Streptonigrin and Related Compounds. 5. Synthesis and Evaluation of Some Isoquinoline Analogues

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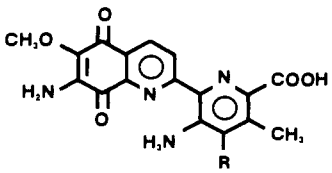
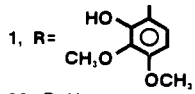
A series of analogues of streptonigrin, in which the quinoline of ring B is replaced by isoquinoline and the substituted pyridine of ring C is replaced by phenyl, nitrophenyl, aminophenyl, or benzyl functions, have been prepared. Thus, 1-substituted isoquinoline-5,8-diones with 7-amino or 6-(alkylamino) groups were prepared. The various quinones were evaluated for antimicrobial activity against *Bacillus subtilis* and root-growth inhibitory activity against *Lepidium sativum*. The effect of specific structural changes on these activities was examined with streptonigrin for comparison. The necessity of an aminoquinone function for activity is confirmed. With regard to the antibacterial activity, the isoquinoline analogues appear to be less active compared to the quinoline derivatives. However, the higher degree of antibacterial activity of the 1-benzylisoquinolines and the 1-nitrophenylisoquinolines compared to the 1-phenyl isoquinolines is noteworthy. In contrast to the results seen with the antibacterial activity, most of the isoquinoline analogues showed activity comparable to, or even higher than, that of streptonigrin in the root-growth inhibition assay. The 1-nitrophenylisoquinolines again appear to be the most active. The equal or greater potency of the benzyl analogue in comparison with the phenyl analogue was unexpected and questions the need for the extended conjugation and the geometry required for metal binding as considered earlier. It also opens up new possibilities for structural variation.

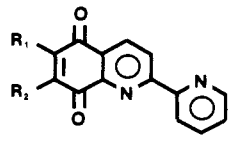
Introduction

Since the discovery of the antitumor antibiotic streptonigrin in 1961,[1] the compound has been the subject of numerous and continuing investigations because of its novel chemical structure[2] 1 and on the broad spectrum of



1, R= 

66, R=H



64, R_1=OCH_3, R_2=NH_2

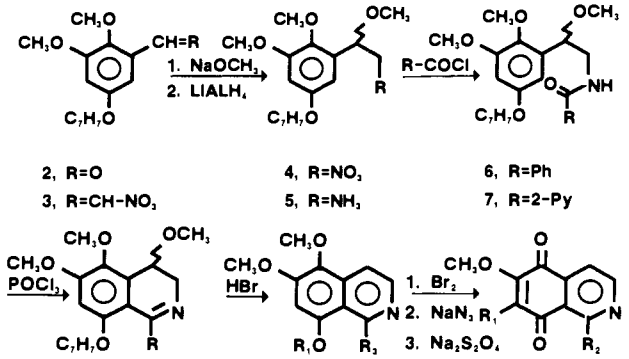
65, R_1=NH_2, R_2=OCH_3

activity against murine tumors.[3,4] Examples of susceptible tumors include sarcoma 180, Lewis lung carcinoma, mammary adenocarcinoma, Flexner–Jobling carcinoma, Walker 256 carcinosarcoma, Iglesias ovarian tumor, and human tumor transplants in immune-suppressed rats. In addition, antiviral activity has been demonstrated against Herpes simplex I and HIV I.[5,6] Clinical studies also indicated activity against human chronic lymphocytic leukemia and malignant lymphoma comparable to that of chlorambucil.[7,8] The drug has been tested in some combination regimens.[9-11]

Many structural variants have been prepared from 1 and testing of some of these led to a number of correlations of structure vs activity.[12] The aminoquinone function on the heterobicyclic system is said to be obligatory for activity. Simpler examples with a 2-phenylquinoline and 2-(2-pyridyl)quinoline-5,8-diones[13] showed that this function was necessary for the microbiological activity, with the activity being subject to modulation by the other substituents.[14] On this premise, analogues with the isoquinoline replacing the quinoline were prepared and their activity examined here.

Although syntheses of various heterocyclic quinone analogues, including the isoquinoline, have been reported for comparison with 1, very few had a substitution pattern equivalent to that of the A/B rings of streptonigrin.[15-23] The comparisons of activity were also made either on test systems in which streptonigrin was not active (e.g. in vitro

Scheme I



2, R=O
3, R=CH-NO_2
4, R=NO_2
5, R=NH_2
6, R=Ph
7, R=2-Py

8, R=Ph
9, R=2-Py
10, R_1=C_7H_7, R_2=Ph
11, R_1=C_7H_7, R_2=2-Py
12, R_1=H, R_2=Ph
13, R_1=H, R_2=2-Py
14, R_1=Br, R_2=Ph
15, R_1=Br, R_2=2-Py
16, R_1=N_3, R_2=Ph
17, R_1=N_3, R_2=2-Py
18, R_1=NH_2, R_2=Ph
19, R_1=NH_2, R_2=2-Py

degradation of DNA)[15] or in systems (e.g. antimicrobial or cytotoxic activity) where the analogues showed little or

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no activity. One possible reason for this low activity (antimicrobial or cytotoxic) is the absence of an aminoquinone system in many of these analogues.

As a rationale for the study of the isoquinoline analogues, it is known that isoquinoline and quinoline possess similar chemical and physical properties[24] and hence may interact similarly at the site of action. Reduction potentials of the quinone systems have been used as a criterion[15] for predicting activity and, in this regard, the two quinones are comparable. On the other hand, in the isoquinoline system, one would lose the theoretically favorable proximity of the quinone carbonyl and the heterocyclic nitrogen as it is present in the quinoline system. Thus, if this proximity is indeed essential for activity, the isoquinoline analogues would be expected to be less potent than the quinoline counterparts, all other factors being equal.

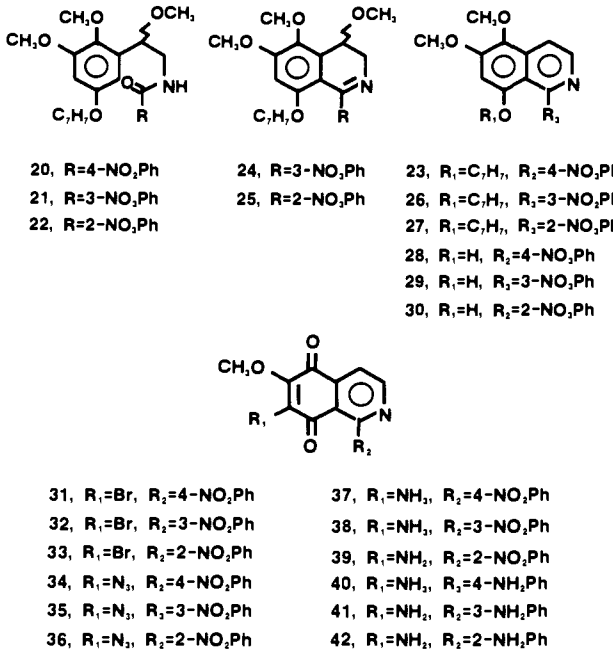
Materials and Methods

Basic Scheme. The reactions used for the formation of the basic aminoisoquinoline-5:8-dione skeleton was shown in Scheme I. 5-(Benzyloxy)-2,3-dimethoxybenzaldehyde (2), prepared from *o*-vanillin[25] was condensed with nitromethane to form nitrostyrene 3. In the usual synthetic scheme, 3 might have been reduced, aroylated, and cyclized to yield a 3,4-dihydroisoquinoline, which would then be converted to the isoquinoline by Pd/C or other agents that can cause dehydrogenation. In the present case, this conversion was found to be slow and incomplete. The modification[26] in which nitrostyrene 3 is reacted with sodium methoxide to yield 4 gave a much more reliable sequence of reactions. Thus, amine 5 readily gave amides 6 and 7, which underwent facile conversion to the corresponding dihydroisoquinoline derivatives 8 and 9. These were readily converted to isoquinolines 10 and 11 by treatment with hydrochloric acid, which also simultaneously effected the desired debenzylation to give 12 and 13.

Addition of bromine to 12 and 13 in acetic acid produced 7-bromoisoquinoline-5,8-diones 14 and 15 in one step. Conversion of 14 and 15 via azide derivatives 16 and 17 to aminoquinones 18 and 19 proceeded smoothly.

C-Ring Substitution. Ring C of streptonigrin is made up of a pyridine with a 5′-amino and a 2′-carboxyl group. The importance of the amine function to the overall activity, specifically its participation in metal chelation, hydrogen bonding, or other interactions, must be assessed. From studies on the aminoquinoline-5,8-diones it appeared that the amine function of ring C might be more important than the pyridine nitrogen.[13] Boger et al.,[15] also from studies of ring C substituents, observed some unusual effects especially due to the carboxyl group.

Scheme II



20, R=4-NO2Ph
21, R=3-NO2Ph
22, R=2-NO2Ph

24, R=3-NO2Ph
25, R=2-NO2Ph

23, R1=C7H7, R2=4-NO2Ph
26, R1=C7H7, R2=3-NO2Ph
27, R1=C7H7, R2=2-NO2Ph
28, R1=H, R2=4-NO2Ph
29, R1=H, R2=3-NO2Ph
30, R1=H, R2=2-NO2Ph

31, R1=Br, R2=4-NO2Ph
32, R1=Br, R2=3-NO2Ph
33, R1=Br, R2=2-NO2Ph
34, R1=N3, R2=4-NO2Ph
35, R1=N3, R2=3-NO2Ph
36, R1=N3, R2=2-NO2Ph

37, R1=NH2, R2=4-NO2Ph
38, R1=NH2, R2=3-NO2Ph
39, R1=NH2, R2=2-NO2Ph
40, R1=NH2, R2=4-NH2Ph
41, R1=NH2, R2=3-NH2Ph
42, R1=NH2, R2=2-NH2Ph

To test the effect of an amine function in ring C, the three (aminophenyl)quinones (40–42) were prepared and their activities compared with those of the phenyl and pyridyl derivatives. Of the three isomers, the ortho isomer would be most effective, if the activity were being expressed through a metal-binding interaction as for example between the NH_2 (of ring C) and the quinoline nitrogen. If, on the other hand, the activity is expressed through a H-bond interaction with DNA, the *m*- and *p*-amino derivatives would be in a better position to participate. It must be stated, however, that because of the nonplanar conformation of ring C (relative to rings A and B) the *o*-amino group may also be available for hydrogen bonding with DNA.

Accordingly, amides 20, 21, and 22 were prepared with, respectively, the *p*-, *m*-, and o-nitrobenzoyl chlorides and amine 5 (Scheme II). Under the conditions of cyclization, *p*-nitro amide 20 directly gave isoquinoline 23, whereas the other two gave the corresponding dihydroisoquinolines 24 and 25, which were subsequently converted to the isoquinolines 26 and 27. After debenzylation, phenols 28–30 were converted to bromoquinones 31–33. Azidoquinones 34 and 35 on reduction with sodium borohydride, yielded the (nitrophenyl)aminoquinones 37 and 38, whereas azidoquinone 36 with the *o*-nitrophenyl function produced the (aminophenyl)aminoquinone 42. The desired (*o*-nitrophenyl)aminoquinone 39 was, however, prepared by the reaction of 36 with triphenylphosphine followed by gentle acid treatment.[27] Reduction of 37 and 38 with sodium dithionite yielded (aminophenyl)aminoquinones 40 and 41.

Effect of the Extended Conjugated System. One of the unique structural features of streptonigrin is the juxtaposition of three aromatic rings, a quinoline, a pyridine, and a benzene ring, with the resulting extended conjugation. Rings C and D are expected to exist in a nonplanar configuration with respect to rings A and B. Many hypotheses on the possible mode of action of streptonigrin,

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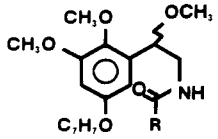
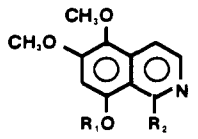
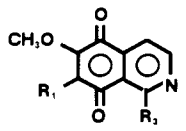
Scheme III



43, R=Benzyl
44, R=2-NO_2 benzyl



45, R_1=C_7H_7, R_2=Benzyl
46, R_1=C_7H_7, R_2=2-NO_2 benzyl
47, R_1=H, R_2=Benzyl
48, R_1=H, R_2=2-NO_2 benzyl



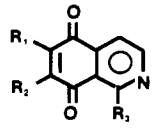
49, R_1=Br, R_2=Benzyl
50, R_1=Br, R_2=2-NO_2 benzyl
51, R_1=N_3, R_2=Benzyl
52, R_1=N_3, R_2=2-NO_2 benzyl
53, R_1=NH_2, R_2=Benzyl
54, R_1=NH_2, R_2=2-NO_2 benzyl
55, R_1=NH_2, R_2=2NH_2 benzyl

particularly those involving the drug's metal-binding ability (which perhaps rests mainly with the rings B, C, and D) have relied heavily on the rigid geometry of this polycyclic system.[15] The effect of disrupting this conjugation would therefore be worth studying. Accordingly, some analogues with the 1-benzylisoquinoline system (**53–55**) were prepared and compared with the corresponding 1-phenylisoquinoline-5,8-diones.

With phenylacetyl chloride and (*o*-nitrophenyl)acetyl chloride and **5**, amides **43** and **44** were respectively prepared (Scheme III). The rest of the scheme proceeded as described to yield, successively, isoquinolines **45** and **46**, isoquinolinols **47** and **48**, bromoquinones **49** and **50**, azidoquinones **51** and **52**, and aminoquinones **53–55**.

Effect of Alkylation of the Aminoquinone. As indicated earlier, the aminoquinone system was found to be essential for activity. Of the analogues made previously, few, if any, contained an alkylamino or a dialkylamino group in place of the amino group of the quinone. Such alkylation of the amino group would be expected to increase the lipid solubility and the stability to hydrolysis, thereby contributing to a higher degree of activity. However, if hydrogen-bonding capability were an important prerequisite, these analogues might be less active.

Accordingly, bromoquinone **31** was treated with methylamine. Instead of the expected 7-(methylamino)-6-methoxyquinone **56**, the product was 6-(methylamino)-7-



56, R_1=OCH_3, R_2=$NHCH_3$, R_3=4-NO_2Ph
57, R_1=$NHCH_3$, R_2=Br, R_3=4-NO_2Ph
58, R_1=$NHCH_3$, R_2=Br, R_3=3-NO_2Ph
59, R_1=$NHCH_3$, R_2=Br, R_3=2-NO_2Ph
60, R_1=$NHCH_3$, R_2=Br, R_3=Benzyl
61, R_1=N-piperidyl, R_2=Br, R_3=4-NO_2Ph
62, R_1=$N(CH_3)_2$, R_2=Br, R_3=2-NO_2Ph
63, R_1=$NHCH_3$, R_2=N_3, R_3=4-NO_2Ph

bromoquinone **57**. Other quinones of this type, **32**, **33**, and **49**, also behaved in a similar manner and yielded analogous products, **58**, **59**, and **60**. Likewise, reaction of **31** with other amines such as piperidine or dimethylamine gave similar products: **61** and **62**. Although such reactions have been reported in the mitosene[28,29] and in the quinoline[30,31] series, they have not been recorded in the isoquinoline series. In view of the facile conversion of **31** to **34** by sodium azide, these reactions are rather unusual and imply possible involvement of a cyclic intermediate as has been suggested in the quinoline system.[30,31]

Additionally, it was found that the bromine in **57** [6-(methylamino)-7-bromo] was unreactive to sodium azide as was the methoxyl in **37** (6-methoxy-7-amino) to methylamine. On the other hand, reaction of **34** (6-methoxy-7-azido) with methylamine gave 6-(methylamino)-7-azidoquinone **63**. These reactions suggested a possible addition mechanism involving positions 6 and 7 where the group at one position controls whether the reaction will proceed at the other or not. For example, an amino or a methylamino function prevents the reaction whereas a bromine or an azide function facilitates the addition reaction.

Joseph and Joullie[21] reported that isoquinoline-5,8-dione underwent monoaddition with hydrogen chloride or aziridine, postulating that addition took place so as to give the 6-isomer. The evidence, although not direct, was based on the analogy of quinoline-5,8-dione, where the 6-position appears to be more reactive. For example 7-methoxy-2-(2-pyridyl)quinoline-5,8-dione reacts with sodium azide to give the 6-amino-7-methoxyquinone directly.[14] On the other hand, streptonigrin, a 6-methoxy-7-aminoquinoline-5,8-dione, undergoes hydrolysis in alkaline solution to give the 6-methoxy-7-hydroxyquinone.[2]

Additionally in the isoquinoline series, Shaikh et al.[16] reported that treatment of 6,7-dichloroisoquinoline-5,8-dione with aqueous sodium hydroxide gave 6-chloro-7-hydroxyisoquinoline-5,8-dione and suggested a mechanism based on a resonance structure in which a positive charge was placed at 7. However, if this were so, the above reactions would have led to the methylamine substituent at the 7-position instead of the 6-position as seen here. Further clarification of these aspects is, therefore, in order.

Biological Activity. The activity of the various analogues was determined by two in vitro procedures in both of which streptonigrin shows significant activity.

One of these is the antibacterial activity against *B. subtilis*. Since this test has been used in earlier work,[13,14] it can serve for comparison with the quinoline analogues. The compounds were dissolved in DMSO at concentrations of 2000, 1000, 400, 200, 80, and 40 μg/mL. To the antibiotic assay disks (Schleicher and Schuel) was added 50 μL of each of the test solutions. The discs were placed equally spaced on agar plates seeded with *B. subtilis* spores, six per plate, and the plates incubated at 37 °C. After 20 h, the zones of inhibition were measured and the results from four replicates averaged. The diameter of the zone of inhibition varied linearly with the log of the concentration. The concentration that gave a zone diameter of 13 mm was taken as the minimum inhibitory concentration (mic). The data were subjected to linear-regression

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Table I. Antibiotic Assay

compd	mic μg	mic nM	slope ± SE	relative potency to streptonigrin	r
18	7.63	27.25	14.78 ± 0.801	0.05	0.9999
19	11.03	39.25	12.37 ± 0.908	0.03	0.973
53	4.72	16.05	11.31 ± 1.4	0.09	0.932
40	10.54	35.8	12.64 ± 1.1	0.04	0.99
41	9.85	33.4	11.7 ± 0.817	0.04	0.984
42	5.07	17.2	9.20 ± 0.914	0.08	0.97
37	no diffusion	no diffusion			
38	1.69	5.2	4.93 ± 0.50	0.26	0.996
39	2.93	9.0	10.3 ± 0.80	0.15	0.976
1	0.693	1.37	5.51 ± 0.226	1.00	0.971
66	0.088	0.25	4.75 ± 0.26	5.48	0.99
64	3.97	14.13	10.23 ± 0.91	0.10	0.99
60	4.72	16.0	11.31 ± 1.3	0.09	0.932
59	2.4	6.76	5.21 ± 0.45	0.20	0.977

analysis using a calculator program and standard errors for the slope were calculated as per Strike.[32]

The second assay is based on the inhibition of root growth using seedlings of *L. sativum*, similar to the procedure of Noel et al.[33] The activity of streptonigrin on seedling growth has been observed in several systems such as onion, *Vicia faba*, and others.[34] The rationale for this activity is that the apical meristem of the root is composed of actively dividing, undifferentiated cells and compounds such as streptonigrin inhibit this growth through their blocking of DNA synthesis.

Cress seeds (*L. sativum*), soaked in water for 15–20 min, were placed (two seeds/square) on 0.6% agar in phage-typing petri dishes (Falcon) and were allowed to germinate in the dark at 27 °C. After 24 h, the radicals were ca. 1 cm long.

The compounds to be tested were dissolved in DMSO to give concentrations of 2000, 1000, 400, 200, 80, 40 μg/mL. Each of these solutions (200 μL) was added to 20 mL of hot 0.6% agar and the agar solutions poured into square phage-typing dishes and the agar was allowed to cool.

Those seedlings with 1-cm radicals were placed on the test agar plates so that the root tips were at the intersection of two grid lines (eight seedlings/plate). Controls were set up with 0.2 mL of DMSO/20 mL of agar. The plates were placed horizontally in an incubator at 27 °C, in the dark for 24 h, after which the root lengths (new growth starting from the intersection of the grids) were measured and averaged to give a value for the root length of each. Each compound was run in duplicate and the data for the two runs (a total of 16 seedlings) were averaged. Percent inhibition (%*I*) was calculated with the formula $1 - (T/C) \times 100 = \%I$, where T = test data and C = control data. The data were analyzed by using probit analysis[35] and subjected to linear regression (calculator program). The concentration at which 50% inhibition of growth occurs (I_{50}) was determined with the same program. Standard errors and confidence limits were calculated as per Strike.[32]

Figure 1 shows a graphical (bar) comparison of the antibacterial and the root growth inhibitory activities of the new analogues with those of streptonigrin.

Results

Antibacterial Activity. Table I shows the antibacterial activity of the various analogues with the relevant statistical parameters. Three of the compounds were too insoluble for evaluation. As was seen before, only those compounds with an aminoquinone structure showed antibacterial activity, with the methoxy, the methoxybromo- and the methoxyazidoquinones being inactive.

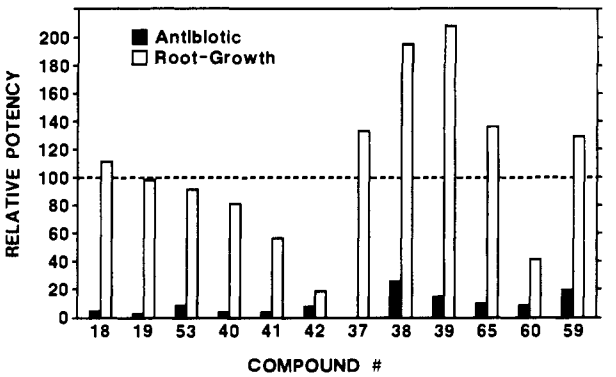

Figure 1. Comparison of the activities of streptonigrin and of the newly prepared analogues. The dotted line represents the level of activity of streptonigrin taken as 100.

The isoquinoline derivatives were all generally less potent than streptonigrin or the analogue without the ring D, **66**. A comparison of the pyridylisoquinoline, phenylisoquinoline, and benzylisoquinoline quinones showed benzylisoquinoline **53** (mic = 16 nM) to be the most active of the group, followed by phenylisoquinoline **18** (mic = 27 nM) and pyridylisoquinoline **19** (mic = 39 nM). Among the aminophenyl-substituted aminoquinones, *o*-aminophenyl derivative **42** was 1.5 times as active as phenyl-substituted quinone **18** whereas the *p*-aminophenyl (**40**) and the *m*-aminophenyl (**41**) derivatives were less active (0.7 and 0.8 times, respectively).

In the nitrophenyl series, the *o*-nitro- (**39**) and the *m*-nitrophenyl (**38**) derivatives were 3 and 5 times more active than phenyl-substituted quinone **18**. *p*-Nitro derivative **37** was too insoluble in this test to be evaluated. The methylamino and the dimethylamino derivatives, in general, were also too insoluble for evaluation. Only two were sufficiently soluble: (methylamino)benzylisoquinoline **60** and (methylamino)(*o*-nitrophenyl)isoquinoline **59** were both less active than the corresponding aminoquinones **53** and **39** but more potent than phenyl-substituted quinone **18**.

Root Growth Inhibition Assay. Comparison of the I_{50} values (Table II) in the root growth inhibition assay for 1-(2-pyridyl)isoquinoline **19**, 1-phenylisoquinoline **18**, benzylisoquinoline **53**, 6-amino-7-methoxy-2-(2-pyridyl)-quinoline (**65**),[13] and streptonigrin (**1**) indicate only slight

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Table II. Root Inhibition Assay

compd	I_{50} μg/mL	I_{50} nM/mL	95% confidence, nM/mL	relative potency to Streptonigrin	r
18	3.15	11.25	7.46–17.09	1.12	0.99
19	3.57	12.7	8.15–19.5	0.99	0.97
53	4.05	13.7	9.15–20.97	0.92	0.97
40	4.57	15.49	10.05–24.0	0.82	0.95
41	6.6	22.37	15.85–31.6	0.57	0.99
42	19.26	65.0	32.37–125.94	0.19	0.98
37	3.06	9.41	6.43–14.06	1.34	0.97
38	2.09	6.43	4.77–8.68	1.96	0.98
39	1.96	6.03	4.55–7.91	2.09	0.99
1	6.4	12.65	8.23–19.76	1.0	0.98
66	1.95	5.5	3.57–8.53	2.3	0.99
64	2.69	9.18	6.11–11.22	1.37	0.94
60	10.7	30.05	24.26–36.72	0.42	0.96
59	3.86	10.0	7.76–12.30	1.3	0.94

differences in their potencies (**65** was tested earlier as part of a related project and found to be ca. 1.2–1.4 times as active as **1**; Rao, K. V., unpublished results) but following the order: **65** > **18** > **1** > **19** > **53**. This is not very surprising since data generated by Boger et al.[15] indicated that **1** and 6-methoxy-7-amino-2-(2-pyridyl)quinoline (**64**)[13] were of approximately equal potency in L1210 cell culture (I_{50}: **1**, 1.2 nM/mL; **64**, 1.06 nM; potency ratio 1.12). This comparison did not, however, hold true for all cell cultures tested in their study. In some cell lines, **1** was substantially more active than **64**, whereas in others, **64** was more potent. The differences are probably attributable to differences in cellular uptake of the compounds as well as the sensitivity of the cell lines.

Destrioxyphenylstreptonogrin **66**[14] was found to be more than twice as potent as **1** in the root growth inhibition assay. The increase in potency of this compound could be attributable to the lack of the D ring, which could impart greater lipid solubility and hence greater uptake by the root.

The 1-(nitrophenyl)-7-aminoquinones (**37–39**) and the 1-(aminophenyl)-7-aminoquinones (**40–42**) were all less potent than the phenyl-substituted isoquinoline quinone **18**. Within the group, *p*-aminophenyl derivative **40** with an I_{50} of 15 nM/mL was the most potent, followed by *m*-aminophenyl **41** with an I_{50} of 22 nM/mL. *o*-Aminophenyl **42** was the least potent of the group with an I_{50} of 65 nM/mL.

The (nitrophenyl)isoquinoline quinones were again found to be more active than the phenylisoquinoline quinone **18**; the *o*-nitro (**39**) and the *m*-nitrophenyl (**38**) derivatives showing I_{50} values of 6 and 6.4 nM/mL, respectively, were equipotent. The decrease in potency observed for *p*-nitro compound **37** (I_{50}, 9 nM/mL) may be partly attributed to the problem of low solubility in water.

Of the methylamino derivatives tested, only **59** showed activity in the plant assay system comparable to or even slightly better than that of **1**. The rest of them were either weakly active (**60**) or not active. Their poor solubility in the assay medium is at least partly responsible. The dialkylamino derivatives were inactive at the levels tested.

Discussion and Conclusions

In the antibiotic assay system, the importance of the aminoquinone function for activity is again confirmed. Substitution of the quinoline ring with an isoquinoline does lower the antibacterial activity to 3–30% of that of **1** but does not abolish it. The apparently higher activity of phenyl derivative **18** over that of pyridyl derivative **19** may not be significant, but it does suggest that the pyridyl nitrogen may play only a minor role in the activity. This is not entirely unexpected because the pyridine nitrogen in **1** is rather hindered. The activity shown by benzylisoquinoline derivative **53** in comparison with those of the 1-phenyl and the 1-pyridyl derivatives appears to be significant and indicates that the triaryl system with its extended conjugation can be replaced. Also, it can be seen from models that substituents such as the *o*-amino group of the C ring of the benzylisoquinoline can more easily participate in metal-binding activity than the corresponding group of a phenylisoquinoline.

Unexpectedly, introduction of an amine function in the phenyl ring did not enhance the activity substantially. Acetylation of the amine function does, however, abolish the activity. The fact that the *o*-aminophenyl derivative was more active than the corresponding meta and the para derivatives lends some credence to the metal-binding hypothesis as discussed earlier. It is also highly significant that the nitro derivatives were considerably more active than the phenyl or the aminophenyl derivatives.

Although some of the alkylamino and dialkylamino analogues were too insoluble for evaluation, the fact that **59** [the methylamino 1-(*o*-nitrophenyl)] and **60** (the methylamino 1-benzyl) were more active than **18** (the amino 1-phenyl) against *B. subtilis* suggests that greater lipid solubility may be an important factor in enhancing activity.

The root growth inhibitory activity, which perhaps indirectly reflects the inhibition of DNA synthesis, showed some parallels to the antibacterial activity. Analogues such as *o*-nitrophenyl **39** and *m*-nitrophenyl **38** showed double the potency compared to that of streptonigrin. In this respect, these are closer in activity to destroxyphenylstreptonigrin **66**,[14] which was 2–5 times more potent than **1** in the antibacterial and the root growth inhibition assays. Once again, the importance of the aminoquinone function is confirmed. However, in contrast to the experience with the antibacterial activity, where most of the isoquinoline analogues were much less active than **1**, most were comparable to **1** in their root growth inhibition activity, with some being even more active than **1**. Thus, it is significant to note that most of the changes made, isoquinoline for quinoline, phenyl for pyridyl, substitution of the phenyl ring, and elimination of the triaryl conjugation, have not adversely affected this activity. While most of the analogues were comparable in potency to streptonigrin in the root growth inhibition assay, the three that were once again significantly low in ptoency are the *m*-aminophenyl (**41**, 0.57 of **1**), the *o*-aminophenyl (**42**, 0.19 of **1**), and the 6-(aminomethyl) (**60**, 0.42 of **1**) derivatives.

These experiments illustrate that considerable variation may be made in the structure of streptonigrin and the activity still maintained at least in the test systems studied. Furthermore, whether changing the quinoline of the B ring of streptonigrin to isoquinoline and the highly substituted

pyridine of the C ring to the phenyl or even benzyl and eliminating the D ring altogether makes these compounds still related to streptonigrin or prototypes of a new series is a point to be considered.

It would be of interest to see if the activities of the analogues seen in these two assay systems will continue to parallel that against in vitro and in vivo tumor systems. Work along these lines and testing of the analogues in cell culture assays are currently in progress.

Experimental Section

Melting points were determined on Fisher-Johns apparatus and are uncorrected. NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard and a Varian EM-390 (90 MHz) instrument. The chemical shifts are given in ppm units, and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared spectra were recorded on a Beckman Acculab 3 instrument in the form of KBr pellets. Ultraviolet spectra were obtained with a Perkin-Elmer Lambda 3B and methanol as solvent. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, Ga, and were within 0.4% of the theoretical values. Column chromatography and thin-layer chromatography (TLC) were carried out with silica gel (Merck, 100–200 mesh or Merck HF/366/254, respectively.)

5-(Benzyloxy)-2,3-dimethoxybenzaldehyde (2). A mixture of 5-hydroxy-2,3-dimethoxybenzaldehyde[25] (10 g), $C_6H_5CH_2Cl$ (7.6 g), and anhydrous K_2CO_3 (10 g) in DMF (100 mL) was stirred under reflux for 2 h, cooled, and poured into water (250 mL). The solid was filtered, washed and crystallized from ligroin to give **2** as white needles: yield 13.4 g (90%); mp 55–56 °C; NMR δ 10.44 (1 H, s), 7.43 (5 H, bs), 7.00 (1 H, d, J = 5 Hz), 6.86 (1 H, d, J = 5 Hz), 5.07 (2 H, s), 3.94 (3 H, s), 3.87 (3 H, s); IR (cm^{-1}) 2880, 1680, 1600, 1480, 1380, 1340, 1270, 1222, 1189, 1040, 985. Anal. ($C_{16}H_{16}O_4$) C, H.

2,3-Dimethoxy-5-(benzyloxy)-β-nitrostyrene (3). A mixture of **2** (10 g), nitromethane (10 mL), and ammonium acetate (10 g) in HOAc (50 mL) was heated (70 °C) with stirring (1 h). After cooling, dilution with water, and extraction with C_6H_6, the solvent layer on concentration gave **3** as bright yellow needles (MeOH): yield 10 g (87%); mp 105–107 °C; NMR δ 8.20 (1 H, d, J = 22.5 Hz), 7.72 (1 H, d, J = 22.5 Hz), 7.45 (5 H, bs), 6.75 (1 H, d, J = 5 Hz), 6.62 (1 H, d, J = 5 Hz), 5.08 (2 H, s), 3.85 (6 H, s); IR (cm^{-1}) 2950, 1530, 1580, 1510, 1480, 1320, 1285, 1180, 1040, 960, 820, 680. Anal. ($C_{17}H_{17}NO_5$) C, H, N.

2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxy-1-nitroethane (4). Sodium methoxide (20% in MeOH, 18 mL) was added to **3** (10 g) in MeOH at −10 °C until a clear and colorless solution resulted. It was acidified and extracted with C_6H_6. Concentration gave **4** as an off-white solid: yield 10.5 g (95%); mp 95–96 °C; NMR δ 7.43 (5 H, bs), 6.60 (2 H, s), 5.28 (1 H, t, J = 10 Hz), 5.05 (2 H, s), 4.59 (2 H, d, J = 10 Hz), 3.84 (6 H, s), 3.30 (3 H, s); IR (cm^{-1}) 3005, 2980, 2970, 2920, 1590, 1550, 1490, 1460, 1380, 1370. Anal. ($C_{18}H_{21}NO_6$) C, H, N.

2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethylamine (5). To lithium aluminum hydride (LAH, 3 g) in dry THF (50 mL) was added a solution of **4** (10 g) in THF (150 mL) dropwise. The reaction mixture was boiled under reflux for 6–8 h. The excess LAH was decomposed by careful addition of EtOAc–MeOH and the suspension filtered (Celite) and washed with THF. Concentration gave the oily amine which was converted to the oxalate salt, a colorless crystalline solid (MeOH–C_3H_6O): yield 9.4 g (80%); mp 175–178 °C; NMR (DMSO-d_6) δ 7.44 (5 H, m), 6.75 (1 H, d, J = 5 Hz), 6.53 (1 H, d, J = 5 Hz), 5.65 (3 H, bs), 5.10 (2 H, s), 4.77 (1 H, t, J = 11 Hz), 3.81 (3 H, s), 3.69 (3 H, s), 3.69 (3 H, s), 3.16 (3 H, s), 2.97 (2 H, d, J = 11 Hz); IR (cm^{-1}) 3450, 3004, 1710–1690. Anal. ($C_{20}H_{25}NO_8$) C, H, N.

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]benzamide (6).** A stirred mixture of **5** (2 g) and triethylamine (1.5 g) in CH_2Cl_2 (50 mL) was treated dropwise with benzoyl chloride. After 2 h, addition of water, extraction with ether, and washing with aqueous acid and $NaHCO_3$ gave an extract which was chromatographed over SiO_2 in C_6H_6 to give **6** as an oil: yield 2 g (97%); NMR δ 7.82 (2 H, m), 7.43 (8 H, m), 6.92 (2 H, s), 5.03 (2 H, s), 4.85, 4.77 (1 H, dd, J = 4 Hz), 3.85 (3 H, s), 3.83 (3 H, s), 3.6, 3.4 (2 H, d, J = 4.5 Hz), 3.27 (3 H, s); IR (cm^{-1}) 3080, 1650, 1490, 1460, 1430, 1350, 1325, 1270, 1230, 1190, 1150, 1110, 1050, 1005, 830. Anal. ($C_{25}H_{27}NO_5$) C, H, N.

The same procedure was used for the preparation of the following amides: **7**, **20**, **21**, **22**, **43**, and **44** (from **5** and the appropriate acid chlorides).

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]picolinamide (7).** From **5** was obtained product **7** as a viscous oil: yield 92%; NMR δ 8.57 (1 H, dd, J = 7.5, 1.5 Hz), 7.85 (1 H, td, J = 7.5, 1.5 Hz), 7.43 (5 H, m), 6.64 (2 H, s), 5.17 (1 H, dd, J = 7.5, 2.5 Hz), 5.13 (2 H, s), 4.07, 4.0 (2 H, dd, J = 7.5, 2.5 Hz), 3.83 (6 H, s), 3.33 (3 H, s); IR (cm^{-1}) 2940, 1675, 1595, 1520, 1490, 1460, 1440, 1330, 1220, 1190, 1150, 1105, 1040, 1000. Anal. ($C_{24}H_{26}N_2O_5$) C, H, N.

Compound **7** was also prepared by adding *N*-bromosuccinimide (1.8 g) to **5** (2 g), triethylamine (2.5 g), and picolinoyl hydrazide in CH_2Cl_2 (50 mL) at −10 °C. After 15 min, diluting with ether, washing with aqueous acid and aqueous $NaHCO_3$, and concentration of the ether gave **7** as a viscous oil: yield 1.6 g (80%).

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]-4-nitrobenzamide (20).** From **5** was obtained product **20** as a pale yellow crystalline solid (ether–ligroin): yield 90%; mp 133–134 °C; NMR δ 8.30 (2 H, d, J = 8.4 Hz), 7.94 (2 H, d, J = 8.4 Hz), 7.41 (5 H, bs), 6.59 (2 H, s), 5.04 (2 H, s), 4.84, 4.76 (1 H, dd, J = 4.5 Hz), 3.85 (6 H, s), 3.64, 3.52 (1 H, 2 d, J = 4.5 Hz), 3.28 (3 H, s); IR (cm^{-1}) 3340, 3120, 1640, 1590, 1520, 1480, 1425, 1340, 1320, 1250, 1225, 1185, 1135, 1105, 1060, 1040, 1000. Anal. ($C_{25}H_{26}N_2O_7$) C, H, N.

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]-3-nitrobenzamide (21).** From **5** was obtained product **21** as a pale yellow crystalline solid (ether): yield 88%; mp 90–92 °C; NMR δ 8.62 (1 H, m), 8.23 (2 H, m), 7.65 (1 H, m), 7.43 (5 H, m), 6.61 (2 H, s), 5.06 (2 H, s), 4.87, 4.77 (1 H, dd, J = 4.85 Hz), 3.88 (3 H, s), 3.87 (3 H, s), 3.67, 3.58 (2 H, d, J = 4.5 Hz), 3.30 (3 H, s); IR (cm^{-1}) 3440, 1660, 1600, 1530, 1495, 1470, 1430, 1350, 1270, 1220. Anal. ($C_{25}H_{26}N_2O_7$) C, H, N.

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]-2-nitrobenzamide (22).** From **5** was obtained product **22** as a viscous oil: yield 95%; NMR δ 8.03 (1 H, m), 7.66–7.30 (8 H, m), 6.93 (2 H, s), 5.05 (2 H, s), 4.87, 4.77 (1 H, dd, J = 4.5 Hz), 3.83 (6 H, s), 3.54, 3.46 (2 H, dd, J = 4.5 Hz), 3.26 (3 H, s); IR (cm^{-1}) 3330, 3100, 1660, 1600, 1530, 1490, 1450, 1350, 1320, 1190, 1170, 1150, 1110, 1050, 1000. Anal. ($C_{25}H_{26}N_2O_7$) C, H, N.

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]phenylacetamide (43).** From **5** was obtained **43** as a viscous oil: yield 94%; NMR δ 7.42 (5 H, m), 7.31 (5 H, m), 6.56 (1 H, d, J = 3 Hz), 6.47 (1 H, d, J = 3 Hz), 5.01 (2 H, s), 4.66, 4.57 (1 H, dd, J = 4.5 Hz), 3.25 (2 H, d, J = 4.5 Hz), 3.17 (3 H, s); IR (cm^{-1}) 2940, 1640, 1580, 1480, 1445, 1420, 1315, 1210, 1180, 1160, 1140, 1010, 990. Anal. ($C_{26}H_{29}NO_3$) C, H, N.

***N*-[2-[2,3-Dimethoxy-5-(benzyloxy)phenyl]-2-methoxyethyl]-2-nitrophenylacetamide (44).** From **5** was obtained product **44** as a beige crystalline solid (ether–ligroin): yield 93%; mp 95–95 °C; NMR δ 8.05 (1 H, m), 7.62–7.26 (8 H, m), 6.89 (2 H, s), 5.04 (2 H, s), 4.69, 4.59 (1 H, dd, J = 4.5 Hz), 3.3 (2 H, d, J = 4.5 Hz), 3.23 (3 H, s); IR (cm^{-1}) 3380, 1680, 1590, 1520, 1490, 1350, 1140. Anal. ($C_{26}H_{28}N_2O_7$) C, H, N.

1-Phenyl-5,6-dimethoxy-8-(benzyloxy)isoquinoline (10) and 1-Phenyl-4,5,6-trimethoxy-8-(benzyloxy)-3,4-dihydroisoquinoline (8). A mixture of **6** (4 g) and $POCl_3$ (15 mL) in C_6H_6 (50 mL) was stirred under reflux for 4 h. Ice was added and after 30 min the mixture was washed with ether. The aqueous layer was extracted with $CHCl_3$ at pH 10 and the extract concentrated to give a mixture of **8** and **10** (1:3; 3 g, 82%). After chromatography on alumina (C_6H_6), **10** was obtained as a colorless solid: mp 117–119 °C; NMR δ 8.51 (1 H, d, J = 6 Hz, C-4), 7.86 (1 H, d, J = 6 Hz, C-3), 7.43 (3 H, m, ArH), 7.23 (5 H, m, ArH), 6.86 (2 H, m, ArH), (1 H, s, C-7), 4.84 (2 H, s, CH_2Ph), 3.95 (3 H, s, OCH_3); IR (cm^{-1}) 3080, 1610, 1550, 1440, 1390, 1360, 1340, 1235, 1190, 1125, 1050. Anal. ($C_{24}H_{21}NO_3$) C, H, N.

The HCl salt of **8** was a greenish-yellow crystalline solid (C_3H_6O): mp 195–200 °C; NMR (DMSO-d_6) δ 7.53 (5 H, m, ArH), 7.17 (4 H, m, ArH), 6.73 (1 H, s, ArH), 6.63 (1 H, s, ArH), 5.05 (2 H, s, $ArCH_2$), 4.83 (1 H, s), 4.40 (1 H, s), 4.23 (1 H, s), 4.07 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 3.30 (3 H, s, OCH_3); IR (cm^{-1})

1610, 1585, 1495, 1455, 1425, 1340, 1320, 1270, 1245, 1220, 1135, 1080, 1040, 980, 810, 740, 690. Anal. ($C_{25}H_{26}ClNO_4$) C, H, N, Cl.

With a similar procedure, dihydroisoquinolines **9**, **24**, and **25**, and isoquinolines **11**, **23**, **26**, **27**, **45**, and **46** were prepared.

1-(2-Pyridyl)-5,6-dimethoxy-8-(benzyloxy)isoquinoline (11). From **7** was obtained a mixture of **11** and **9** (3:1), yield 88%. Of these, **11** was a tan crystalline solid (ether): yield 0.9 g (66%); mp 130–132 °C; NMR δ 8.48 (1 H, d, J = 6 Hz), 8.41 (1 H, dd, J = 4.5, 1.5 Hz), 7.90 (1 H, d, J = 6 Hz), 7.43 (2 H, m), 7.25 (3 H, m), 6.96 (4 H, m), 6.70 (1 H, s), 4.86 (2 H, s), 3.93 (3 H, s), 3.90 (3 H, s); IR (cm^{-1}) 3080, 1610, 1580, 1550, 1450, 1390, 1370, 1360, 1340, 1265, 1240, 1198, 1120, 1110, 1070, 1050, 1040, 990. Anal. ($C_{23}H_{20}N_2O_3$) C, H, N.

1-(4-Nitrophenyl)-5,6-dimethoxy-8-(benzyloxy)isoquinoline (23). From **20** was obtained **23** as a bright yellow crystalline solid (ether): yield 72%; mp 164–166 °C; NMR δ 8.49 (1 H, d, J = 6 Hz), 7.95 (1 H, d, J = 6 Hz), 7.90 (2 H, d, J = 8.7 Hz), 7.43 (2 H, d, J = 8.7 Hz), 7.22 (3 H, m), 6.92 (2 H, m), 6.79 (1 H, s), 4.80 (2 H, s), 4.05 (3 H, s), 3.97 (3 H, s); IR (cm^{-1}) 3000, 1610, 1555, 1520, 1450, 1400, 1350, 1235, 1130, 1110, 1040, 990, 935, 850. Anal. ($C_{24}H_{20}N_2O_5$) C, H, N.

1-(3-Nitrophenyl)-5,6-dimethoxy-8-(benzyloxy)isoquinoline (26) and 1-(3-Nitrophenyl)-4,5,6-trimethoxy-8-(benzyloxy)-3,4-dihydroisoquinoline (24). From **21** was obtained a mixture of **26** and **24**; yield 99%. After chromatography (alumina, C_6H_6), **26** appeared as orange-crystals (ether): mp 124–126 °C; NMR δ 8.53 (1 H, d, J = 6 Hz), 8.20 (1 H, m), 7.96 (1 H, d, J = 6 Hz), 7.77 (2 H, m), 7.28 (4 H, m), 6.93 (2 H, m), 6.82 (1 H, s), 4.83 (2 H, s), 4.06 (3 H, s), 3.99 (3 H, s); IR (cm^{-1}) 3030, 1605, 1550, 1515, 1440, 1385, 1340, 1240, 1115, 1090, 1000. Anal. ($C_{24}H_{20}N_2O_5$) C, H, N.

From **24** was prepared a bright yellow HCl salt (ether): mp 195–199 °C; NMR (DMSO-d_6) δ 8.34 (1 H, m), 8.03 (2 H, m), 7.62 (1 H, m), 7.19 (4 H, m), 6.8 (2 H, m), 5.01 (2 H, s), 4.89 (1 H, bs), 4.45 (1 H, bs), 4.27 (1 H, bs), 4.12 (3 H, s), 3.87 (3 H, s), 3.35 (3 H, s); IR (cm^{-1}) 2950, 1615, 1585, 1520, 1480, 1340, 1320, 1290, 1260, 1230, 1080, 1035, 980, 690. Anal. ($C_{25}H_{25}ClN_2O_6$) C, H, N.

1-(2-Nitrophenyl)-5,6-dimethoxy-8-(benzyloxy)isoquinoline (27) and 1-(2-Nitrophenyl)-4,5,6-trimethoxy-8-(benzyloxy)-3,4-dihydroisoquinoline (25). From **22** was prepared a mixture of **27** and **25**; yield 96%. After chromatography, **27** was obtained as a bright yellow crystalline solid (ether): mp 188–190 °C; NMR δ 8.49 (1 H, d, J = 6 Hz), 7.93 (1 H, d, J = 6 Hz), 7.66 (1 H, m), 7.30 (7 H, m), 7.00 (3 H, m), 6.70 (1 H, s), 4.75 (2 H, s), 3.99 (3 H, s), 3.97 (3 H, s); IR (cm^{-1}) 2960, 1605, 1550, 1510, 1450, 1350, 1230, 1110, 990, 930, 830. Anal. ($C_{24}H_{20}N_2O_5$) C, H, N.

Compound **25** was obtained as a pale yellow crystalline solid (ether): mp 197 °C dec; NMR δ 7.57 (2 H, m), 7.33 (4 H, m), 6.87 (3 H, m), 6.60 (1 H, s), 4.90 (1 H), 4.67 (2 H, s), 4.33 (1 H), 4.00 (3 H, s), 3.93 (3 H, s), 3.46 (3 H, s); IR (cm^{-1}) 2960, 1610, 1580, 1510, 1480, 1420, 1380, 1340, 1320, 1290, 1260, 1240, 1130, 1080, 1040, 970, 890, 840, 740, 690. Anal. ($C_{25}H_{24}N_2O_6$) C, H, N.

1-Benzyl-5,6-dimethoxy-8-(benzyloxy)isoquinoline (45). From **43** was obtained **45** as a tan crystalline solid (ether–ligroin): mp 126–128 °C; NMR δ 8.43 (1 H, d, J = 6 Hz), 7.80 (1 H, d, J = 6 Hz), 7.25 (8 H, m), 6.93 (2 H, m), 6.64 (1 H, s), 5.10 (2 H, s), 4.85 (2 H, s), 3.87 (6 H, s); IR (cm^{-1}) 3080, 1610, 1560, 1450, 1350, 1240, 1210, 1190, 1120, 1100, 1060, 1010, 960. Anal. ($C_{25}H_{23}NO_3$) C, H, N.

1-(2-Nitrobenzyl)-5,6-dimethoxy-8-(benzyloxy)isoquinoline (46). From **44** was obtained **46** as a greenish-yellow crystalline solid (ether): mp 143–145 °C; NMR δ 8.36 (1 H, d, J = 6 Hz), 7.96 (1 H, m), 7.79 (1 H, d, J = 6 Hz), 7.46–6.89 (8 H, m), 6.71 (1 H, s), 5.16 (2 H, s), 5.10 (2 H, s), 3.92 (6 H, s); IR (cm^{-1}) 1605, 1555, 1515, 1350, 1230, 1120, 1060, 1000, 750, 720. Anal. ($C_{25}H_{22}N_2O_5$) C, H, N.

1-Phenyl-5,6-dimethoxy-8-hydroxyisoquinoline (12). The mixture of **8** and **10** (2 g) was boiled under reflux with 6 N MeOH–HCl (50 mL) for 16 h. It was cooled and extracted at pH 7 with $CHCl_3$ and the extract concentrated to give **12**, as an orange-yellow solid: yield 85%; mp 225–227 °C; NMR δ 8.35 (1 H, d, J = 6 Hz), 7.34 (1 H, d), 7.38 (5 H, m), 6.77 (1 H, s), 3.90 (3 H, s), 3.83 (3 H, s); IR (cm^{-1}) 1610, 1560, 1495, 1475, 1450, 1400, 1360, 1265, 1225, 1170, 1175, 1100, 1030, 990, 845, 815, 770, 665. Anal. ($C_{17}H_{15}NO_3$) C, H, N.

With the same procedure, the following compounds were prepared: **13**, **28–30**, **47**, and **48**.

1-(2-Pyridyl)-5,6-dimethoxy-8-hydroxyisoquinoline (13). From **9** and **11** was prepared **13**, which was an orange crystalline solid (ether): yield 77%; mp 102–103 °C; NMR (DMSO-d_6) δ 8.65 (1 H, dd, J = 7.5, 1.5 Hz), 8.43 (1 H, d, J = 6 Hz), 8.03 (1 H, td, J = 7.5, 1.5), 7.91 (1 H, d, J = 6 Hz), 7.52 (2 H, m), 6.86 (1 H, s), 3.97 (3 H, s), 3.86 (3 H, s); IR (cm^{-1}) 1620, 1590, 1550, 1480, 1440, 1410, 1390, 1370, 1320, 1280, 1235, 1200, 1130, 1110, 1055, 1010, 980. Anal. ($C_{16}H_{14}N_2O_3$) C, H, N.

1-(4-Nitrophenyl)-5,6-dimethoxy-8-hydroxyisoquinoline (28). From **23** was prepared **28**. It was an orange crystalline solid (ether): yield 83%; mp 205 °C; NMR δ 8.44 (1 H, d, J = 6 Hz), 8.28 (2 H, d, J = 8.7 Hz), 7.83 (1 H, d, J = 6 Hz), 7.71 (2 H, d, J = 8.7 Hz), 6.86 (1 H, s), 3.94 (3 H, s), 3.84 (3 H, s); IR (cm^{-1}) 3480, 1610, 1520, 1400, 1350, 1240, 1220, 1200, 1130, 1110, 1050. Anal. ($C_{17}H_{14}N_2O_5$) C, H, N.

1-(3-Nitrophenyl)-5,6-dimethoxy-8-hydroxyisoquinoline (29). From **24** and **26** was obtained **29** as an orange crystalline solid (ether): yield 60%; mp 215 °C; NMR (DMSO-d_6) δ 8.37 (1 H, d, J = 6 Hz), 8.27 (1 H, m), 8.16 (1 H, m), 7.87 (1 H, m), 7.83 (1 H, d, J = 6 Hz), 7.58 (1 H, m), 6.82 (1 H, s), 3.94 (3 H, s), 3.87 (3 H, s); IR (cm^{-1}) 3090, 1610, 1560, 1530, 1485, 1455, 1400, 1350, 1235, 1110, 1050, 1000, 950, 940, 830, 810, 740, 710. Anal. ($C_{17}H_{14}N_2O_5$) C, H, N.

1-(2-Nitrophenyl)-5,6-dimethoxy-8-hydroxyisoquinoline (30). From **27** and **25** was prepared **30**. It was an orange-yellow crystalline solid (ether): yield 0.655 g (60%); mp 165–167 °C; NMR (DMSO-d_6) δ 8.35 (1 H, d, J = 6 Hz), 8.13 (1 H, dd, J = 7.5, 1.5 Hz), 7.81 (1 H, d, J = 6 Hz), 7.65 (2 H, td, J = 7.5, 1.5 Hz), 7.43 (1 H, dd, J = 7.5, 1.5 Hz), 6.71 (1 H, s), 3.90 (3 H, s), 3.87 (3 H, s); IR (cm^{-1}) 1605, 1550, 1510, 1460, 1440, 1380, 1340, 1230, 1120, 1100, 1035. Anal. ($C_{17}H_{14}N_2O_5$) C, H, N.

1-Benzyl-5,6-dimethoxy-8-hydroxyisoquinoline (47). From **45** was obtained **47** as a tan crystalline solid (ether): yield 71%; mp 224–226 °C; NMR (DMSO-d_6) δ 8.25 (1 H, d, J = 6 H), 7.65 (1 H, d, J = 6 Hz), 7.20 (5 H, bs), 6.84 (1 H, s), 4.85 (2 H, s), 3.90 (3 H, s), 3.78 (3 H, s); IR (cm^{-1}) 1610, 1560, 1480, 1450, 1350, 1250, 1220, 1195, 1060, 1010, 820, 736, 720. Anal. ($C_{18}H_{17}NO_3$) C, H, N.

1-(2-Nitrobenzyl)-5,6-dimethoxy-8-hydroxyisoquinoline (48). From **45** was prepared **48**. It was a tan crystalline solid (ether–ligroin): yield 72%; mp 123–125 °C; NMR (DMSO-d_6) δ 8.14 (1 H, m), 6.89 (1 H, s), 5.11 (2 H, s), 3.92 (3 H, s), 3.80 (3 H, s); IR (cm^{-1}) 3100, 1610, 1560, 1515, 1350, 1260, 1200, 1150, 820. Anal. ($C_{18}H_{16}N_2O_5$) C, H, N.

1-Phenyl-6-methoxy-7-bromoisoquinoline-5,8-dione (14). Bromine (0.3 mL) was added to **12** (0.32 g) in AcOH (5 mL). After 20 h, dilution with $CHCl_3$ and washing with cold aqueous $NaHSO_3$, aqueous $NaHCO_3$, and water gave $CHCl_3$ layer which was concentrated to give **14** as a bright yellow solid: yield 0.357 g (91%); mp 160 °C dec; NMR δ 9.04 (1 H, d, J = 6 Hz), 7.97 (1 H, d, J = 6 Hz), 7.47 (5 H, s), 4.31 (3 H, s); IR (cm^{-1}) 3080, 1670, 1650, 1585, 1555, 1435, 1390, 1330, 1245, 1100, 1030, 910, 835, 750, 740, 690. Anal. ($C_{16}H_{10}BrNO_3$) C, H, N, Br.

The same procedure was used to prepare the following bromoquinones: **15**, **31–33**, **49**, and **50**.

1-(2-Pyridyl)-6-methoxy-7-bromoisoquinoline-5,8-dione (15). From **13** was obtained **15** as a bright orange-yellow solid: yield 90%; mp 155–160 °C dec; NMR δ 9.05 (1 H, d, J = 6 Hz), 8.66 (1 H, dd, J = 7.5, 1.5 Hz), 8.02 (1 H, d, J = 6 Hz), 7.90 (1 H, td, J = 7.5, 1.5 Hz), 7.47 (2 H, m), 4.32 (3 H, s); IR (cm^{-1}) 1680, 1655, 1580, 1560, 1440, 1400, 1325, 1245, 1120, 1020. Anal. ($C_{15}H_9BrN_2O_3$) C, H, N, Br.

1-(4-Nitrophenyl)-6-methoxy-7-bromoisoquinoline-5,8-dione (31). From **28** was obtained **31**. It was a bright yellow crystalline solid: yield 85%; mp 195 °C dec; NMR (DMSO-d_6) δ 9.08 (1 H, d, J = 6 Hz), 8.30 (2 H, d, J = 8.7 Hz), 8.00 (1 H, d, J = 6 Hz), 7.69 (2 H, d, J = 8.7 Hz), 4.25 (3 H, s); IR (cm^{-1}) 1665, 1580, 1550, 1500, 1435, 1340, 1250, 1100, 1020, 920, 850, 830. Anal. ($C_{16}H_9BrN_2O_5$) C, H, N, Br.

1-(3-Nitrophenyl)-6-methoxy-7-bromoisoquinoline-5,8-dione (32). From **29** was obtained **32** as a bright yellow crystalline solid (ether): yield 89%; mp 198–200 °C; NMR (DMSO-d_6) δ 9.14 (1 H, d, J = 6 Hz), 8.34 (2 H, m), 8.05 (1 H, d, J = 6 Hz), 7.83 (2 H, m), 4.26 (3 H, s); IR (KBr) (cm^{-1}) 3120, 2980, 1670, 1580,

1525, 1350, 1340, 1280, 1120, 1075, 1025, 930, 860, 830. Anal. ($C_{16}H_9BrN_2O_5$) C, H, N.

1-(2-Nitrophenyl)-6-methoxy-7-bromoisoquinoline-5,8-dione (33). From **30** was prepared **33**. It was a greenish-yellow crystalline solid (ether): yield 82%; mp 172–174 °C; NMR δ 9.05 (1 H, d, J = 6 Hz), 8.3 (1 H, dd, J = 7.5, 1.5 Hz), 8.03 (1 H, d, J = 6 Hz), 7.72 (2 H, m), 7.33 (1 H, dd, J = 7.5, 1.5 Hz), 4.33 (3 H, s); IR (cm^{-1}) 1675, 1595, 1560, 1520, 1440, 1400, 1345, 1320, 1240, 1110, 1070, 1020, 920, 850, 830, 780, 750, 710, 690. Anal. ($C_{16}H_9BrN_2O_5$) C, H, N.

1-Benzyl-6-methoxy-7-bromoisoquinoline-5,8-dione (49). From **47** was prepared **49**. It was a greenish-yellow crystalline solid (ether): yield 85%; mp 85–88 °C; NMR δ 8.98 (1 H, d, J = 6 Hz), 7.85 (1 H, d, J = 6 Hz), 7.28 (5 H, m), 4.81 (2 H, s), 4.28 (3 H, s); IR (cm^{-1}) 1650, 1565, 1320, 1225, 1035. Anal. ($C_{17}H_{12}BrNO_3$) C, H, N.

1-(2-Nitrobenzyl)-6-methoxy-7-bromoisoquinoline-5,8-dione (50). From **48** was obtained **50** as a dark yellow-brown crystalline solid (ether): yield 91%; mp 153–155 °C; NMR δ 8.73 (1 H, d, J = 6 Hz), 8.26 (1 H, dd, J = 7.5, 1.5 Hz), 7.79 (1 H, d, J = 6 Hz), 7.51 (2 H, td, J = 7.5, 1.5 Hz), 7.31 (1 H, dd, J = 7.5, 1.5 Hz), 5.12 (2 H, s), 4.30 (3 H, s); IR (cm^{-1}) 1670, 1650, 1570, 1510, 1335, 1230, 1040, 780, 740, 725, 715. Anal. ($C_{17}H_{11}BrN_2O_5$) C, H, N.

1-Phenyl-6-methoxy-7-azidoisoquinoline-5,8-dione (16). Sodium azide (0.041 g) was added to a stirred solution of **14** (0.2 g) in DMF (5 mL). After 15 min, addition of water, extraction with EtOAc, and concentration of the EtOAc layer gave **16** as an orange~yellow crystalline solid (ether): yield 88%; mp 130 °C dec; NMR δ 9.0 (1 H, d, J = 6 Hz), 7.97 (1 H, d, J = 6 Hz), 7.45 (5 H, s), 4.15 (3 H, s); IR (cm^{-1}) 2120, 1660, 1585, 1555, 1435, 1390, 1330, 1245, 1100. The compound was unstable and was directly used in the next step.

A similar procedure was used to prepare the following azidoquinones: **16**, **17**, **34–36**, **51**, and **52**.

1-(2-Pyridyl)-6-methoxy-7-azidoisoquinoline-5,8-dione (17). From **15** was obtained **17** as an orange-red crystalline solid which slowly darkened on standing: yield 86%; mp 140 °C dec; NMR δ 9.0 (1 H, d, J = 6 Hz), 8.63 (1 H, dd, J = 7.5, 1.5 Hz), 7.98 (1 H, d, J = 6 Hz), 7.90 (1 H, td, J = 7.5, 1.5), 7.53 (2 H, m), 4.18 (3 H, s); IR (cm^{-1}) 3080, 2125, 1655, 1585, 1560, 1480, 1445, 1410, 1320, 1285, 1250, 1180, 1140, 1130, 1090, 1050, 960, 920, 750.

1-(4-Nitrophenyl)-6-methoxy-7-azidoisoquinoline-5,8-dione (34). From **31** was prepared **34**. It was an orange-brown unstable crystalline solid (ether): yield 85%; mp 150 °C dec; NMR (DMSO-d_6) δ 9.12 (1 H, d, J = 6 Hz), 8.33 (2 H, d, J = 8.7 Hz), 8.02 (1 H, d, J = 6 Hz), 7.84 (2 H, d, J = 8.7 Hz), 4.09 (3 H, s); IR (cm^{-1}) 3090, 2120, 1660, 1600, 1560, 1510, 1440, 1350, 1280, 1240, 1100, 855, 740.

1-(3-Nitrophenyl)-6-methoxy-7-azidoisoquinoline-5,8-dione (35). From **32** was obtained **35** as a red-orange crystalline solid (ether): yield 93%; mp 130 °C dec; NMR (DMSO-d_6) δ 9.10 (1 H, d, J = 6 Hz), 8.4 (2 H, m), 8.06 (1 H, m), 8.00 (1 H, d, J = 6 Hz), 7.78 (1 H, m), 4.08 (3 H, s); IR (cm^{-1}) 3100, 2960, 2120, 1675, 1595, 1550, 1525, 1435, 1340, 1280, 1240, 1110, 1070, 1040, 720.

1-(2-Nitrophenyl)-6-methoxy-7-azidoisoquinoline-5,8-dione (36). From **33** was prepared **36**. It was an orange-red unstable crystalline solid (ether): yield 83%; mp 115 °C dec; NMR δ 9.03 (1 H, d, J = 6 Hz), 8.30 (1 H, m), 8.03 (1 H, d, J = 6 Hz), 7.73 (2 H, m), 7.36 (1 H, m), 4.23 (3 H, s); IR (cm^{-1}) 3140, 2120, 1660, 1600, 1550, 1440, 1395, 1350, 1330, 1240, 1070, 940, 730. Anal. ($C_{16}H_9N_5O_5$) C, H, N.

1-Benzyl-6-methoxy-7-azidoisoquinoline-5,8-dione (51). From **49** was obtained **51** as an orange-red crystalline solid (ether): yield 90%; mp 84–86 °C; NMR δ 8.97 (1 H, d, J = 6 Hz), 7.86 (1 H, d, J = 6 Hz), 7.29 (5 H, m), 4.79 (2 H, s), 4.18 (3 H, s); IR (cm^{-1}) 2120, 1750, 1585, 1550, 1315, 1225, 1055. Anal. ($C_{17}H_{12}N_4O_3$) C, H, N.

1-(2-Nitrobenzyl)-6-methoxy-7-azidoisoquinoline-5,8-dione (52). From **50** was prepared **52**. It was a red-brown crystalline solid (ether): yield 85%; mp 115 °C dec; NMR δ 8.76 (1 H, d, J = 6 Hz), 8.11 (1 H, dd, J = 7.5, 1.5 Hz), 7.83 (1 H, d, J = 6 Hz), 7.56 (2 H, td, J = 7.5, 1.5 Hz), 7.36 (1 H, dd, J = 7.5, 1.5 Hz), 5.12 (2 H, s), 4.73 (3 H, s); IR (cm^{-1}) 3095, 2120, 1660, 1590, 1520, 1340, 1300, 1240, 1080, 860, 840, 730. Anal. ($C_{18}H_{12}NO_3$) C, H, N.

1-Phenyl-6-methoxy-7-aminoisoquinoline-5,8-dione (18). To a solution of **16** (0.1 g) in THF–MeOH (10 mL/0.5 mL) was added $NaBH_4$ (0.1 g). Addition of water, extraction with EtOAc, and concentration of the solvent layer gave **18** as a bright red crystalline solid (ether–ligroin): yield 0.081 g (90%); mp 168–170 °C dec; NMR (DMSO-d_6) δ 8.96 (1 H, d, J = 6 Hz), 7.89 (1 H, d, J = 6 Hz), 7.0 (2 H, b), 3.82 (3 H, s); IR (cm^{-1}) 3480, 3280, 3140, 2950, 1680, 1635, 1610, 1550, 1360, 1290, 1230, 1100. Anal. ($C_{16}H_{12}N_2O_3$) C, H, N.

With a similar procedure, the following aminoquinones were prepared: **19**, **37**, **38**, **42**, **53**, and **54**.

1-(2-Pyridyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (19). From **17** was prepared **19**. It was a blue-black solid: yield 82%; mp 160–162 °C; NMR (DMSO-d_6) δ 8.99 (1 H, d, J = 6 Hz), 8.56 (1 H, dd, J = 7.5, 1.5 Hz), 7.95 (1 H, d, J = 6 Hz), 7.93 (1 H, td, J = 7.5, 1.5 Hz), 7.52 (2 H, m), 7.03 (2 H, bs), 3.83 (3 H, s); IR (cm^{-1}) 3440, 3320, 1675, 1630, 1590, 1440, 1410, 1240. Anal. ($C_{15}H_{11}N_3O_3$) C, H, N.

1-(4-Nitrophenyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (37). From **34** was obtained **37** as a reddish-purple crystalline solid (ether): yield 90%; mp 270–271 °C dec; NMR (DMSO-d_6) δ 9.02 (1 H, bs), 8.31 (2 H, d, J = 8.7 Hz), 7.95 (1 H, d, J = 6 Hz), 7.79 (2 H, d, J = 8.7 Hz), 7.04 (2 H, bs), 3.83 (3 H, s); IR (cm^{-1}) 3480, 3365, 3090, 2968, 1675, 1620, 1560, 1510, 1440, 1410, 1350, 1240, 1100, 1000, 920, 860, 800, 740, 690. Anal. ($C_{16}H_{11}N_3O_5$) C, H, N.

1-(3-Nitrophenyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (38). From **35** was prepared **38**. It was a red crystalline solid (ether): yield 90%; mp 264–266 °C; NMR (DMSO-d_6) δ 9.03 (1 H, d, J = 6 Hz), 8.43 (2 H, m), 7.97 (1 H, d, J = 6 Hz), 7.71 (2 H, m), 7.11 (2 H, bs), 3.83 (3 H, s); IR (cm^{-1}) 3500, 3380, 1675, 1595, 1550, 1520, 1435, 1400, 1340. Anal. ($C_{16}H_{11}N_3O_5$) C, H, N.

1-(2-Aminophenyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (42). From **36** was obtained **42** as a red crystalline solid (ether): yield 90%; mp 220 °C (subl) and 250 °C; NMR (DMSO-d_6) 9.34 (1 H, d, J = 6 Hz), 9.02 (1 H, m), 8.77 (1 H, d, J = 6 Hz), 7.97 (2 H, bs), 3.92 (3 H, s); IR (cm^{-1}) 3480, 3360, 1630, 1610, 1540, 1440, 1220, 760, 720. Anal. ($C_{16}H_{13}N_3O_3$) C, H, N.

1-Benzyl-6-methoxy-7-aminoisoquinoline-5,8-dione (53). From **51** was prepared **53**. It was a bright red crystalline solid (ether): yield 90%; mp 163–165 °C; NMR (DMSO-d_6) δ 8.90 (1 H, d, J = 6 Hz), 7.82 (1 H, d, J = 6 Hz), 7.25 (5 H, s), 7.05 (2 H, b), 4.65 (2 H, 2), 3.80 (3 H, s); IR (cm^{-1}) 3420, 3350, 1670, 1650, 1610, 1560, 1220, 1010, 700. Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

1-(2-Nitrobenzyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (54). From **52** was obtained **54** as a dark red crystalline solid: yield 90%; mp 237–240 °C; NMR (DMSO-d_6) δ 7.61 (2 H, td, J = 7.5, 1.5 Hz), 7.37 (1 H, dd, J = 7.5, 1.5 Hz), 7.06 (2 H, bs), 4.97 (2 H, s), 3.83 (3 H, s); IR (cm^{-1}) 3480, 3380, 1670, 1640, 1600, 1550, 1520, 1350, 1235. Anal. ($C_{17}H_{13}N_3O_5$) C, H, N.

1-(2-Nitrophenyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (39). To **36** (0.1 g) in CH_2Cl_2 (8 mL) was added triphenylphosphine (0.075 g 1.1 equiv). After 30 min, the dark purple mixture was chromatographed (alumina) to obtain the triphenylphosphinimine which was stirred in THF (1 mL) and HOAc (1 mL). After 1 h, dilution with H_2O, extraction with $CHCl_3$, and chromatography of the extract (alumina) gave **39** as a purple solid (ether): yield 60%; mp 198–200 °C; NMR δ 8.99 (1 H, d, J = 6 Hz), 8.77 (1 H, m), 7.97 (1 H, d, J = 6 Hz), 7.81 (2 H, m), 7.50 (1 H, m), 6.96 (2 H, bs), 3.85 (3 H, s); IR (cm^{-1}) 3500, 3390, 1660, 1620, 1585, 1540, 1510, 1430, 1400, 1350, 1330, 1295, 1230, 1050. Anal. ($C_{16}H_{11}N_3O_5$) C, H, N.

1-(4-Aminophenyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (40). A mixture of **34** (0.2 g) and sodium dithionite (2 g) in 1:1 MeOH–H_2O was boiled under reflux for 30 min. Extraction with $CHCl_3$ gave **40** as a red crystalline solid (ether): yield 50%; mp 253–255 °C; NMR (DMSO-d_6) δ 8.85 (1 H, d, J = 6 Hz), 7.72 (1 H, d, J = 6 Hz), 7.30 (2 H, d), 6.97 (2 H, bs), 6.61 (2 H, d, J = 8.7 Hz), 5.41 (2 H, bs), 3.81 (3 H); IR (cm^{-1}) 3460, 3365, 1660, 1630, 1610, 1560, 1555, 1440, 1395, 1360, 1295, 1230, 1175, 1100. Anal. ($C_{16}H_{13}N_3O_3$) C, H, N.

A similar procedure was used for the preparation of the following: **41** and **55**.

1-(3-Aminophenyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (41). From **35** was obtained **41** as a red crystalline solid (ether): yield 46%; mp 215–217 °C; NMR (DMSO-d_6) δ 8.47 (1

H, d, J = 6 Hz), 7.79 (1 H, d, J = 6 Hz), 7.0 (4 H, m), 6.93 (2 H, b), 5.03 (2 H, bs), 3.78 (3 H, s); IR (cm^{-1}) 3480, 3360, 1675, 1630, 1595, 1550, 1445, 1400, 1360, 1310, 1240, 1195, 1090, 1000. Anal. ($C_{16}H_{13}N_3O_3$) C, H, N.

1-(2-Aminobenzyl)-6-methoxy-7-aminoisoquinoline-5,8-dione (55). From **52** was prepared **55**. It was a dark red crystalline solid (ligroin): yield 54%; mp 135 °C (subl) and 198 °C; NMR δ 8.83 (1 H, d, J = 6 Hz), 7.77 (1 H, d, J = 6 Hz), 7.50 (4 H, bm), 7.17 (2 H, bs), 4.03 (2 H, s); IR (cm^{-1}) 3405, 3320, 1620, 1590, 1520, 1430, 1240, 1040. Anal. ($C_{17}H_{15}N_3O_3$) C, H, N.

1-(4-Nitrophenyl)-6-(1-piperidinyl)-7-bromoisoquinoline-5,8-dione (61). Piperidine (0.1 mL) was added to **31** (0.1 g) in DMF (5 mL). The dark purple mixture was diluted with H_2O and extracted with $CHCl_3$. Concentration of the extract gave **61** as a violet crystalline solid (ether): yield 83%; mp 168–170 °C; NMR δ 9.06 (1 H, d, J = 6 Hz), 8.35 (2 H, d, J = 9 Hz), 8.0 (1 H, d, J = 6 Hz), 7.63 (2 H, d, J = 9 Hz), 3.6 (4 H, m), 1.78 (6 H, m); IR (cm^{-1}) 2950, 2860, 1670, 1640, 1570, 1540, 1510, 1340. Anal. ($C_{20}H_{16}BrN_3O_4$) C, H, N.

A similar procedure was used to prepare the quinones **57–60, 62,** and **63.**

1-(4-Nitrophenyl)-6-(methylamino)-7-bromoisoquinoline-5,8-dione (57). From **31** and methylamine was prepared **57**. It was a red solid (ether): yield 80%; mp 245 °C subl; NMR (DMSO-d_6) δ 9.0 (1 H, m), 8.3 (2 H, d, J = 9 Hz), 7.97 (1 H, m), 7.67 (2 H, d, J = 9 Hz), 3.3 (3 H, s); IR (cm^{-1}) 3360, 1670, 1640, 1600, 1500, 1340, 740. Anal. ($C_{16}H_{10}BrN_3O_4$) C, H, N.

1-(3-Nitrophenyl)-6-(methylamino)-7-bromoisoquinoline-5,8-dione (58). From **32** was obtained **58** as a red solid (ether): mp 250 °C dec; NMR (DMSO-d_6) δ 9.03 (1 H, d, J = 6 Hz), 8.3 (2 H, m), 7.97 (1 H, d, J = 6 Hz), 7.76 (2 H, m), 3.26 (3 H, s); IR (cm^{-1}) 3360, 1670, 1600, 1520, 1340, 740, 670. Anal. ($C_{16}H_{10}BrN_3O_4$) C, H, N.

1-(2-Nitrophenyl)-6-(methylamino)-7-bromoisoquinoline-5,8-dione (59). From **33** was prepared **59**. It was a dark red unstable solid (ether): yield 75%; mp 195 °C dec; NMR (DMSO-d_6) δ 8.99 (1 H, d, J = 6 Hz), 8.23 (1 H, dd, J = 7.5, 1.5 Hz), 7.97 (1 H, d, J = 6 Hz), 7.83 (2 H, td, J = 7.5, 1.5 Hz), 7.4 (1 H, dd, J = 7.5, 1.5 Hz), 3.3 (3 H, s); IR (cm^{-1}) 3360, 1670, 1590, 1510, 1340, 740. Anal. ($C_{16}H_{10}BrN_3O_4$) C, H, N.

1-Benzyl-6-(methylamino)-7-bromoisoquinoline-5,8-dione (60). From **49** was obtained **60** as a red solid (ether): yield 85%; mp 95–98 °C; NMR (DMSO-d_6) δ 8.87 (1 H, d, J = 6 Hz), 7.75 (1 H, d, J = 6 Hz), 7.23 (5 H, s), 4.75 (2 H, s), 3.23 (3 H, s); IR (cm^{-1}) 3360, 1675, 1640, 1600, 1520, 1300, 1110, 700, 690. Anal. ($C_{17}H_{13}BrN_2O_2$) C, H, N.

1-(2-Nitrophenyl)-6-(dimethylamino)-7-bromoisoquinoline-5,8-dione (62). From **33** was prepared **62**. It was a dark purple crystalline solid (ether–ligroin): yield 77%; mp 127 °C; NMR δ 8.97 (1 H, d, J = 6 Hz), 8.26 (1 H, dd, J = 7.5, 1.5 Hz), 7.93 (1 H, d, J = 6 Hz), 7.67 (2 H, td, J = 7.5, 1.5 Hz), 7.33 (1 H, dd, J = 7.5, 1.5 Hz), 3.23 (6 H, s); IR (cm^{-1}) 1670, 1630, 1570, 1510, 1340, 1210. Anal. ($C_{17}H_{12}O_4N_3Br$) C, H, N.

1-(4-Nitrophenyl)-6-(methylamino)-7-azidoisoquinoline-5,8-dione (63). From **34** was obtained **63** as a dark blue crystalline solid (THF): yield 50%; mp 200 °C dec; IR (cm^{-1}) 3360, 2120, 1665, 1600, 1510, 1340. Anal. ($C_{16}H_{10}N_6O_4$) C, H, N.

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2′-Deoxy-2′-methylenecytidine and 2′-Deoxy-2′,2′-difluorocytidine 5′-Diphosphates: Potent Mechanism-Based Inhibitors of Ribonucleotide Reductase

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It has been found that 2′-deoxy-2′-methyleneuridine (MdUrd), 2′-deoxy-2′-methylenecytidine (MdCyd), and 2′-deoxy-2′,2′-difluorocytidine (dFdCyd) 5′-diphosphates (MdUDP (1) MdCDP (2) and dFdCDP (3), respectively) function as irreversible inactivators of the *Escherichia coli* ribonucleoside diphosphate reductase (RDPR). **2** is a much more potent inhibitor than its uridine analogue **1**. It is proposed that **2** undergoes abstraction of H3′ to give an allylic radical that captures a hydrogen atom and decomposes to an active alkylating furanone species. RDPR also accepts **3** as an alternative substrate analogue and presumably executes an initial abstraction of H3′ to initiate formation of a suicide species. Both **2** and **3** give inactivation results that differ from those of previously studied inhibitors. The potent anticancer activities of MdCyd and dFdCyd indicate a significant chemotherapeutic potential. The analogous RDPR of mammalian cells should be regarded as a likely target and/or activating enzyme for these novel mechanism-based inactivators.

Introduction

The most potent drug available for the treatment of adult acute leukemia is 1-(β-D-arabinofuranosyl)cytosine (araC).[1,2] Problems associated with araC arise from its rapid deamination to the therapeutically inactive uridine derivative and its ineffectiveness against solid tumors. Novel 2′-substituted cytidine derivatives have been synthesized recently and their antitumor activity investigated in the hope of finding an efficatious drug. Ueda and coworkers[3] and Samano and Robins[4] have recently reported

(1) Abbreviations used are as follows: araC, 1-(β-D-arabinofuranosyl)cytosine; MdCyd, 2′-deoxy-2′-methylenecytidine; MdUrd, 2′-deoxy-2′-methyleneuridine; dFdCyd, 2′-deoxy-2′,2′-difluorocytidine; MdCDP (**2**), MdUDP (**1**), and dFdCDP (**3**) are the corresponding nucleoside 5′-diphosphates, respectively; RDPR, ribonucleoside diphosphate reductase; XdNDP, 2′-deoxy-2′-halonucleoside 5′-diphosphate; TEAB, triethylammonium bicarbonate.

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