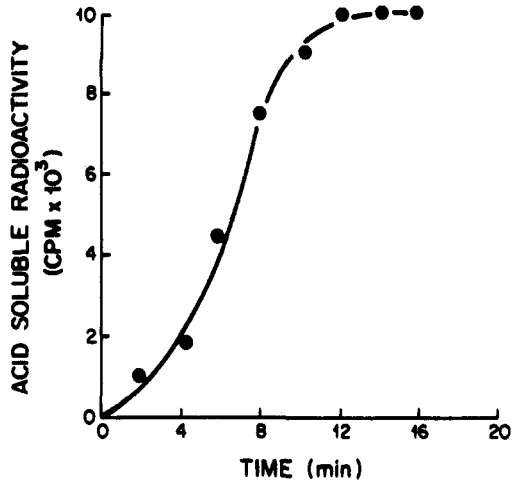


Figure 3. Time course of DNA degradation by streptonigrin. Triple the amount of the standard reaction mixture was incubated in this case, i.e., 300-μL total volume, was used. Aliquots (30 μL) were removed at intervals and processed as described in the assay section.

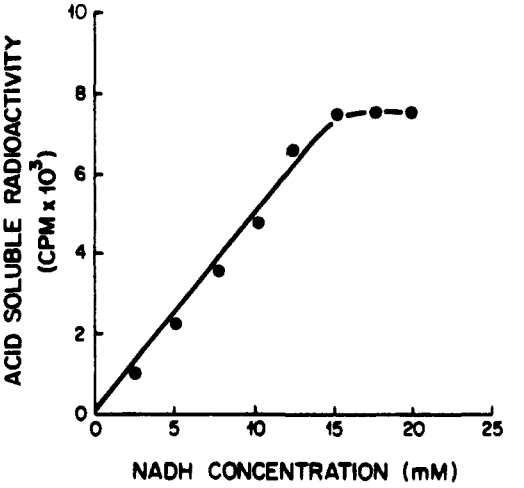


Figure 4. Effects of varying concentrations of NADH on the cleavage of DNA by streptonigrin. Standard reaction mixtures were incubated with different concentrations of NADH. After 8 h they were processed as described in thhe assay section.

tected by Co^{2+} and other free radical scavengers such as sodium benzoate or by chelating agents such as EDTA.

(2) The time course of DNA degradation (Figure 3) appears to be slightly sigmoidal with perhaps a small induction period. The general behavior however shows that the optimal time for DNA degradation by 1 under standard experimental conditions is 8 h.

(3) Figures 4–6 show the effect of varying the concentrations of NADH, Cu^{2+}, and streptonigrin, respectively, on strand scissions. It is quite evident that optimal concentrations of these components are 15 mM, 100 μM, and 200 μM, respectively, against a fixed concentration of DNA (200 μM).

(4) The effect of pH on the degradation of DNA is shown in Figure 7. Little degradation is seen below pH 6, but the level rises rapidly in the pH range 6–8, becoming optimal at pH 8.5. The lack of significant degradation below pH 6 almost certainly is due to the rapid decomposition of NADH in the acid medium.

V. Structure-Activity Relationships among the Synthetic Compounds

In examining the relationship of changes in structure on the redox potential of the quinone moiety and the consequences for ability to effect DNA strand cleavage, we began our studies with one of the most active analogues reported to date, namely, Lown's tricyclic compound (**4**, X = Y = Cl, Z = NO_2), followed by some of its derivatives.[7]

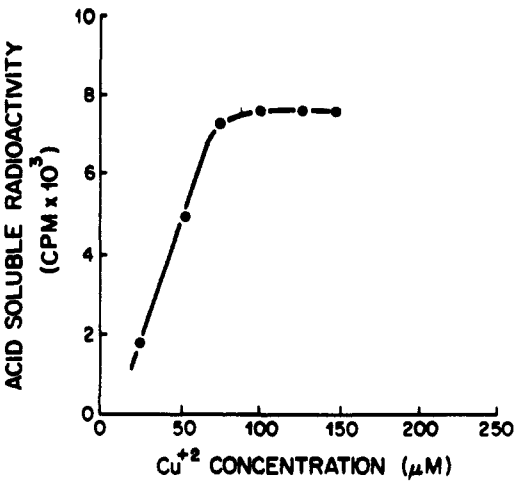


Figure 5. Effects of varying concentrations of Cu^{2+} on the degradation of DNA by streptonigrin. Standard reaction mixtures were incubated with different concentrations of Cu^{2+}. After 8 h they were processed as described in the assay section.

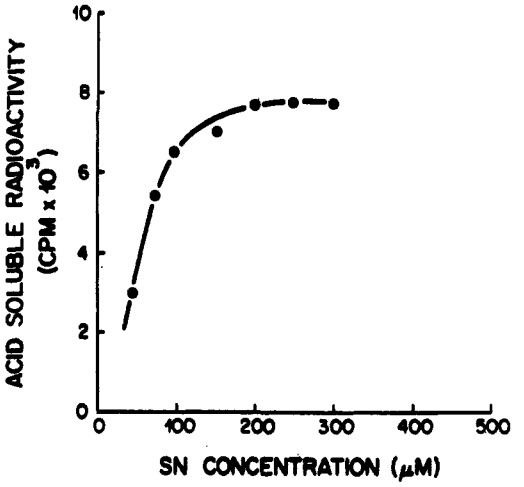


Figure 6. Effect of varying concentrations of streptonigrin on strand scissions of DNA. Standard reaction mixtures were incubated with different concentrations of SN and processed after 8 h as described in the assay section.

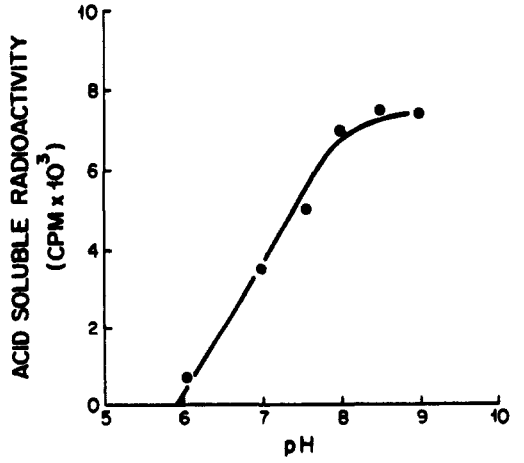
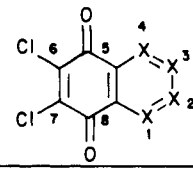


Figure 7. Effect of variations of pH on strand scission of DNA by streptonigrin. Standard reaction mixtures were incubated with phosphate buffers of different pH values. After 8 h they were processed as described in the assay section.

Table III



no.	X1	X2	X3	X4	E, V	% cleavage
6	CH	CH	CH	CH	−0.604	0
11	N	CH	CH	CH	−0.510	16
23	CH	N	CH	CH	−0.418	42
25	N	CH	CH	N	−0.404	51
26	N	CH	N	CH	−0.348	66
27	CH	N	N	CH	−0.232	98

Thereafter we investigated the contribution of the 2-nitrophenyl ring by examining 6,7-dichloro-5,8-quinolinedione (11) and a series of simple compounds (8–10 and 12–21) derived from it. This was followed by a study to determine the importance of the quinoline nitrogen atom and its position on the activity of interest. For this purpose several naphthaquinones (5–7) analogous to the simple quinolines above were synthesized. These however had no activity and suggested that the presence of nitrogen was important for activity. The isoquinolinediones (22–24) were therefore synthesized for comparison with the corresponding quinolines and these proved to have enhanced activity. This led to the synthesis of the diaza analogues (25–27) of 6 and these were found to be the most active compounds that we had so far examined. The relative ability of the foregoing compounds to cleave DNA under a standard set of conditions is shown in ascending order of activity both among and within the classes: naphthalenes < quinolines < isoquinolines < diazanaphthalenes.

Having, from the beginning, suspected that the reduction potential of the quinone moiety played a major role in controlling the rate of degradation of DNA, we submitted each of the compounds of Table II to cyclic voltammetry. This was carried out in the same 2% v/v DMF, phosphate buffer (pH 8.5) as was used in the DNA-degradation assay. As expected, the reductions were completely reversible and involved two electrons. Half-wave potentials ($E_{1/2}$) were measured with reference to a saturated calomel electrode and are reported in Table II. A plot of $E_{1/2}$ vs. percent degradation of DNA under standard conditions is shown in Figure 8. A least-squares analysis[28] of the 22 data points yields a straight-line relationship (eq 1) between the counts per minute (C) and the half-wave potential ($E_{1/2}$) in which a = 143.0 (deviation ± 5.0) and

$$C = aE_{1/2} + b \quad (1)$$

b = 80.83 (deviation ± 2.35). A more convenient form (eq 2) relates the cleavage fraction F to $E_{1/2}$, the deviations

$$F = 2.90E_{1/2} + 1.633 \quad (2)$$

of the a and b coefficients being ±0.10 and ±0.048, respectively. Calculation of the correlation coefficient[28] r gives a value of 0.991, indicating a confidence level of >99% in the linearity of the relationship. From this essentially linear relationship it is evident that the activity of any one compound is directly proportional to its ease of reduction. Within any one of the quinoline or isoquinoline classes, changing the quinone-ring substitution pattern from chloro hydroxy to dichloro and then to cyano hydroxy shows comparable changes in both the reduction potential (higher) and the rate of DNA degradation (greater). However, the nitro hydroxy compounds, although exhibiting parallel behavior, show slightly lower activity then might be expected on the basis of their reduction potential values.

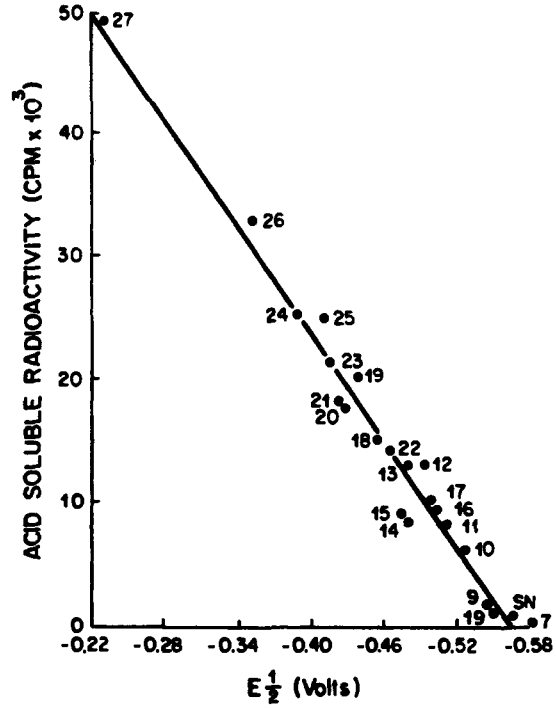


Figure 8. Reduction potentials and activities of SN and its analogues.

A second effect that can be observed is the pronounced impact of the presence and position of the heteroatom (N) in the molecule. This is more easily seen from Table III.

In comparing the lack of activity of the naphthalene derivatives with the other compounds, it is evident that either a nitrogen must be present or much more likely the reduction potential of the compound must be −0.56 (streptonigrin) or less negative in order to have DNA-degrading ability. The effect on both activity and reduction potential of changing the position of the nitrogen atom (11 vs. 23) is even more pronounced than the effect of changing the weak electron-withdrawing group chlorine for the more powerful electron-withdrawing groups nitro or cyano at position 7 (cf. 8 with 13 and 14, 16 with 19 and 21, and 22 with 24). The changes become even more dramatic with the double shift of nitrogen in going from the quinoxalinedione 25 to the phthalazinedione 26 but parallel nicely the changes in the mononitrogen series (18 vs. 23). The intermediate position of 26 with respect to 25 and 27 is logical on the basis of the position of quinoline 11 vs. isoquinoline 23.

(28) Perrin, C. L. *Mathematics for Chemists*; Wiley-Interscience: New York, 1970; Chapter 6, pp 161–162.

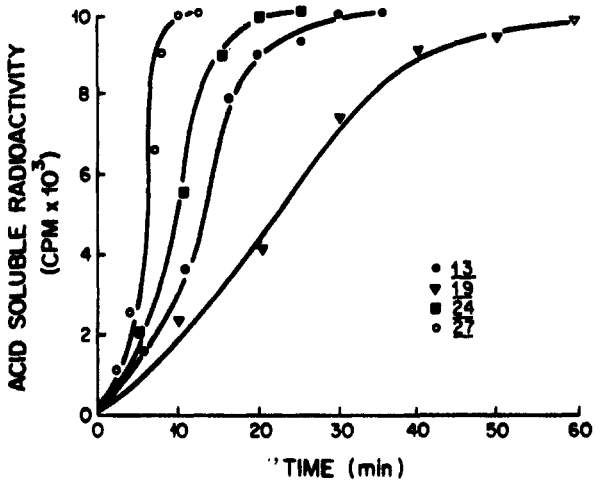


Figure 9. Time course of DNA degradation by 13, 19, 24, and 27. Conditions were the same as in Figure 1.

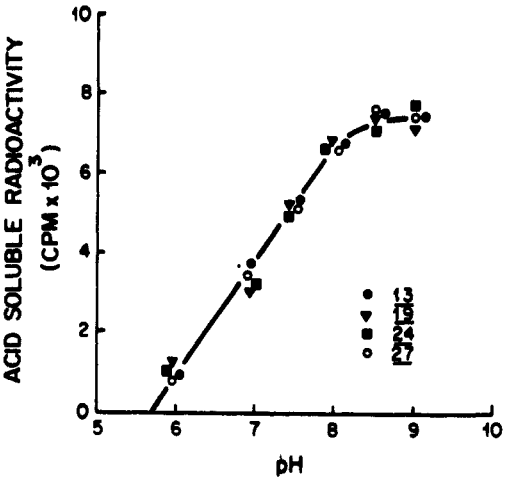


Figure 10. Effect of variations of pH on strand scission of DNA by 13, 19, 24, and 27. Conditions were the same as in Figure 5 except that time intervals for 19, 13, 24, and 27 were 30, 15, 12, and 6 min, respectively.

One outstanding question with regard to the above structure–activity relationship (SAR) still remains, and this concerns whether or not the simpler analogues degrade DNA by the same mechanism as streptonigrin. Although we cannot answer this question unequivocally, the following series of tests with the most active compounds (13, 19, 24, and 27) suggest that they do. Strictly in conformity with strand scissions induced by 1, these four compounds failed to break DNA in the presence of (a) radical inhibitors such as Co^{2+} or sodium benzoate or (b) chelating agents like EDTA. As in the case of streptonigrin, the presence of the specific reducing agent NADH was crucial, and the synergistic effects of copper were evident.

Further, the time-course curves (Figure 9) of all of these four compounds are similar in shape to that displayed by streptonigrin although on a much shorter time scale. In addition, the pH/degradation curves (Figure 10) are strikingly similar. Finally, the influence of various metals on the process of strand scission, whether by 1 or by 13, is exactly the same.[29]

VI. Conclusions

(1) The presence of a *p*-quinone in the nonheterocyclic ring is essential for DNA-cleaving activity. Hydroquinones were found to be inactive[29] in the system used.

(2) The redox potential of the quinone must be in the region of −0.56 V or less negative in order to display DNA strand-breaking activity.

(3) The presence and position of the nitrogen atom play an important role in determining the reduction potential and hence the activity of the compound. Isoquinolines are more active than the corresponding quinolines, whereas the related naphthoquinones are inactive.

(4) Introduction of a second nitrogen atom in the heteroring increases both the reduction potential and the activity of the compound. The position of the second nitrogen atom is also important.

(5) The substitution of electron-withdrawing groups at positions 2, 6, or 7 and possibly *N*-oxide formation (one example only) make the reduction potential less negative and correspondingly increases the DNA strand-breaking capability of the compound.

(6) The almost marginal ability of streptonigrin to cleave DNA, compared with some of the quinones tested, may simply reflect the necessity of low chemical reactivity, to avoid reaction with structures encountered in a biological system, prior to a rendezvous with cellular DNA. This of course assumes that there is a relationship between the ability of 1 to cleave DNA and its antitumor activity.

(29) These and related results will be the subject of a future publication by the authors.

Experimental Section[30]

3-Chloro-2-hydroxy-1,4-naphthalenedione (6). To a warm solution of NaOH (0.5 N, 5 mL) was added 2,3-dichloro-1,4-naphthalenedione[14] (226 mg, 1 mM) in small amounts over a period of 20 min while heating and stirring were continued. The reaction mixture was stirred for 1 more h, cooled to room temperature, and acidified with concentrated HCl to yield a yellow precipitate of pure 43, which was recrystallized from EtOH (190 mg, 91%): mp 218–220 °C (lit.[15] mp 215 °C); IR (Nujol) 3300 (OH), 1680, 1660, 1640 (CO) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.47–7.12 (br s, 1 H, OH), 7.92 (m, 4 H, aromatic H); MS, *m/z* (relative intensity) 210, 208 (33, 100; M^+), 182, 180 (26, 79; $M^+ - CO$), 173 (33, $M^+ - Cl$), 145 (32, $M^+ - CO - Cl$), 117 (17, $M^+ - 2CO - Cl$); HRMS calcd for $C_{10}H_5ClO_3$ 207.9927, found 207.9934.

A. 6,7-Disubstituted 5,8-Quinolinediones. 6,7-Dichloro-5,8-quinolinedione (11). Sodium chlorate (53 g, 0.5 M) was added over a period of 1 h to a stirred solution of 8-hydroxyquinoline (14.5 g, 0.1 M) in concentrated HCl (600 mL) at 50–60 °C. The reaction mixture then was allowed to stir for 2 h and thereafter diluted to 2 L with distilled water. The yellow precipitate that formed was removed by filtration and discarded. The filtrate was extracted with CH_2Cl_2 (3 × 500 mL), and the organic phases were combined, washed with water, and set to crystallize after reducing the volume and adding MeOH. First and second crops yielded pure bright yellow crystals of 11 (6.8 g, 30%): mp 221–223 °C (lit.[22] mp 220–222 °C); IR (Nujol) 1690, 1675 (C=O) cm^{-1}; ^1H

(30) Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined with a Perkin-Elmer 727B spectrometer. The spectra were calibrated against a polystyrene standard and only significant absorption bands are recorded. The ^1H NMR spectra were recorded on a Varian HFT-80 spectrometer and tetramethylsilane was used as an internal standard. Low-resolution mass spectra were recorded on a Hewlett-Packard 5982 mass spectrometer at 70 eV and only the parent and most intense ions are reported. High-resolution mass spectra were obtained on an AEI MS-30 instrument. For column chromatography, silica gel 60, particle size 0.063–0.200 mm (70–230-mesh ASTM), WM EM Reagents, was used. Precoated plates (silica gel GF, thickness 250 μm), supplied by Analtech, Inc., were used for TLC analyses. All new compounds were shown to be homogeneous in two solvent systems (a) CH_2Cl_2–acetone, 95:5, and (b) THF–ether, 65:35, each on a volume/volume basis. Cyclic voltammetry was accomplished with a "Princeton Applied Research Model 170 Electrochemistry System".

NMR (Me_2SO-d_6) δ 9.05 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 8.46 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H 4), 7.88 (dd, $J_{3,4}$ = 8.0 Hz, 1 H, H3); MS, m/z (relative intensity), 231, 229, 227 (10, 50, 80; M^+), 203, 201, 199 (10, 50, 80%; M^+ - CO), 192 (20, M^+ - Cl), 171 (30, M^+ - 2CO), 164 (40, M^+ - Cl - CO), 136 (100, M - Cl - 2CO).

7(6)-Chloro-6(7)-hydroxy-5,8-quinolinediones (8 and 9). (a) A solution of NaOH (0.5 N, 4 mL) was stirred with **11** (227 mg, 1 mM) for 30 min at room temperature when all of it dissolved, giving a blood-red solution. Keeping this solution in the refrigerator overnight afforded the mixed sodium salts of **8** and **9** (173.5 mg, 83%). Repeated fractional crystallization from MeOH led to their separation. Because the parent hydroxy compounds **8** and **9** tended to decompose at room temperature, the sodium salts were left intact for further experimental use. **8**: mp above 300 °C; IR (Nujol) 1665 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.78 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 7.72 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3); MS, m/z (relative intensity) 211, 209 (17, 50, M^+), 183, 181 (34, 100, M^+ - CO), 174 (25, M^+ - Cl), 153 (25, M^+ - 2CO); HRMS calcd for $C_9H_4ClNO_3$ 208.9880, found 208.9882. **9**: mp 205 °C dec; IR (Nujol) 1665 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.78 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 831 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H4), 7.72 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3); MS, m/z (relative intensity) 211, 209 (17, 50, M^+), 183, 181 (34, 100, M^+ - CO), 174 (25, M^+ - Cl), 153 (25, M^+ - 2CO); HRMS calcd for $C_9H_4ClNO_3$ 208.9882, found 208.9896.

(b) To a boiling solution of **11** (227 mg, 1 mM) in MeOH (50 mL) was added a solution of NaOCN (195 mg, 3 mM) in water (10 mL). Boiling was continued for 10 min and the reaction mixture was allowed to stand overnight at room temperature. Dark-red crystals of the sodium salt of **8** separated (203 mg, 88%) identical in physical properties with that prepared under method (a) above.

7(6)-Hydroxy-6(7)-nitro-5,8-quinolinediones (14 and 15). To a boiling solution of **11** (227 mg, 1 mM) in MeOH (50 mL) was added a solution of $NaNO_2$ (207 mg, 3 mM) in water (10 mL). Boiling was continued for 10 min and then the reaction mixture was left at room temperature overnight. This yielded orange crystals of the sodium salt of **14** (109 mg, 45%); the second crop proved to be the sodium salt of **15** (75 mg, 31%). **14**: mp above 300 °C; IR (KBr) 1640 (C=O), 1480, 1330 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.85 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 8.39 (dd, $J_{2,3}$ = 9.0 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H4), 7.78 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3); MS, m/z (relative intensity) 220 (32%, M^+), 192 (48%, M^+ - CO), 164 (68%, M^+ - 2CO). **15**: mp above 300 °C; IR (KBr) 1640 (C=O), 1480, 1330 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.85 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 8.39 (dd, $J_{2,4}$ = 8.0 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H4), 7.78 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3); MS, m/z (relative intensity) 220 (32, M^+), 192 (48, M^+ - CO), 164 (68, M^+ - 2CO); HRMS. This compound was difficult to vaporize and therefore was characterized as its amino derivative (**34**), as detailed in the following experiment.

6-Amino-5,7,8-trihydroxyquinoline (34). Sodium dithionite (435 mg, 2.5 mM) was added to a solution of the sodium salt of **14** (121 mg, 0.5 mM) in water (10 mL) at room temperature and reaction mixture was stirred for 1 h when pure **34** was collected as an orange precipitate (74 mg, 77%): mp above 300 °C; IR (Nujol) 3500, 3400 (NH_2 and OH) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.30 (m, 2 H, H2 + H4), 7.18 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3), 5.3 (br m, 5 H, NH_2 + 3-OH); MS, m/z (relative intensity) 192 (100, M^+); HRMS calcd for $C_9H_8N_2O_3$ 192.0535, found 192.0540.

6,7-Dicyano-5,8-dihydroxyquinoline (32). A saturated solution of NaCN (245 mg, 5 mM) in water (1 mL) was added to a boiling solution of **11** (227 mg, 1 mM) in THF-MeOH (20 mL, 1:1) and boiling was continued for 2 min. The reaction mixture was evaporated to dryness in vacuo and the resultant dark-green mass was dissolved in the minimum amount of water (10 mL). Acidification of this solution yielded a buff-colored precipitate of pure **32** (172 mg, 82%), which becomes dark if subjected to recrystallization: mp above 300 °C; IR (Nujol) 3400 (OH), 2200 (CN) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.14 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 8.676 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H4), 7.88 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3), 9.3–7.5 (br s, 2 H, OH); MS, m/z (relative intensity) 211 (100, M^+), 184 (35, M^+ - HCN), 157 (20, M^+ - 2HCN), 128 (84, M^+ - 2HCN - CHO); HRMS calcd for $C_{11}H_5N_3O_2$ 211.0382, found 211.0383.

7-Cyano-6-hydroxy-5,8-quinolinedione (13). Concentrated nitric acid (2 mL) was added over a period of 30 min to a stirred suspension of **32** (211 mg, 1 mM) in glacial acetic acid (5 mL) at 100 °C. The blood-red solution that resulted was left at room temperature overnight to obtain dark-red crystals of pure **13** (100 mg, 50%): mp above 300 °C; IR (Nujol) 3400 (OH), 2200 (CN), 1720 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.00 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 8.77 (dd, $J_{3,4}$ = 8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H4), 8.12 (dd, $J_{3,4}$ = 8 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3), 6.04 (s, 1 H, OH); MS, m/z (relative intensity) 200 (100, M^+), 172 (55, M^+ - CO), 144 (55, M^+ - 2CO); HRMS calcd for $C_{10}H_4N_2O_3$ 200.0222, found 200.0208.

6,7-Dicyano-5,8-quinolinedione (33). To a suspension of **32** (211 mg, 1 mM) in acetic anhydride (3 mL) at 100 °C was added concentrated HNO_3 (0.5 mL) over a period of 30 min when the suspension gave way to an orange-red solution. This was left overnight at room temperature under anhydrous conditions to yield bright green crystals of **33** (48 mg, 23%): mp 238 °C dec; IR (Nujol) 1700, 1680 (CO) cm^{-1}; ^1H NMR (acetone-d_6) δ 9.17 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1 H, H2), 8.01 (dd, $J_{3,4}$ = 8 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H_3); MS, m/z (relative intensity) 209 (87, M^+), 181 (100, M^+ - CO), 153 (75, M - 2CO); HRMS calcd for $C_{11}H_3N_3O_2$ 209.0225, found 209.0232.

2-Methyl-6,7-dichloro-5,8-quinolinedione (10). Sodium chlorate (5.3 g, 50 mM) was added over a period of 1 h to a stirred suspension of 2-methyl-8-hydroxyquinoline (1.59 g, 10 mM) in concentrated HCl (50 mL), and the reaction mixture was stirred for further 2 h at room temperature. The orange precipitate that formed when the reaction mixture was diluted with water (100 mL) was removed by filtration and discarded. The filtrate was extracted with CH_2Cl_2 (3 × 150). The organic phases were combined and washed with water, and the undried solution was set to crystallize (the crystals are impure if the solution is dried) by reducing the volume and leaving at room temperature overnight. First and second crops afforded pure **10** (313.3 mg, 13%): mp 180–181 °C; IR (Nujol) 1690, 1640 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.39 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 7.59 (d, $J_{3,4}$ = 8 Hz, 1 H, H3), 2.80 (s, 3 H, CH_3); MS, m/z (relative intensity) 245, 243, 241 (12, 67, 100, M^+), 217, 215, 213 (10, 60, 96, M^+ - CO), 189, 187, 185 (8, 30, 45, M^+ - 2CO), 180, 178 (18, 55, M^+ - CO - Cl), 152, 150 (22, 65, M^+ - 2CO - Cl); HRMS calcd for $C_{10}H_5Cl_2NO_2$ 240.9697, found 240.9713.

6,7-Dichloro-5,8-quinolinedione *N*-Oxide (12). (a) To a suspension of 8-hydroxyquinoline *N*-oxide (1.61 g, 10 mM) in concentrated HCl (10 mL) was added concentrated HNO_3 (2 mL) over a period of 30 min. The reaction mixture was then poured over aqueous chipped ice (100 mL) and the orange precipitate that resulted was removed by filtration and discarded. The bright orange-red filtrate was extracted with CH_2Cl_2 (3 × 100 mL). The organic phases were combined, washed with water and dried ($MgSO_4$), and reduced in volume and then allowed to crystallize after dilution with MeOH. The resulting bright-red crystals (0.455 g) showed three spots when analyzed by TLC. These crystals were dissolved in a minimum amount of CH_2Cl_2 and loaded on two silica gel preparative platelets (20 × 20 cm, 1500 μm). After development with a solution of CH_2Cl_2–THF–HCO_2H, 90:5:5, **12** (R_f 0.5) was extracted with CH_2Cl_2 and allowed to crystallize. First and second crops yielded bright-orange crystals of pure **12** (243 mg, 10%): mp 209–210 °C; IR (Nujol) 1725 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.50 (dd, $J_{3,4}$ = 8 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H4), 7.50 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 4.8 Hz, 1 H, H3); MS, m/z (relative intensity) 247, 245, 243 (10, 30, 50, M^+), 231, 229, 227 (10, 30, 50, M^+ - O), 203, 201, 199 (10, 30, 50, M^+ - O - CO), 164 (45, M^+ - O - CO - Cl), 136 (99, M^+ - O - 2CO - Cl); HRMS calcd for $C_9H_3Cl_2NO_3$ 242.9490, found 242.9498.

(b) To a magnetically stirred solution of **11** (227 mg, 1 mM) in CF_3COOH (3 mL) at 5 °C (ice bath) was added 35% H_2O_2 (10 mL) dropwise over a period of 10 min. The reaction mixture was stirred for an additional 30 min when it was brought to room temperature and neutralized with a saturated solution of $NaHCO_3$. Methylene chloride (3 × 25 mL) extraction led to a bright orange-red solution, which was washed successively with $NaHSO_3$ solution and water and then dried ($MgSO_4$). The volume of the organic phase was reduced in vacuo to 3 mL and the solution was then loaded on two silica gel preparative plates (20 × 20 cm, 1500

μm). After development (CH_2Cl_2–THF–HCO_2H, 90:5:5) **12** (R_f 0.5) was extracted with CH_2Cl_2 and allowed to crystallize. Two crops of pure **12** (34 mg total, 14%) as orange crystals were obtained. It was identical in all respects with a specimen prepared as in (a) above.

B. 6,7-Disubstituted 2-(*o*-Nitrophenyl)-5,8-quinolinediones. 2-(*o*-Nitrophenyl)-5,8-dimethoxyquinoline (28). Nitrocinnamaldehyde (35.4 g, 0.2 mol) was added over 15 min to a well-stirred solution of 2,5-dimethoxyaniline (30.6 g, 0.2 mol) and sodium *m*-nitrobenzenesulfonate (67.0 g, 0.3 mol) in glacial acetic acid (700 mL) at 60–70 °C. The mixture was heated at 100–115 °C for 1.5 h and then poured over a mixture of chipped ice and water (2 L). The resulting dark-brown oily material was taken up in CH_2Cl_2 (3 × 1 L) and the organic layer was washed successively with water and $NaHCO_3$ solution and dried ($MgSO_4$). Concentration of the solution then gave a thick black liquid, which was loaded on a neutral alumina column (500 g) and eluted with 30 L of 2:1 hexane–CH_2Cl_2. Reducing the volume to 300 mL gave yellow crystals of **28** (7 g, 10%): mp 143–145 °C (lit.[7] mp 142–144 °C); IR ($CHCl_3$) 1515, 1340, (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.54 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 7.0–8.0 (m, 5 H, aromatic H), 6.78 (dd, J = 8 Hz, 2 H, H6, H7), 4.05 (s, 3 H, CH_3O), 3.94 (s, 3 H, CH_3O); MS, m/z (relative intensity), 310 (20, M^+).

2-(*o*-Nitrophenyl)-6,7-dichloro-5,8-quinolinedione (18). Sodium chlorate (30 g, 282 mM) was added to a stirred suspension of **28** (3.1 g, 10 mM) in concentrated HCl (300 mL) at 25 °C over a period of 1 h. After 2 further h of stirring, the reaction mixture was diluted to 1 L when crude **18** came out of a solution as a yellow precipitate (2.8 g). Crystallization from CH_2Cl_2–MeOH afforded bright yellow crystalls of pure **18** (2.6 g, 75%): mp 232–234 °C (lit.[7] mp 231–233 °C); IR (Nujol) 1690, 1670 (C=O), 1525, 1370 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.59 (d, J = 8 Hz, 1 H, H4), 7.81 (d, J = 8 Hz, 1 H, H3), 7.69 (m, 4 H, Ar H); MS, m/z (relative intensity), 352 (2.5, M^+), 350 (15, M^+), 348 (25, M^+), 320 (40, M^+ – CO), 302 (50, $M^+ - NO_2$).

2-(*o*-Nitrophenyl)-7(6)-chloro-6(7)-hydroxy-5,8-quinolinediones (16 and 17). To a warm solution of sodium hydroxide (0.5 N, 20 mL) was added **18** (349 mg, 1 mM) in small amounts over a period of 30 min. The blood-red reaction mixture was cooled to room temperature after all of **18** had gone into solution. Acidification yielded a yellow precipitate of pure **16** and **17** (297 mg, 90%). The precipitate was dissolved in MeOH and treated with a quantitative amount of $NaHCO_3$ (76 mg, 0.9 mM), and the resulting salts were subjected to fractional crystallization. Acidification (HCl) then gave the desired free acids **16**: mp 241–242 °C; IR (KBr) 3400 br (OH), 1620 (C=O), 1525, 1340 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) 8.50 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 8.10 (d, $J_{3,4}$ = 8 Hz, 2 H, H3), 7.85 (m, 3 H, aromatic H), 4.18 (br s, 1 H, OH); MS, m/z (relative intensity), 332 (25, M^+), 330 (70, M^+), 304 (30, M – CO), 302 (90, M – CO), 295 (20, M – Cl); HRMS calcd for $C_{15}H_9ClN_2O_3$ 330.0044, found 330.0082. **17**: mp 234–236 °C; IR (KBr) 3400 br (OH), 1680, 1620 (C=O), 1525, 1340 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.50 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 8.10 (d, $J_{3,4}$ = 8 Hz, 2 H, H3, and aromatic H), 7.85 (m, 3 H, aromatic H), 4.18 br (s, 1 H, OH); MS, m/z (relative intensity), 332 (25, M^+), 330 (70, M^+), 304 (30, M^+ – CO), 302 (90, M^+ – CO), 295 (20, M^+ – Cl); HRMS calcd for $C_{15}H_9ClN_2O_3$ 330.0044, found 330.0064.

2-(*o*-Nitrophenyl)-7(6)-hydroxy-6(7)-nitro-5,8-quinolinediones (20 and 21). To a boiling solution of **18** (349 mg, 1 mM) in acetone (15 mL) was added a concentrated aqueous solution of $NaNO_2$ (207 mg, 3 mM), and boiling was continued for 15 min. After evaporation in vacuo, the resultant orange mass was dissolved in MeOH and crystallized by dilution with water at room temperature. The yellow crystals (205 mg, 60%) were a mixture of sodium salts of **20** and **21**, the separation of which was affected by repeated fractional crystallization from the same solvent system. **20**-: mp 230 °C dec; IR (Nujol) 3500 (OH), 1710, 1650 (C=O), 1520, 1380 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.52 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 8.08 (d, $J_{3,4}$ = 8 Hz, 2 H, H3, and aromatic H), 7.84 (m, 3 H, aromatic H); MS, m/z (relative intensity), 341 (4, M^+), 340 (11, $M^+ - 1$). **21**: mp 210 °C dec; IR (Nujol) 3500 (OH), 1710, 1650 (C=O), 1520, 1380 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.52 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 8.08 (d, $J_{3,4}$ = 8 Hz, 2 H, H3, and aromatic H), 7.84 (m, 3 H, aromatic); MS, m/z (relative intensity) 341 (4, M^+), 340 (11, M – 1). This substance could not be vaporized properly to give an HRMS. Therefore it was characterized as the diamino derivative (**29**) as detailed in the following experiment.

2-(*o*-Aminophenyl)-6-amino-7-hydroxy-5,8-quinolinedione (29). To a solution of **20** (34.1 mg, 0.1 mM) in methanol (10 mL) there was added a freshly prepared solution of sodium dithionite (105 mg, 0.6 mM) in water (4 mL). The reaction mixture was stirred for 1 h at room temperature, filtered to remove inorganic salts, and then left overnight in the refrigerator. This afforded violet crystals of **29** (21.6 mg, 77%): mp above 300 °C; IR (KBr) 3500, 3400 (NH_2 and OH), 1620 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.50 (m, 6 H, H4 + H3 + aromatic H), 3.75 (br s, 5 H, NH and OH); MS, m/z (relative intensity) 281 (100, M^+), 252 (30, M^+ – CO); HRMS calcd for $C_{15}H_{11}N_3O_3$ 281.0800, found 281.0794.

2-(*o*-Nitrophenyl)-6,7-dicyano-5,8-dihydroxyquinoline (30). A solution of **18** (175 mg, 0.5 mM) in THF–MeOH (50 mL, 50:50) was brought to reflux at which point a solution of NaCN (123 mg, 2.5 mM) in H_2O (1 mL) was added. The reaction mixture was allowed to boil for 2 min and then evaporated to dryness in vacuo. The dark green solid of the sodium salt of **30** thus obtained was dissolved in the minimum amount of hot water (10 mL) and acidified to provide a buff-colored precipitate of **30** (143 mg, 86%) which could not be recrystallized from any solvent: mp above 300 °C; IR (Nujol) 3500 (OH), 2200 (CN), 1520, 1380 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.85 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 7.91 (m, 7 H, H3 + OH); MS, m/z (relative intensity) 332 (100, M^+), 314 (30, $M^+ - H_2O$), 286 (60, $M^+ - NO_2$); HRMS calcd for $C_{17}H_8N_4O_4$ 332.0546, found 332.0526.

2-(*o*-Nitrophenyl)-6,7-dicyano-5,8-quinolinedione (31). Concentrated nitric acid (1 mL) was added dropwise over a period of 15 min to a suspension of **30** (240 mg, 0.7 mM) in glacial acetic acid (5 mL) at 100 °C. The bright, orange-red solution that resulted was left at room temperature overnight to afford yellow crystals of **31** (156 mg, 65%): mp 244–245 °C; IR (Nujol) 1700 (C=O), 1520, 1370 (NO_2) cm^{-1}; ^1H NMR (acetone-d_6) δ 8.72 (d, $J_{3,4}$ = 8 Hz, 1 H, H4), 8.29 (d, $J_{3,4}$ = 8 Hz, 1 H, H3), 8.06 (m, 1 H, aromatic H), 7.89 (m, 3 H, aromatic H); MS, m/z (relative intensity) 330 (17, M^+), 302 (20, M^+ – CO), 284 (100, $M^+ - NO_2$); HRMS calcd for $C_{17}H_6N_4O_4$ 330.0309, found 330.0378.

C. 6,7-Disubstituted 5,8-Isoquinolinediones. 6,7-Dichloro-5,8-isoquinolinedione (23). Concentrated HNO_3 (5 mL) was added in portions over a period of 1 h to a stirred suspension of 5-hydroxyisoquinoline (5.8 g, 40 mM) in concentrated HCl (100 mL) at 80–90 °C. The resulting yellow solution was diluted to 1 L by water and extracted with ether (3 × 300 mL). The organic phases were combined, washed with water, and dried ($MgSO_4$) and the desired product **34** (1.2 g) was obtained crystalline by reducing the volume and adding MeOH. Recrystallization from the same solvent system afforded pale-green crystals of pure **23** (1.0 g, 11%): mp 180–181 °C; IR (Nujol) 1700 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.45 (s, 1 H, H1), 9.13 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H3), 7.98 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H4); MS, m/z (relative intensity) 231, 229, 227 (20, 66, 100; M^+), 201, 199 (18, 25; M^+ – CO), 194, 192 (30, 88; M^+ – Cl), 166, 164 (32, 95; M^+ – Cl – CO), 138, 136 (24, 72; M^+ – Cl – 2CO); HRMS calcd for $C_9H_3Cl_2NO_2$ 226.9541, found 226.9539.

6-Chloro-7-hydroxy-5,8-isoquinolinedione (22). A solution of NaOH (0.2 N, 2 mL) was stirred with **23** (22.7 mg, 0.1 mM) for 30 min at room temperature when all of it dissolved, giving a blood-red solution. Acidification with concentrated HCl (20 L) and chilling afforded dark-red crystals of **22** (18.2 mg, 87%): mp 167 °C dec; IR (Nujol) 3350 (OH), 1670 (CO) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.15 (s, 1 H, H1), 8.98 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H3), 7.90 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H4), 6.02 (s, 1 H, OH); MS, m/z (relative intensity) 211, 209 (7, 21, M^+); HRMS calcd for $C_9H_4Cl_2NO_3$ 208.9880, found 208.9874.

6,7-Dicyano-5,8-dihydroxyisoquinoline (35). To a boiling solution of **23** (227 mg, 1 mM) in THF–MeOH (20 mL, 50:50) was added a saturated solution of NaCN (0.92 g, 5 mM) in water (1 mL) and boiling was continued for 2 min. After evaporation of the organic solvents, the resulting dark-green mass was dissolved in the minimum amount of water (10 mL) and acidified with 3 N HCl to obtain a buff-colored precipitate of pure **35** (102 mg), which tended to darken when subjected to recrystallization. The red-colored filtrate was extracted with EtOAc (3 × 30 mL) to isolate the remainder of the **35** from the aqueous phase. The

organic layers were combined, washed with water, dried ($MgSO_4$), and evaporated to dryness in vacuo to yield additional **35** (86 mg) (total yield, 89%): mp above 300 °C; IR (Nujol) 3600 (OH), 2200 (CN) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.64 (s, 1 H, H1), 8.83 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H3), 8.17 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H4), 5.2–3.7 (br s, 2 H, OH); MS, m/z (relative intensity) 211 (15, M^+), HRMS calcd for $C_{11}H_5N_3O_2$ 211.0382, found 211.0366.

6-Cyano-7-hydroxy-5,8-isoquinolinedione (24). A suspension of **35** (105 mg, 0.5 mM) in acetic acid (3 mL) was stirred and heated at 100 °C when concentrated HNO_3 (0.5 mL) was added in portions over a period of 10 min. The resulting bright orange-red solution was diluted with water (5 mL) and left overnight at room temperature to deposit dark-red crystals of pure **24** (38 mg, 37%); mp 130 °C dec; IR (Nujol) 3500 (OH), 2200 (CN), 1700 (CO) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.09 (m, 2 H, H1 + H3), 7.92 (d, $J_{3,4}$ = 5.0 Hz, 1 H, H4), 3.79 (s, 1 H, OH); MS, m/z (relative intensity) 200 (88, M^+), 172 (25, M^+ – CO), 144 (25, M^+ – 2CO); HRMS calcd for $C_{10}H_4N_2O_3$ 200.0222, found 200.0200.

D. 6,7-Dichlorodiazanaphthalene-5,8-diones. 1,4-Dimethoxy-2,3(2,5)-dinitrobenzenes. To a solution of 1,4-dimethoxybenzene (13.8 g, 0.1 M) in glacial acetic acid (35 mL) was added concentrated nitric acid (18 mL). After the vigorous reaction had subsided, a further 18 mL of nitric acid was added. The mixture was then heated for 5 min at 80–90 °C, cooled, and diluted with water (350 mL) to obtain a mixture of the desired isomers as a yellow crystalline solid (18.2 g, 80%): mp 155–160 °C (lit.[22] mp 156–160 °C). The separation of the two isomers, unreported previously, was accomplished by column chromatography. 1,4-Dimethoxy-2,3-dinitrobenzene (**47**): mp 184–186 °C; IR (KBr) 1550, 1310 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.21 (s, 2 H, H5 and H6), 3.93 (s, 6 H, CH_3O); MS, m/z (relative intensity) 228 (100, M^+). 1,4-Dimethoxy-2,5-dinitrobenzene: mp 200–202 °C; IR (KBr) 1550, 1310 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (s, 2 H, H3 and H5), 3.98 (s, 6 H, CH_3O); MS, m/z (relative intensity) 228 (100, M^+).

1,2(1,4)-Diamino-3,6-dimethoxybenzenes. A solution of the mixed 1,4-dimethoxy-2,3- and -2,5-dinitrobenzenes (22.8 g, 0.1 M) in THF–MeOH (300 mL, 50:50) was boiled while sodium dithionite (174 g, 1.0 M) was added in small portions over 30 min. The reaction mixture was boiled for a further 1 h and diluted with water (100 mL). The organic solvents were evaporated in vacuo, and the violet aqueous solution was acidified with concentrated HCl to yield violet crystals of the pure 1,4-diamino compound (6.8 g); basicification of mother liquor gave pinkish-grey crystals of the 1,2-diamino compound (2.7 g). 1,2-Diamino isomer: mp 68–70 °C; IR (Nujol) 3380, 3150 (NH_2) cm^{-1}; ^1H NMR δ ($CDCl_3$) 6.34 (s, 2 H, H1 and H5), 3.77 (s, 6 H, CH_3O), 1.60 (br s, 4 H, NH_2); MS, m/z (relative intensity) 168 (85, M^+), 153 (100, $M^+ - CH_3$), 138 (45, $M^+ - 2\ CH_3$), 110 (99, $M^+ - 2CH_3 - N_2$).

5,8-Dimethoxyquinoxaline (36). A solution of the mixed dinitro derivatives (9.1 g, 40 mM) prepared according to the penultimate experiment above in absolute EtOH (250 mL) containing platinum oxide (300 mg) as catalyst was stirred with H_2 (50 lb/in.^{2}) for 4 h. After filtration through a Celite bed to remove the catalyst, the dark-brown solution was evaporated in vacuo to obtain a deep violet residue, which was immediately added to a warm saturated solution of glyoxal sodium bisulfite adduct (12 g, 45 mM) in water (100 mL). The reaction mixture was stirred and heated at 70 °C for 45 min and filtered to remove a black residue. The filtrate was basified with NaOH pellets and filtered again to obtain a clear blood-red solution, which was extracted with CH_2Cl_2 (3 × 250 mL). The organic phases were combined, washed with water, dried ($MgSO_4$), and percolated through a column of Woehlm neutral alumina (activity 1). The bright yellow eluent was concentrated to afford greenish-yellow crystals of pure **36** (4.7 g, 62%): mp 147–148 °C (lit.[31] mp 148–149 °C); IR (KBr) 1612, 1490, 1270. 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.89 (s, 2 H, NCH=), 7.02 (s, 2 H, aromatic H), 4.06 (s, 6 H), OCH_3); MS, m/z (relative intensity) 190 (68, M^+), 175 (100, $M^+ - CH_3$), 161 (58, $M^+ - 2CH_3$).

6,7-Dichloro-5,8-quinoxalinedione (25). A solution of **36** (1.9 g, 10 mM) in concentrated HCl (100 mL) was stirred while sodium chlorate (1.06 g, 10 mM) was added in portions over 30 min at room temperature. The reaction mixture was immediately diluted (to avoid the formation of *N*-oxides) with distilled water (200 mL) and filtered. The light-orange solid residue (1.6 g) was discarded and the blood-red filtrate was extracted with CH_2Cl_2 (3 × 50 mL). The organic phases were combined, washed with water, dried ($MgSO_4$), reduced in volume in vacuo, and chromatographed over silica. Fractions containing pure **25** as identified by TLC were mixed, evaporated to reduce the volume diluted with ether, and allowed to crystallize. First and second crops yielded yellow, sandlike crystals of pure **25** (25 mg, 1.1%): mp 171–175 °C dec; IR (Nujol) 1690 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.95 (s, 2 H, H2 + H3); MS, m/z (relative intensity), 232, 230, 228 (15, 66, 100; M^+), 204, 202, 200 (10, 60, 91; M – CO); HRMS calcd for $C_8H_2Cl_2N_2O_2$ 227.9493, found 227.9484.

2,4-Dimethoxy-6-nitrobenzaldehyde. Finely powdered 2,5-dimethoxybenzaldehyde (3.3 g, 20 mM) was added with cooling and stirring to cold concentrated nitric acid (12 mL). After standing at room temperature overnight, the reaction mixture was diluted with water (100 mL) and the resulting yellow solid (3.5 g), a mixture of 2,4-dimethoxy-6-nitrobenzaldehyde and its 3-nitro isomer, was collected. The mixture, mp 135–155 °C (lit.[26,27] mp 130–153 °C), was separated by column chromatography to yield light yellow crystals (from EtOH) of the pure 6-nitro isomer (2.8 g, 66%): mp 159–160 °C (lit.[24,32] mp 159 °C); IR (KBr) 1730 (C=O), 1550, 1350 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.35 (s, 1 H, CHO), 7.30 (d, $J_{3,4}$ = 9.8 Hz, 1 H, H3), 7.09 (d, $J_{3,4}$ = 9.8 Hz, 1 H, H4), 3.94 (s, 3 H, 5-CH_3), 3.86 (s, 3 H, 2-CH_3); MS, m/z (relative intensity) 211 (100, M^+).

5,8-Dimethoxyquinazoline (37). A solution of 2,4-dimethoxy-6-nitrobenzaldehyde (10.5 g, 50 mM) in formamide (150 mL) was heated at 80 °C while dry HCl was bubbled through for 1 h. The solution was cooled and diluted with water (150 mL) to yield, after crystallization from aqueous ethanol, yellow crystals of the crude *N*,*N*^{1}-diformamido acetal of 2,5-dimethoxy-6-nitrobenzaldehyde (12 g, 85%): mp 242–244 °C (lit.[23] mp 244 °C). This intermediate was used as such in the following procedure.

To a suspension of the above compound (6 g, 21 mM) in ice (70 g) and glacial acetic acid (27 mL) was added Zn metal (18 g) in small portions during 1.5 h while the reaction mixture was vigorously stirred in an ice bath. The temperature of the reaction mixture then was allowed to rise to room temperature, and additional Zn (5 g) was added in small portions, stirring being continued for a further 1.5 h. The reaction mixture then was filtered directly into a solution of NaOH (50%, 125 mL). The yellow suspension that resulted was extracted with ether (3 × 250 mL). The organic phases were combined, washed with water, dried ($MgSO_4$), and reduced in volume (150 mL) to yield light yellow crystals of pure **37** (1.64 g, 41%): mp 119 °C (lit.[23] mp 119 °C); ^1H NMR (Me_2SO-d_6) δ 9.63 (s, 1 H, H2), 9.27 (s, 1 H, H4) 7.38 (d, $J_{6,7}$ = 8.8 Hz, 1 H, H7), 7.07 (d, $J_{6,7}$ = 8.8 Hz, 1 H, H6), 3.97 (s, 3 H, 5-OCH_3), 3.94 (s, 3 H, 8-OCH_3); MS, m/z (relative intensity) 190 (29, M^+), 175 (100, $M^+ - CH_3$), 160 (15, $M^+ - 2CH_3$).

6,7-Dichloro-5,8-quinazolinedione (26). Concentrated HNO_3 (1 mL) was added in portions over 15 min to a stirred solution of **37** (360 mg, 2 mM) in concentrated HCl (10 mL) at 80–90 °C. The solution was cooled, diluted with water (100 mL), and extracted with ether (3 × 100 mL). The organic phases were combined, washed with water, dried ($MgSO_4$), and reduced in volume (50 mL) whereupon a small quantity of a yellow crystalline of impurity was deposited. This was removed by filtration. The filtrate then was reduced to 20 mL and left overnight in the refrigerator to yield light yellow crystals of pure **26** (45 mg, 10%): mp 135 °C dec; IR (KBr) 1690 (C=O) cm^{-1}; ^1H NMR ($CHCl_3$) δ 9.73 (s, 1 H, H2), 9.62 (s, 1 H, H4); MS, m/z (relative intensity) 232, 230, 228 (15, 72, 100, M^+), 204, 202, 200 (5, 23, 35, M^+ – CO), 167, 165 (14, 43, M^+ – CO – Cl); HRMS calcd for $C_8H_2Cl_2N_2P_2$ 227.9493, found 227.9492.

Phthalazine. A mixture of 1,2-dicyanobenzene (5.12 g, 40 mM), dioxane (20 mL), and hydrazine (95%, 10 mL) was treated with acetic acid (1.8 mL) below 30 °C and then heated on the steam bath for 3 h. The resulting orange paste was filtered and the collected solid was washed with ethanol and air-dried to yield

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crude 1,4-dihydrazinophthalazine (6.3 g, 83%): mp 185–190 °C dec. (lit.[24] mp 185–190 °C dec for crude and 190 °C dec for pure). This intermediate was used as such in the following procedure.

Oxygen (CO_2 free) was bubbled through a stirred suspension of **60** (5.7 g, 30 mM) in ethanol (400 mL) and NaOH solution (10 N, 30 mL) until all of the orange starting material had disappeared (about 2.5 h). EtOH was removed from the deep purple reaction mixture in vacuo, water (50 mL) was added to the residue and the pH was adjusted to 9 with concentrated HCl. The product was extracted with CH_2Cl_2 (3 × 200 mL), and the combined extracts were washed with distilled water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure to yield the dark-brown crude phthalazine. This material was recrystallized from MeOH to give two crops of phthalazine as dark yellow crystals (2.65 g, 68%): mp 87–89 °C (lit.[25] mp 88–89 °C); IR (KBr) 1610, 1485, 1430, 1370, 1280, 1240, 900 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.73 (s, 2 H, H1 and H4), 8.13 (m, 4 H, H5–H8); MS, *m/z* (relative intensity) 130 (100, M^+), 103 (24, M^+ – HCN), 76 (19, M^+ – 2HCN).

5-Nitrophthalazine (38). To a solution of phthalazine (2.6 g, 20 mM) in concentrated H_2SO_4 (96%, 10 mL) was added KNO_3 (3.03 g, 30 mM) in small portions to avoid the reaction becoming too vigorous. The mixture then was heated on a steam bath for 12 h, cooled, diluted with distilled water (40 mL), and treated with a solution of NaOH (10 N, 35 mL) to obtain a yellow precipitate of **38** (0.7 g). This was recrystallized from EtOH to give the pure compound as yellow prismatic crystals (0.6 g, 17%): mp 188–189 °C (lit.[26] mp 187–188 °C); IR (Nujol) 1525, 1340 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 10.15 (s, 1 H, H4), 9.90 (s, 1 H, H1), 8.73 (m, 2 H, H6 and H8), 8.23 (m, 1 H, H7); MS, *m/z* (relative intensity) 175 (100; M^+).

5-Aminophthalazine (39). A freshly prepared solution of sodium dithionite (1.74 g, 10 mM) in water (4 mL) was quickly added to a boiling solution of **38** (350 mg, 2 mM) in THF–MeOH (20 mL, 1:2) and boiling was continued for 5 min. The reaction mixture was diluted with water (30 mL), evaporated in vacuo to remove organic solvents, and extracted with EtOAc (2 × 50 mL). The organic extracts were combined, washed with water, and evaporated in vacuo, yielding a yellow residue of **39** (272 mg) which was crystallized from MeOH (212 mg, 73%): mp 223–224 °C; IR (Nujol) 3350, 3150 (NH_2) cm^{-1}; ^1H NMR (acetone-d_6) δ 9.72 (s, 1 H, H4), 9.37 (s, 1 H, H1), 7.58 (m, 1 H, H7), 7.20 (m, 2 H, H6 and H8), 2.80 (s, 2 H, NH_2); MS, *m/z* (relative intensity) 145 (100, M^+); HRMS calcd for $C_8H_7N_3$ 145.0640, found 145.0639.

6,7-Dichloro-5,8-phthalazinedione (27). Sodium chlorate (1.06 g, 10 mM) was added in small amounts over a period of 30 min to a magnetically stirred solution of **39** (145 mg, 1 mM) in concentrated HCl (10 mL) at room temperature. The reaction mixture was allowed to stir for an additional hour and then diluted with distilled water (20 mL) and filtered. The light-orange solid residue was discarded and the blood-red filtrate was extracted with methylene chloride (3 × 30 mL). The combined organic extracts were washed with water and reduced to 10 mL in vacuo when excess of ether was added to precipitate crude **27** as a brick-red solid (52 mg), which was recrystallized as brown crystals from MeOH (29.6 mg, 13%): mp 225 °C dec; IR (KBr) 1690 (=O), 1550, 1350, 1290, 1125, 825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.92 (s, 2 H, H1 and H4); MS, *m/z* (relative intensity) 232, 230, 228 (15, 71, 100; M^+), 204, 202, 200 (5, 25, 42, M^+ – CO), 195, 193 (5, 25, M^+ – Cl); HRMS calcd for $C_8H_2Cl_2N_2O_2$ 227.9493, found 227.9507.

Assay Procedure for Determination of Extent of DNA Cleavage. The test compound in dimethylformamide (DMF) solution (10 mM) was added to NADH (150 mM) in 0.5 sodium phosphate buffer (pH 8.5). This was followed by the addition of aqueous copper sulfate (500 μM), nonradioactive calf thymus DNA (750 μM), and tritium-labeled Hela cell DNA in solution (250 μM) in the order given. Experiments were performed at 37 °C in a total volume of 100 μL in most instances, and the final concentrations of reactants were as follows: streptonigrin or lower homologue (200 μM), NADH (15 mM), sodium phosphate buffer (50 mM), copper sulfate (100 μM), calf thymus DNA (150 μM), radioactive DNA (*DNA; 50 M; 10000 cpm), and 2% DMF.

At timed intervals the reaction mixture was cooled in an ice bath, and an aqueous solution of bovine serum albumin (100 μL; 1 mg/mL) was added followed by the addition of 1 N perchloric acid (200 μL). After standing in the ice bath for 3 min to complete the precipitation of the uncleaved DNA, the mixture was centrifuged at room temperature for 3 min. The sample (100 μL) of the supernatant solution was then mixed with a liquiscintillation cocktail (National Diagnostic) and the radioactivity of the acid-soluble degraded DNA was measured.

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Registry No. 1, 3930-19-6; 5, 1526-73-4; 6, 117-80-6; 7, 1015-84-5; 8·Na, 102072-64-0; 9·Na, 102072-65-1; 10, 98994-93-5; 11, 6541-19-1; 12, 84289-01-0; 13, 102072-69-5; 14·Na, 102072-66-2; 15-Na, 102072-67-3; 16, 102072-71-9; 17, 102072-72-0; 18, 61472-28-4; 19, 102072-86-6; 20·Na, 102072-73-1; 21, 102072-74-2; 22, 102072-79-7; 23, 84289-03-2; 24, 102072-81-1; 25, 102072-82-2; 26, 102072-83-3; 27, 102072-85-5; 28, 61472-25-1; 29, 102072-75-3; 30, 102072-77-5; 30·Na, 102072-76-4; 31, 102072-78-6; 32, 53159-50-5; 33, 102072-70-8; 34, 102072-68-4; 35, 102072-80-0; 36, 19506-19-5; 37, 17944-05-7; 38, 89898-86-2; 39, 102072-84-4; $NaO_3SCH(OH)CH(OH)SO_3Na$, 517-21-5; 8-hydroxyquinoline, 148-24-3; 2-methyl-8-hydroxyquinoline, 826-81-3; 8-hydroxyquinoline *N*-oxide, 1127-45-3; nitrocinnamaldehyde, 1466-88-2; 2,5-dimethoxyaniline, 102-56-7; 5-hydroxyisoquinoline, 2439-04-5; 1,4-dimethoxybenzene, 150-78-7; 1,4-dimethoxy-2,3-dinitrobenzene, 6945-76-2; 1,4-dimethoxy-2,5-dinitrobenzene, 56741-26-5; 1,4-diamino-3,6-dimethoxybenzene, 17626-02-7; 1,2-diamino-3,6-dimethoxybenzene, 40328-95-8; 2,5-dimethoxybenzaldehyde, 93-02-7; 2,4-dimethoxy-6-nitrobenzaldehyde, 15994-96-4; 2,4-dimethoxy-3-nitrobenzaldehyde, 91004-45-4; formamide, 75-12-7; 2,5-dimethoxy-6-nitro-(*N*,*N*′-diformamido)benzaldehyde acetal, 19819-19-3; 1,2-dicyanobenzene, 91-15-6; 1,4-dihydrazinophthalazine, 484-23-1; phthalazine, 253-52-1.